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[Authors]. [Abstract Title]. Program No. XXX.XX. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.1

Topic: B.07. Synaptic Transmission

Support: NSF CMMI 08-00870

NSF ECCS 08-01928

NIH 5R01NS063405-02

Title: Compressive forces disrupts vesicle dynamics in growth cones

Authors: *W. AHMED, S. RUBAKHIN, J. SWEEDLER, T. SAIF;
U. Illinois, Urbana, IL

Abstract: Mechanical cues from the cellular micro-environment such as external forces and substrate stiffness influence neuronal functions including neurite growth and pathfinding. It has recently been shown that the mechanical tension in neurons in vivo contributes to neurotransmitter clustering at the presynaptic terminal, suggesting a steady-state tension may be necessary for neuronal function [Siechen et al. PNAS 2009]. To investigate the effect of mechanical strain on in vitro vesicle dynamics, we use deformable cell culture substrates to apply large deformations to neurons of the Aplysia californica while observing with live-imaging using high-resolution optics. Specifically, we modified cell culturing protocols to allow Aplysia californica neurons to be cultured on thin polydimethylsiloxane substrates. The cells adhere well to the substrate and remain adhered and viable under large stretches. The substrate stretch generates controllable forces on the cell processes. We show that mechanical strain has a significant effect on vesicle motion in the growth cones of Aplysia neurons. More specifically, vesicle run length decreases severely upon application of compressive strain or relaxation of rest tension in the neurites. These results show that mechanical strain affects vesicle dynamics of cultured neurons, which may be due to the perturbation of the molecular mechanisms of vesicular transport and/or anchoring.

Disclosures: W. Ahmed, None; **S. Rubakhin**, None; **J. Sweedler**, None; **T. Saif**, None.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.2

Topic: B.07. Synaptic Transmission

Support: ISF Grant 993/08

ISF Grant 170/08

BSF Grant 2007199

Title: Basal GABA regulates GABA(B)R conformation and release probability at single hippocampal synapses

Authors: *I. SLUTSKY[1], T. LAVIV[1], I. RIVEN[1], I. RIVEN[1], I. DOLEV[1], I. VERTKIN[1], B. BALANA[2], P. SLESINGER[2];
[1]Physiol. & Pharmacol., Tel Aviv Univ., Tel Aviv, Israel; [2]Peptide Biol. Lab., The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Presynaptic $GABA_B$ receptor ($GABA_BR$) heterodimers are composed of GB_{1a}/GB_2 subunits and critically influence synaptic and cognitive functions. Here we explored local $GABA_BR$ activation by integrating optical tools for monitoring receptor conformation and synaptic vesicle release at individual presynaptic boutons of hippocampal neurons. Utilizing fluorescence resonance energy transfer (FRET) spectroscopy, we detected a wide range of FRET values for CFP/YFP-tagged GB_{1a}/GB_2 receptors in excitatory boutons that negatively correlated with release probabilities at single synapses. High FRET of $GABA_BRs$ associated with low release probability. Notably, pharmacological manipulations that either reduced or increased basal receptor activation decreased inter-synapse variability of GB_{1a}/GB_2 receptor conformation. Despite variability along axons, presynaptic $GABA_BR$ tone was dendrite-specific, having a greater impact on synapses at highly innervated proximal branches. Prolonged neuronal inactivity reduced basal receptor activation, leading to homeostatic augmentation of release probability. Our findings suggest that local variations in basal GABA concentration are a major determinant of GB_{1a}/GB_2 conformational variability, which contributes to heterogeneity of neurotransmitter release at hippocampal synapses.

Disclosures: I. Slutsky, None; **T. Laviv**, None; **I. Riven**, None; **I. Riven**, None; **I. Dolev**, None; **I. Vertkin**, None; **B. Balana**, None; **P. Slesinger**, None.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.3

Topic: B.07. Synaptic Transmission

Support: NIH grants

Title: Cathepsin v participates in proneuropeptide processing to generate the enkephalin opioid peptide for neurotransmission

Authors: *L. M. FUNKELSTEIN[1], T. TONEFF[1], D. LU[1], C. MOSIER[1], T. REINHECKEL[2], C. PETERS[2], V. HOOK[1];
[1]Skaggs Sch. Pharm., UCSD, LA JOLLA, CA; [2]Inst. fur Molekulare Medizin und Zellforschung, Freiburg, Germany

Abstract: This study demonstrates the novel role of the cysteine protease cathepsin V as a candidate proneuropeptide processing enzyme for production of the neurotransmitter enkephalin. Proneuropeptide precursors are processed by proteases to produce active enkephalin and related opioid neuropeptides in the nervous system for analgesia. We have demonstrated that the related cathepsin L functions in secretory vesicles as a significant proneuropeptide processing enzyme to generate endogenous opioid peptides, illustrated by gene knockout and expression studies combined with molecular neurobiological strategies (Yasothornsrikul et al., 2003; Funkelstein et al., 2008; Minokadeh et al., 2010).
The human cathepsin V cysteine protease shares high homology with mouse cathepsin L (75% sequence identity). Cathepsin V shares similar functions with mouse cathepsin L, as shown in genetic rescue (Hageman et al, 2004; Sevenich et al., 2010) and biochemical (Bromme et al., 1999) studies. Therefore, we investigated cathepsin V as a candidate proneuropeptide processing enzyme to produce the enkephalin neuropeptides. Expression of cathepsin V in neuroendocrine cell lines resulted in conversion of proenkephalin (PE) to a 23 kDa high molecular weight intermediate and production of (Met)enkephalin. In human neural cells, reduction of endogenous cathepsin V by siRNA led to reduced levels of (Met)enkephalin. In vitro PE processing assays also showed that cathepsin V converts PE to a 23 kDa PE-derived intermediate that is present in human brain. In addition, cathepsin V also participates in production of neuropeptide Y (NPY) from proNPY in human neuroblastoma cells. The colocalization of cathepsin V with enkephalin in neuropeptide secretory vesicles is consistent with the candidate proneuropeptide processing function of cathepsin V. Moreover, cathepsin V is present in isolated human adrenal medullary chromaffin secretory vesicles and in human brain. These findings illustrate the novel biological

role of cathepsin V, like cathepsin L, in secretory vesicles for enkephalin and neuropeptide production for neurotransmission.

Disclosures: L.M. Funkelstein, None; **T. Toneff**, None; **D. Lu**, None; **C. Mosier**, None; **T. Reinheckel**, None; **C. Peters**, None; **V. Hook**, None.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.4

Topic: B.07. Synaptic Transmission

Support: NIH Grant NS052664-01

Title: dMiro's control of kinesin- and dynein-driven mitochondrial movements in axons of Drosophila

Authors: *M. BABIC[1], G. J. RUSSO[2], A. WELLINGTON[3], J. ZHANG[3], K. E. ZINSMAIER[3]; [1]Grad. Interdisciplinary Program in Neurosci., [2]Grad. Program in Biochem. and Mol. & Cell. Biol., [3]Dept. of Neurosci., Univ. of Arizona, TUCSON, AZ

Abstract: Neuronal excitability and synaptic function critically depend on local energy production by properly localized mitochondria. Thus, neuronal mitochondria are constantly transported into axons and dendrites, and back into the cell body. In axons, transport proceeds along uniformly plus-end-out oriented microtubules, and employs plus-end directed kinesin and minus-end directed dynein motors. While net anterogradely (AM) or retrogradely moving (RM) mitochondria exhibit saltatory motions in both directions due to the competing actions of these opposing motors, their net movement is strongly biased towards the desired direction. Axonal AM mitochondria spend over 60% of their time employing kinesin motions, and less than 10% in dynein motions. RM mitochondria exhibit the opposite. Hence, effective transport in a given direction is largely determined by a directional program that defines how long a mitochondrion recruits a particular motor activity. The molecular nature of this program is poorly understood. Our previous work showed that the mitochondrial Rho-like GTPase dMiro is critical for executing directional programming by increasing basic motor activities of dynein or kinesin (1,2). To determine how dMiro controls both motors mechanistically, we generated mutations in each of dMiro's two GTPase domains, locking the domain in a GTP-, GDP-, or no nucleotide-bound form and examined which defects of the null mutant can be rescued (or not) by mutant protein expression. Additionally, we introduced mutations in both EF-hand domains of dMiro

that abolish Ca^{2+} binding.
Expression of mutant dMiro containing a GDP-locked GTPase domain 1 restores the directional program and net velocity of RM (but not AM) mitochondria by permitting normal dynein-driven motions. In contrast, a GTP-locked form partially restores the directional program and net velocity of AM (but not RM) mitochondria by increasing the number of kinesin motions such that the resulting series of motions produces a normal net velocity. Mutations in the 2nd GTPase domain also impair AM and RM transport, mostly by interfering with stop and reversal frequency of both motors. Expression of EF-hand mutant dMiro restores an essentially normal directional program and net velocity of both antero- and retrograde transport. However, axonal mitochondria gradually become dysfunctional and degraded such that only very few mitochondria return to the cell body. We suggest that dMiro may regulate kinesin and dynein motors in a switch-like manner to achieve effective net-directional mitochondrial transport.

References

1. Guo et al (2005) Neuron 47: 379.
2. Russo et al (2009) J Neurosci 27: 5443.

Disclosures: M. Babic, None; **G.J. Russo**, None; **A. Wellington**, None; **J. Zhang**, None; **K.E. Zinsmaier**, None.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.5

Topic: B.02. Ligand Gated Ion Channels

Support: NIMH Grant MH066892

AHA Grant 09PRE2080126

Title: Modulation of NMDA receptor gating through the linkers connecting the ligand-binding and ion channel domains

Authors: *I. TALUKDER[1], P. BORKER[2], L. P. WOLLMUTH[1];
[1]Dept. of Neurobio. and Behavior, [2]Stony Brook Univ., Stony Brook, NY

Abstract: Gating in the NMDA receptor is initiated in the extracellular ligand-binding domain (LBD) and is ultimately propagated via three linkers—S1-M1, M3-S2 and S2-M4—to the ion channel. M3-S2, through its connection to the transmembrane segment M3, the main channel

gating element, directly couples LBD movements into channel gating, but the functional contributions of S1-M1 and S2-M4 to the overall gating process are unknown. We therefore made cysteine substitutions, one at a time, in the S1-M1 and S2-M4 linkers of NMDA receptors composed of GluN1-GluN2C and probed these cysteine-substituted receptors with the bulky cysteine-reactive reagent—3-(Triethylammonium)propyl Methanthiosulfonate bromide (PTrEA). Covalent modifications of certain positions with PTrEA yielded receptors with potentiated ligand-activated whole-cell currents. All PTrEA-modified receptors that showed current potentiation were more rapidly blocked by MK801, an irreversible open channel pore blocker whose reaction kinetics can index channel open probability, than their unmodified counterparts. These results suggest that whole-cell current potentiation is driven by an increase in channel open probability. Indeed, when tested, PTrEA modification at the potentiation positions caused single channel activity to increase, without affecting channel conductances. Additionally, the mean open times of the unmodified and PTrEA-modified receptors were comparable, implying that the increase in channel open probability arises from a destabilization of the closed state. We conclude that PTrEA-induced potentiation through these select sites within S1-M1 and S2-M4 is driven by a gating effect of shifting the energetic equilibrium towards the channel open state. The magnitude of this gating effect was dependent on the intrinsic gating properties of the NMDA receptor subunit composition, being more pronounced on the inherently low open probability GluN2C- than the much higher open probability (and more generally expressed in the CNS) GluN2A- containing receptors. For the majority of these positions, we propose that alteration of gating is elicited by steric destabilization of contact interfaces where close apposition of the contacting partners is necessary for efficient channel closure. This study, for the first time, implicate the dynamics of the NMDA receptor S1-M1 and S2-M4 linkers as modulators of the overall gating process and also conceptualize a non-competitive and subunit-specific mechanism to affect receptor function.

Disclosures: I. Talukder, None; **P. Borker**, None; **L.P. Wollmuth**, None.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.6

Topic: B.02. Ligand Gated Ion Channels

Support: ANR

HFSP

FRM

Conseil Regional Aquitaine

BBSCR

Title: Functional role of synaptic NMDA receptor dynamics

Authors: *L. G. GROC[1], M. SAINLOS[2], L. LADEPECHE[1], L. MIKASOVA[1], S. COUSINS[3], F. STEPHENSON[3], B. IMPERIALI[2], D. CHOQUET[1], L. BARD[1];
[1]CNRS-Unive Bordeaux 2, Bordeaux, France; [2]MIT, Cambridge, MA; [3]Univ. of London, London, United Kingdom

Abstract: The NMDA receptor (NMDAR) plays a key role in synaptic maturation and plasticity. The mechanism that regulate the synaptic content of the different NR2A- or NR2B-NMDAR subtypes are however still unclear. We previously reported that surface NMDARs have differential surface mobility depending on their NR2 subunit composition. By engineering a divalent membrane permeable peptide carrying the last amino acids of the C-terminal domain of NR2A subunit, we showed that such biomimetic ligand significantly increased the surface mobility of 2A-NMDARs and reduced their synaptic content. These effects were specific for 2A-NMDARs since there was no effect on other glutamatergic receptors or on potassium channels bearing similar C-terminus sequence. Using this unique cellular tool, we then explored the role of 2A-NMDAR during synaptic maturation and plasticity processes. Preventing the clustering of 2A-NMDARs reduced the number of mature spines in developing hippocampal neurons. In addition, long-term plasticity was affected in these conditions. All together, these data indicate that NR2A-NMDARs are dynamically retained within the synapse and that controlling 2A-NMDAR synaptic retention impacts on the dynamic range of synaptic adaptation.

Disclosures: L.G. Groc, None; **M. Sainlos**, None; **L. Ladepeche**, None; **L. Mikasova**, None; **S. Cousins**, None; **F. Stephenson**, None; **B. Imperiali**, None; **D. Choquet**, None; **L. Bard**, None.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.7

Topic: B.07. Synaptic Transmission

Support: National Institute for Neurological Disorders and Stroke R21NS06637

Title: Screening for marine neuromodulatory drugs using Drosophila melanogaster

Authors: *M. MEJIA[1], M. D. HEGHINIAN[2], F. MARI[2], C. ARMISHAW[4], T. A. GODENSCHWEGE[3];
[1]Florida Atlantic Univ., BOCA RATON, FL; [2]Chem. and Biochem., [3]Biol. Sci., Florida Atlantic Univ., Boca Raton, FL; [4]Torrey Pines Inst. for Mol. Studies, Port Saint Lucie, FL

Abstract: The venom of marine cone snails is comprised of a large mélange of neuroactive peptides known as conotoxins. Isolated conotoxins have been shown to elicit a wide range of physiological effects and are of great scientific interest. Currently, there are around 500,000 different conopeptides that can be isolated from several species, but only 200 have been characterized, such as the powerful painkiller PrialtTM - an ω-conotoxin that targets voltage-gated calcium channels. Previous screening methods for conotoxins include patch clamping and in vivo injections in vertebrate animals. However, such techniques are not optimal as fast and reliable screening methods. We have perfected an unbiased screening method for novel potential drugs, such as conotoxins, for effects on a variety of molecular targets on a simple neuronal circuit in Drosophila melanogaster, known as the Giant Fiber System (GFS). The GFS encompasses electrical synapses and a variety of chemical synapses as well as different types of neurons and muscles. Non-invasive administration of nanomolar quantities of conopeptides by injections into the animal while simultaneous monitoring for changes of circuit function using electrophysiology allows us to rapidly detect bioactivity in vivo. Here, we describe the effects of the previously well-characterized α-conotoxin, ImI, on the function of the GFS and compare it with chemically-modified analogues constructed to have a higher affinity for human α7 nicotinic acetylcholine receptor. In addition, we have screened several HPLC- separated fractions of the venom of Conus brunneus. Here we describe several novel conopetides isolated from the venom that revealed bioactivity in our assay.

Disclosures: M. Mejia, None; **T.A. Godenschwege**, None; **M.D. Heghinian**, None; **F. Mari**, None; **C. Armishaw**, None.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.8

Topic: B.07. Synaptic Transmission

Support: NIH Fellowship F32DA024960

NIH Grant R01DA04271

Title: Enkephalin neuropeptide biosynthesis via proteolytic processing of proenkephalin by cathepsin L and cathepsin H for neurotransmission

Authors: *W. D. LU[1], L. FUNKELSTEIN[1], C. DIEHL[2], V. HOOK[1,2,3];
[1]Sch. of Pharm., Univ. Calif. San Diego, LA JOLLA, CA; [2]Sch. of Med., [3]Depts. of Neurosci., Univ. Calif. San Diego, La Jolla, CA

Abstract: Active secreted neuropeptides involved in cell-cell communication in neuroendocrine systems are produced from larger, inactive proneuropeptide precursors through proteolytic processing of multiple dibasic cleavage sites (e.g. Lys-Arg, Arg-Arg) by a sequence of secretory vesicle proteases. Herein, we identified cathepsin H as a secretory vesicle aminopeptidase that produces (Met)enkephalin (ME) peptides from proenkephalin cleavage intermediates that model post cathepsin L endoproteolytic processing. Initial endoproteolytic cleavage by the cysteine protease cathepsin L at paired basic residues generates peptide intermediates that possess basic residue extensions at the amino (NH_2) terminus. Removal of these extensions is required to produce active neuropeptides and is facilitated by aminopeptidase enzymes. The role of localized aminopeptidase enzymes in chromaffin granules were previously described by this group. The intent of this study was to characterize the role of cathepsin H as a secretory vesicle aminopeptidase that processes enkephalin intermediates with NH_2-terminal arginine and/or lysine extensions to the active ME neuropeptide. Within, is described the co-localization of the cysteine protease cathepin H with the neuropeptide ME in secretory vesicles through immunostaining of primary culture of bovine adrenal medulla chromaffin cells. Moreover, the kinetic parameters determined in this study for cathepsin H aminopeptidase activity on NH_2-terminal arginine and lysine fluorogenic substrates are comparable to those previously found for aminopeptidase activity in bovine adrenal medulla chromaffin granules. Cathepsin H aminopeptidase activity was selective for arginine and lysine NH_2-terminal fluorogenic substrates (R-MCA and K-MCA, respectively) among the 20 aminoacyl-methylcoumarylamide (MCA) substrates. The inhibition profile of cathepsin H by aminopeptidase inhibitors armastatin, bestatin, and arphamenine was comparable to the profile previously observed for the aminopeptidase(s) in bovine adrenal medulla chromaffin granule. In vitro studies of cathepsin H and NH_2-terminal monobasic and dibasic arginine and lysine on ME peptide (K-ME, R-ME, KR-ME, and KK-ME) demonstrated cleavage of the model peptide intermediates in a sequential and temporal manner to the active ME peptide as analyzed by HPLC and mass spectrometry (MS) and cleavage products sequenced and identified through tandem MS. The data suggest a role for cathepsin H as a secretory vesicle aminopeptidase involved with secondary processing of neuropeptide intermediates to active neuropeptides involved in cell-cell communication.

Disclosures: W.D. Lu, None; **L. Funkelstein**, None; **C. Diehl**, None; **V. Hook**, None.

Nanosymposium

10. Modulation of Synaptic Transmission

Location: Room 25A

Time: Saturday, November 13, 2010, 1:00 pm - 3:15 pm

Program Number: 10.9

Topic: B.07. Synaptic Transmission

Title: Fluoxetine prevents stimulation-dependent fatigue of synaptic vesicle exocytosis

Authors: *A. W. HENKEL[1,2], O. WELZEL[2], T. W. GROEMER[2], J. KORNHUBER[2];
[1]Physiol., Kuwait Univ., Safat, Kuwait; [2]Psychiatry, Univ. Hosp., Erlangen, Germany

Abstract: Effects of the antidepressant fluoxetine on stimulation-dependent synaptic vesicle exocytosis were analyzed in cultured primary hippocampal neurons. Exocytosis was triggered by electric field stimulation and imaged by fluorescence microscopy. Synaptic vesicles were fluorescently labeled and destained with FM 1-43 in two consecutive cycles and several kinetic parameters from both trials were compared. In control preparations, the second staining-destaining cycle caused a significant reduction of relative fluorescence loss, number of active synapses and fluorescence half-decay time. These fatigue effects were largely prevented by short-term administration of 1 μM fluoxetine, which was present before and during the second stimulation cycle. Fluoxetine concentrations above 10 μM inhibited exocytosis almost completely but showed no other toxic effects on neurons. Stressed neurons, grown under hyperosmotic conditions, were even more fatigue-protected by fluoxetine. These observations support the idea that therapeutic concentrations of fluoxetine enhance the recovery of neurotransmission and this effect might contribute to the abuse of fluoxetine (Prozac) as psychostimulant.

Disclosures: A.W. Henkel: None. **O. Welzel:** None. **T.W. Groemer:** None. **J. Kornhuber:** None.

Nanosymposium

11. APP Metabolism and Processing

Location: Room 31C

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 11.1

Topic: C.02. Alzheimer's disease and other dementias

Support: CIHR

Title: Amyloid precursor protein is rapidly internalized to the lysosome in a novel clathrin-independent lipid-raft associated pathway

Authors: *W. TANG[1,2], S. O. MEAKIN[2], S. H. PASTERNAK[3,2];
[1]Mol. Brain Res. Group, [2]Robarts Res. Inst., London, ON, Canada; [3]Clin. Neurolog. Sciences, Cognitive Neurol. and Alzheimer Res. Ctr., London, ON, Canada

Abstract: Alzheimer's Disease (AD) is the most common form of neurodegenerative illness in adults. It is characterized by the deposition of large Beta-Amyloid (Aβ) peptide plaques in the brain, resulting in neuronal dysfunction and death. Currently, there is no consensus as to the intracellular compartment(s) in which APP is processed into Aβ. Several studies have suggested that lipid rafts and their associated proteins, such as flotillin, may play a key role in mediating APP internalization in neuronal cells. Previous studies in our lab have shown that lysosomes are highly enriched in APP and the gamma-secretase complex responsible for Aβ production, suggesting that lysosomes participate in the processing of APP. Furthermore, we have recently uncovered a novel sorting pathway that rapidly traffics APP directly to the lysosome from the cell surface, bypassing the early and late endosome stages. This pathway is highly selective in that it excludes APP bearing either the London or Swedish mutations associated with familial AD. We hypothesize that wt-APP is internalized directly to the lysosomes via a clathrin-independent lipid-raft associated mechanism. To characterize the internalization of APP, we used APP constructs with an N-terminal HA-tag, allowing us to label APP at the cell surface with a fluorescent antibody. Time course experiments were then performed in SN56 cells using confocal microscopy to observe the transport of surface-labeled APP to early endosomes and lysosomes, identified using fluorescent protein-tagged Rab5 and LAMP1 respectively. Here, we show that cell surface-labeled wt-APP is rapidly trafficked to LAMP1-positive compartments from the cell surface. This phenomenon persists when clathrin-dependent endocytosis is blocked by treating cells with the potent dynamin inhibitor dynasore. Confocal microscopy analysis of cells co-transfected with green fluorescent protein-tagged flotillin-1 and the lysosome marker LAMP1 demonstrates that internalized APP colocalizes with flotillin-1 in LAMP1-positive vesicles at very early time points. Taken together, this suggests that wt-APP is internalized directly to the lysosomes via a clathrin-independent lipid-raft associated mechanism.

Disclosures: W. Tang: None. **S.O. Meakin:** None. **S.H. Pasternak:** None.

Nanosymposium

11. APP Metabolism and Processing

Location: Room 31C

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 11.2

Topic: C.02. Alzheimer's disease and other dementias

Support: DFG PI 379/5-1

Title: APP dimer formation in an oxidizing environment, provided by the endoplasmatic reticulum

Authors: S. ISBERT[1], D. KADEN[2], G. MULTHAUP[2], S. WEGGEN[3], S. KINS[4], *C. U. PIETRZIK[5];
[1]Inst. of Pathobiochemistry, Univ. Med. Ctr. of the Johannes Gutenberg-University, Mainz, Germany; [2]Inst. of Chem. and Biochem., Freie Univ. Berlin, Berlin, Germany; [3]Dept. of Neuropathology, Heinrich-Heine-University, Duesseldorf, Duesseldorf, Germany; [4]Dept. of Human Biol. and Human Genet., Tech. Univ. of Kaiserslautern, Kaiserslautern, Germany; [5]Univ. Mainz, Mainz, Germany

Abstract: The Amyloid precursor protein (APP) is part of a larger gene family, which has been found to form homo- or hetero-complexes with its homologues, although the exact molecular mechanism of dimer formation remains elusive. Our study was designed to address the origin of APP/APLP dimer formation and to elucidate the consequences on mutual processing. To define the subcellular compartment where APP dimerization might originate, we fused dilysine based retention motifs (KKAA-Endoplasmatic Reticulum ER; KKFF-Golgi) to the C-terminus of APP isoforms, inhibiting trafficking to the plasma membrane and retaining APP in intracellular compartments. After generating stable overexpressing cell lines, retention was verified biochemically and via confocal images. Interestingly we detected an explicit slower migrating band under non reducing conditions in cells stable expressing the ER retention construct APP695KKAA, compared to APP695wt cells, or the *Golgi* retained APP, representing intracellular SDS stable APP homodimers. At the same time we monitored a drastic drop of APP metabolites (A-beta/AICD/APPs), indicating that dimerization occurs before *secretase* cleavage and full maturation, accompanied by the absence of fully glycosylated APP. However reducing agents and simultaneous heat denaturing effectively disrupted dimer formation. Additionally we demonstrate that APP homodimerization was prevented already in the ER when APP ectodomain E1 deletion constructs (APPΔE1KKAA) were used. Consequently we reason APP dimerization is initiated and sustained via intermolecular disulfide linkage, mediated by the E1 APP ectodomain. Therefore we postulate that cysteine residues, only present in the E1 domain, can form dimers in an oxidative environment as provided by the ER. Moreover we provide evidence that APLPs dimerize in the same fashion as APP. Additionally we investigated the dimerization properties of different APP isoforms. We could clearly show that APP isoforms expressing the (Kunitz Protease Inhibitor) KPI domain exhibit a dramatically reduced capacity for dimerization compared to non-KPI containing APP695 isoform. Therefore we show for the first time that dimerization properties differ between neuronal APP695 and the KPI-domain containing peripheral isoforms and that dimer formation occurs en route to the cell surface, initiated in the ER.

Disclosures: S. Isbert, None; **C.U. Pietrzik**, None; **D. Kaden**, None; **G. Multhaup**, None; **S. Weggen**, None; **S. Kins**, None.

Nanosymposium

11. APP Metabolism and Processing

Location: Room 31C

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 11.3

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Clinical Pharmacology Training Grant T32 GM08685

HL-53524

Mayo Foundation

Title: Endothelial nitric oxide modulates expression and processing of amyloid precursor protein

Authors: *S. A. AUSTIN, A. V. SANTHANAM, Z. S. KATUSIC;
Departments of Anesthesiol. and Mol. Pharmacol. and Exp.Therap., Mayo Clin., Rochester, MN

Abstract: Endothelial dysfunction, specifically loss of endothelial-derived nitric oxide (NO), is a common feature of several cardiovascular risk factors associated with developing mild cognitive impairment and Alzheimer's disease (AD). Therefore, we sought to determine the relationship between endothelial derived NO and the expression and processing of amyloid precursor protein (APP). Utilizing human brain microvascular endothelial cells (BMEC), we inhibited generation of NO by treating with N-Nitro-L-Arginine Methyl Ester (L-NAME, $3x10^{-4}$ M), a nitric oxide synthase (NOS) inhibitor. Inhibition of endothelial NOS led to increased expression of APP and beta-site APP cleaving enzyme (BACE)1, the enzyme involved in generating the amyloidogenic peptide, beta amyloid (Aβ). Furthermore, NOS inhibition of BMEC led to significantly increased secretion of both Aβ1-40 (L-NAME 168 $\pm$ 27.38 pg/mL vs. control 10.93 $\pm$ 0.70 pg/mL; n=4; $P<0.001$) and Aβ1-42 (L-NAME 208 $\pm$ 31.73 pg/mL vs. control 0.76 $\pm$ 0.98 pg/mL; n=4; $P<0.001$). To assess whether the effect of NO on APP and BACE1 expression was mediated by guanylyl cyclase generated cGMP, we next treated BMEC with 1*H*-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10^{-6} M), a highly selective inhibitor of soluble guanylyl cyclase. Indeed, inhibition of guanylyl cyclase led to increased APP and BACE1 protein expression similar to that seen with NOS inhibition. Furthermore, BMEC treated with sildenafil (10^{-6} M), a phosphodiesterase 5 inhibitor which increases cGMP levels, led to decreased APP and BACE1 protein expression. To determine if the loss of endothelial-derived NO had any consequence in

vivo, we examined brain and microvascular tissue from wild-type and $eNOS^{-/-}$ mice. Brain tissue from $eNOS^{-/-}$ mice had statistically higher APP, BACE1 and Aβ levels as compared to wild-type mice (n= 6-8). Furthermore, brain microvessels also had statistically higher BACE1 expression as compared to control. Our data suggest that endothelial NO plays an important role in suppressing APP, BACE1 and Aβ levels within the brain and cerebrovasculature. The NO/cGMP pathway may be an important therapeutic target in preventing and treating mild cognitive impairment as well as AD.

Disclosures: S.A. Austin, None; **A.V. Santhanam**, None; **Z.S. Katusic**, None.

Nanosymposium

11. APP Metabolism and Processing

Location: Room 31C

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 11.4

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH 5T32AG000222-18

Funds for Medical Discovery Award from Harvard Medical School and Massachusetts General Hospital

Neurodegenerative Disease Pilot Study Grant Program from Harvard NeuroDiscovery Center and Massachusetts Alzheimer's Disease Research Center

Title: Loss-of-function of atxn1 increases a-beta levels by potentiating beta-secretase processing of the amyloid-beta precursor protein

Authors: *C. ZHANG[1], B. ANDREW[2], J. SUH[2], R. TANZI[2];
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Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease with strong and complex genetic inheritance. Four genes have been established to either cause familial early-onset AD (APP, PSEN1 and PSEN2) or to increase susceptibility for late-onset AD (APOE). To date approximately 80% of the late-onset AD genetic variance remains elusive. Recently our genome-wide association screen (GWAS) identified four novel late-onset AD candidate genes. Ataxin 1 (ATXN1) is one of these four AD candidate genes and has been indicated to be the disease gene for spinocerebellar ataxia type 1 (SCA1), which is also a neurodegenerative disease.

Mounting evidence suggests that the excessive accumulation of Aβ, the proteolytic product of β-amyloid precursor protein (APP), is a primary pathological event in AD. In this study, we asked whether ATXN1 may lead to AD pathogenesis by affecting Aβ and APP processing through its down-regulation in vitro (in human neuronal cell models and mouse primary cortical neurons) and in vivo (in ATXN1 knockout mice). We show that knock-down of ATXN1 significantly increases the levels of both Aβ40 and Aβ42 in vitro. This effect could be rescued with concurrent overexpression of ATXN1. Moreover, overexpression of ATXN1 decreased Aβ levels. Regarding the underlying molecular mechanism, we show that the effect of ATXN1 expression on Aβ levels is modulated by β-secretase cleavage of APP in vitro. Finally, we show that BACE1 and sAPPβ protein levels are up-regulated in vivo. Taken together, ATXN1 functions as a genetic risk modifier for AD; most likely through a loss-of-function mechanism leading to enhanced β-secretase cleavage of APP and elevated Aβ levels.

Disclosures: C. Zhang, None; **J. Suh**, None; **R. Tanzi**, None; **B. Andrew**, None.

Nanosymposium

11. APP Metabolism and Processing

Location: Room 31C

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 11.5

Topic: C.02. Alzheimer's disease and other dementias

Support: CIHR

Title: Live cell imaging demonstrates that APP is transported rapidly and directly from the Golgi to the lysosome

Authors: *J. TAM[1], S. H. PASTERNAK[1,2];
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Abstract: The production of β amyloid (Aβ) through cleavage of amyloid precursor protein (APP) is central to the pathogenesis of Alzheimer's disease (AD). Aβ is produced from the sequential cleavage of APP by the β-secretase and then by the γ-secretase. Although the endosomal/lysosomal system has been implicated in Aβ production, the subcellular site of Aβ production remains contentious. Our lab and others have shown that APP and γ-secretase are resident proteins of the lysosome. We have also recently shown that APP is transported rapidly and directly to the lysosome from the cell surface and that familial early onset AD (FAD) mutations of APP impairs trafficking. While most studies demonstrate the cleavage of APP after

internalization from the cell surface, the transport of APP to intracellular compartments remains virtually unstudied. In order to study the intracellular trafficking pattern of APP, we generated constructs encoding the C-terminal 112 residues of APP fused to photoactivatable GFP (APP-paGFP). APP-paGFP was then co-transfected into neuronal SN56 cells, along with a fluorescent Golgi compartment marker (GalT-CFP) and a fluorescent-tagged lysosomal compartment marker (LAMP1-mRFP), and imaged using a LSM 510 Meta scanning confocal microscope. To visualize APP transport out of the Golgi, we photoactivated APP-paGFP in the Golgi with 405nm light, using GalT-CFP as a target, and recorded video of APP trafficking to downstream compartments. In the present study, we show that APP traffics from the Golgi to the lysosome within minutes and is rapidly cleared from this compartment. Treatment of the cells treated with chloroquine to deacidify lysosomes or γ-secretase inhibitor L685, 485 delays the cleavage of APP in lysosomes. Cleavage of APP bearing the Swedish FAD mutation occurs nearly instantaneously, and results in diffuse fluorescent in the cytosol. However, treatment with chloroquine or L685 485 also delays this cleavage, and results in accumulation of APP in lysosomes. These results suggest that the lysosome may be a site of Aβ production.

Disclosures: J. Tam: None. **S.H. Pasternak:** None.

Nanosymposium

11. APP Metabolism and Processing

Location: Room 31C

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 11.6

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH 5R01NS45860

Cure Alzheimer's Fund

Title: Palmitoylation regulates APP maturation and processing

Authors: *R. BHATTACHARYYA[1,2], C. BARREN[1], D. M. KOVACS[1,2];
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Abstract: Amyloid Precursor Protein (APP) is sequentially cleaved by β-secretase (BACE1) and γ-secretase to generate amyloid β (Aβ) peptides. BACE1 and the γ-secretase components Nct and ApH1 are modified by palmitoylation of cysteine (Cys) residues at or near their C-terminal trans-membrane domains. Here, we asked whether APP is also palmitoylated. To address the question,

we have performed two palmitoylation assays: 1) acyl biotinylation assay and 2) fluorescent detection of protein fatty-acylation using a chemical reporter. Both assays confirmed that APP is palmitoylated in three different cell lines, CHO, B104 and H4. Interestingly, all 16 cysteines of APP are located in the N-terminal lumenal domain, suggesting that the palmitoylation of APP is an example of a lumenal palmitoylation. Although the majority of protein palmitoylation is reported to be cytoplasmic, lumenal palmitoylation has been identified among several secretory proteins, such as Wingless, EGFR ligand spi and Hedgehog. To identify the specific palmitoylation domain(s) in APP, we tested palmitoylation of N-terminal truncation mutants of APP. Our data confirmed that effective palmitoylation of APP occurred in the N-terminal domain between Cys 38 and 187 of APP_{751}. We then began mutating the 12 N-terminal Cys residues to Ser or Ala, either alone or in clusters. Palmitoylation assays of single and double mutants revealed that specific Cys residues were required for effective palmitoylation of APP. Interestingly, the same mutants generated little or no C-terminal fragments (α- and β-CTFs) of APP. Moreover, the palmitoylation deficient mutants of APP were severely defective in maturation. Finally, we observed severe loss of APP processing when cells stably expressing APP_{751} were treated with palmitoylation inhibitors Cerulenin or 2-Bromopalmitate, suggesting a role of palmitoylation in APP processing. In summary, we have identified that, 1) the specific cysteine residues in the N-terminal lumenal domain of APP are required for effective palmitoylation of APP, 2) the mutation of the same residues results in defective maturation and processing of APP, and 3) palmitoylation inhibitors decrease APP processing. These data show for the first time that specific Cys residues play critical roles in the effective palmitoylation, maturation and processing of APP.

Disclosures: R. Bhattacharyya, None; **C. Barren**, None; **D.M. Kovacs**, None.

Nanosymposium

11. APP Metabolism and Processing

Location: Room 31C

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 11.7

Topic: C.02. Alzheimer's disease and other dementias

Support: Cure Alzheimer's Fund

American Health Assistance Foundation (A2010638)

Title: Late-onset Alzheimer’s disease mutations in the ADAM10 prodomain impair non-amyloidogenic cleavage of the amyloid precursor protein in vivo

Authors: *J. SUH, D. ROMANO, R. E. TANZI;
Genet. and Aging Res. Unit-Neurology, Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: Increasing evidence supports the hypothesis that abnormal processing of the amyloid precursor protein (APP) plays a key role in the pathogenesis of Alzheimer's disease (AD). ADAM10, a protease possessing alpha-secretase activity, cleaves APP in the middle of the Abeta domain, precluding the generation of the neurotoxic peptide. Recently, we have identified two missense mutations in the prodomain region of ADAM10 gene (Q170H, R181G) in several late-onset (>60 years) AD families. In the present study, we examined the in vivo effects of these mutations in transgenic mice overexpressing either wild-type (WT) or mutant [Q170H, R181G, dominant-negative (DN)] forms of human ADAM10 behind the prion promoter. Compared to the WT ADAM10 mice, we observed a less pronounced decrease in the mature form of endogenous APP and concomitant increase of its alpha-secretase generated C-terminal fragment (CTFalpha) in the brains of mice expressing the mutant forms of ADAM10. While the expression levels of active form were comparable among different ADAM10 transgenic lines, the ADAM10-CTF levels were significantly reduced in the mutant and DN transgenic mice, suggesting decreased self-processing of ADAM10. Attenuation of alpha-secretase activity by the mutations was more pronounced in double transgenic mice generated by crossing with Tg2576 APPswe-overexpressing mice. Compared to the Tg2576/ADAM10-WT double transgenic control, cerebral APPsbeta and Abeta levels were significantly increased in Tg2576/ADAM10-Q170H or -R181G mice. Collectively, these results suggest that the prodomain of ADAM10 plays a critical role in regulating non-amyloidogenic cleavage of APP in brains and that the two novel late-onset AD-associated ADAM10 prodomain mutations are pathogenic.

Disclosures: J. Suh, None; **D. Romano**, None; **R.E. Tanzi**, None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.1

Topic: A.03. Stem Cells

Support: Foundation Fighting Blindness

Lincy Foundation

Title: Modeling retinal development and disease with human pluripotent stem cells

Authors: *J. S. MEYER[1,2], E. E. CAPOWSKI[3], A. D. VERHOEVEN[3], K. A. WALLACE[3], L. S. WRIGHT[3], S. HOWDEN[4], S. TIAN[4], R. STEWART[4], J. A. THOMSON[4], D. M. GAMM[3,5]; [1]Waisman Ctr., Univ. Wisconsin, MADISON, WI; [2]Dept. of Biol., IUPUI, Indianapolis, IN; [3]Waisman Ctr., [4]Morgridge Inst., [5]Ophthalmology and Visual Sciences, Eye Res. Inst., Univ. of Wisconsin, Madison, WI

Abstract: Established methods for deriving early retinal progenitors from human pluripotent stem cells (hPSCs) typically yield a heterogeneous population of cells, which complicates studies of retinal development. Therefore, we sought to develop a simple method of isolating a highly enriched population of optic vesicle (OV) stage, multipotent retinal progenitor cells from human ES and iPS cells. hPSCs were differentiated toward a retinal lineage using a previously described protocol. Highly enriched populations of OV stage retinal progenitors were manually separated from forebrain progenitor populations and allowed to differentiate for up to 120 days. Differences in gene expression between retinal and forebrain progenitor populations were determined via PCR and microarray analyses. Differentiating OV populations were then examined by qPCR and ICC to ascertain their ability to generate retinal cell types. Upon initial isolation, >90% of all OV stage hPSC populations expressed the definitive neural retinal progenitor marker Chx10. Microarray analysis of retinal and forebrain progenitor populations revealed key differences in the expression of numerous transcription factors, including Rx, Tbx2, Dlx1 and Islet1. In vitro maturation of OV populations produced all major classes of retinal cell types in a manner reminiscent of normal development. Next, we sought to demonstrate that iPS cells could be used to establish retinal disease models. We generated and characterized an iPS cell line derived from a patient with gyrate atrophy, an RPE-based inherited retinal degenerative disease. We then compared iPS-derived RPE cells to RPE cells derived from ES cell and fetal sources, all of which were capable of limited expansion in the presence of mitogens. RPE from all sources were highly similar based on functional assays, as well as gene and protein expression of characteristic markers. However, unlike iPS cell-derived RPE from normal controls, gyrate atrophy patient-derive iPS cell RPE lacked activity of the ornithine aminotransferase enzyme, characteristic of the disease process. Results from this study demonstrate that highly enriched populations of OV stage, multipotent retinal progenitors can be isolated from hPSCs. Furthermore, patient-specific hiPSCs can be used to produce specific retinal cell types that express disease-causing gene mutations. As such, hiPSCs should prove useful for studying the pathophysiology of some human retinal diseases, as well as for screening small molecules for therapeutic effects. These results will facilitate future studies of mechanisms of human retinogenesis and disease as well as efforts to develop hPSC-based therapies.

Disclosures: J.S. Meyer, None; **E.E. Capowski**, None; **A.D. Verhoeven**, None; **K.A. Wallace**, None; **L.S. Wright**, None; **D.M. Gamm**, None; **S. Howden**, None; **R. Stewart**, None; **S. Tian**, None; **J.A. Thomson**, None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.2

Topic: A.03. Stem Cells

Support: CIRM SEED Grant RS1-00205-1

CIRM Postdoctoral Fellowship

Title: Investigating synapse formation and function using human ES and iPS cell-derived forebrain neurons

Authors: *J.-E. KIM[1], C. A. SANCHEZ[1], M. O'SULLIVAN[1], M. ISRAEL[1], K. BRENNAND[2], L. S. B. GOLDSTEIN[1], F. H. GAGE[2], A. GHOSH[1];
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Abstract: A major goal of stem cell research is to identify conditions to reliably regulate their differentiation into specific cell types that are functional. This is particularly important for human stem cells if they are to be used for transplantion studies or for developing platforms for drug development. We have established procedures to direct the differentiation of human embryonic stem (hES) cells and induced pluripotent stem (hiPS) cells into forebrain neurons. hES and hiPS cells were induced to form embryoid bodies using forced aggregation in V-bottom 96-well plates. When these “spin EBs” were cultured in suspension in the presence of the BMP antagonist Noggin and then allowed to attach to a matrigel substrate, numerous neuroepithelial rosettes appeared. These rosettes were subsequently dissected, replated, and expanded in the presence of FGF2 and EGF as neural progenitor cells (NPCs). At the rosette and NPC stage, forebrain markers such as BF1, Pax6, and Emx2 were upregulated, whereas the more caudal markers HoxA4 and HoxB4 were undetectable by RT-PCR. Terminal differentiation of fluorescent reporter-expressing NPCs was initiated by replating them at a lower density on a glial feeder layer and withdrawal of FGF2 and EGF. After 3-4 weeks in the co-culture, the NPCs differentiated into numerous TuJ1-, and MAP2-positive cells with neuronal morphologies.
In vitro differentiated human neurons must further meet the criterion of functionality by demonstrating connectivity with other neurons in a cortical circuit. We set out to investigate neuronal synapse formation in these cells by co-culturing them with embryonic rat neurons. NPCs were co-cultured with E18 rat cortical neurons. After five weeks, fluorescent cells with a neuronal morphology were selectively patch-clamped. Terminally differentiated human NPCs were able to receive synaptic inputs from other neurons and were able to fire action potentials. These observations suggest that human ES and iPS cells exposed to developmentally relevant signals can give rise to cells that display a range of characteristics typical of forebrain neurons.
In a final set of experiments, HEK293T cells expressing Neuroligin(NLGN)-3 and -4X, but not those containing autism-associated mutations, were able to induce presynaptic differentiation in iPS cell-derived neurons. We show that a mutant form of NLGN4X resulting from exon skipping

is unable to localize correctly to dendrites when overexpressed in hiPS cell-derived neurons. These findings establish human stem cell-derived neurons as a viable model for the study of synaptic differentiation and function under normal and disorder-associated conditions.

Disclosures: J. Kim, None; **C.A. Sanchez**, None; **M. O'Sullivan**, None; **A. Ghosh**, None; **M. Israel**, None; **L.S.B. Goldstein**, None; **F.H. Gage**, None; **K. Brennand**, None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.3

Topic: A.03. Stem Cells

Support: NIH Grant R01 NS058784

Title: Genetic engineering of multipotent human neural stem cells for multimodal imaging of structural repair in experimental stroke model

Authors: *M. DAADI[1,2,3], S. HU[1,4], J. KLAUSNER[1,3], Z. LI[1,4], G. SUN[1,3], J. C. WU[1,4], G. K. STEINBERG[1,2,3];
[1]Stanford Univ., STANFORD, CA; [2]Stanford Inst. for Neuro-Innovation and Translational Neurosciences, Stanford, CA; [3]Dept. of Neurosurg., Stanford, CA; [4]Med. & Radiology, Mol. Imaging Program, Stanford, CA

Abstract: Cell transplantation is a promising therapeutic intervention for human neurological disorders. Clinically relevant, is the development of multimodal, non-invasive molecular neuroimaging approaches to monitor the survival and function of transplanted cells.
In the present study, human embryonic stem cells (hESCs) were stably transduced with a self-inactivating lentiviral vector carrying a triple fusion reporter gene construct. The construct consisted of monomeric red fluorescence protein (mRFP), firefly luciferase (Fluc) and herpes simplex virus truncated thymidine kinase (HSV-ttk) reporter genes. Stably transduced hESCs were selected based on mRFP expression using fluorescence activated cell sorting. Multipotent neural stem cells (NSCs) were isolated from these hESCs and perpetuated in vitro using serum free media supplemented with mitogenic growth factors. Sprague Dawley adult male rats were subjected to a transient 65 minute suture occlusion of the middle cerebral artery. The hNSCs were harvested and transplanted in the peri-infarct region. The NSCs uniformly expressed nestin, vimentin and 3CB2. For the MR imaging, cultured NSCs were incubated with MR compatible contrast agent superparamagnetic iron oxides (SPIO).

The survival of graft was monitored using MR imaging on a weekly basis during the first two-weeks and biweekly thereafter for 3 months. Volumetric analysis of the stroke and the NSC grafts demonstrated a significant diminution of the lesion over time. The fate of the grafted cells was analyzed with confocal microscopy. PET imaging was acquired using 18F-fluoro-hydroxymethylbutyl-guanine ([18F]-FHBG) as reporter probe and 18F-Fluorodeoxyglucose ([18F]-FDG) as metabolic probe. PET activity was consistent with the expression of the HSV-ttk reporter gene by the NSCs. Analysis of the stroke and grafted NSCs demonstrated a 2.5 fold increase in metabolic activity in the lesioned hemisphere of transplanted animals. Multimodal imaging provides a reliable means to monitor and analyze in real time the therapeutic effects of NSC grafts.

Disclosures: M. Daadi, None; **S. Hu**, None; **J. Klausner**, None; **Z. Li**, None; **G. Sun**, None; **J.C. Wu**, None; **G.K. Steinberg**, None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.4

Topic: A.03. Stem Cells

Support: Singapore NRF CRP grant NRF2008NRF-CRP002-082

Title: Nanotopographical cues enhance the neuronal differentiation of human embryonic stem cells

Authors: S. ANKAM[1], K. S. LIM[3], A. A. KYWE MOE[3], J. GOH[1], *E. K. YIM[2];
[1]Div. of Bioengineering, [2]Div. of Bioengineering, RCE in Mechanobiology, Dept. of Surgery, Natl. Univ. of Singapore, Singapore, Singapore; [3]Duke-NUS Grad. Med. Sch., Singapore, Singapore

Abstract: Abstract:
Stem cell niche is composed of a spatially arranged extracellular matrix (ECM) which is known to present biochemical and topographical cues for regulating stem cell fate for development and regeneration. Synthetic substrate topography in micro- or nano-scale has been shown to direct stem cell differentiation in previous in vitro studies. We hypothesize that topographical cues could enhance the differentiation of human embryonic stem cells (ESCs) to neurons. The objective of this study is to determine the optimal topographical pattern that could enhance the neuronal differentiation. The neuronal differentiation of hESCs on gratings, pillars and wells of

micro- and nanometers were compared. Soft lithography was used to fabricate these different patterns onto polydimethylsiloxane (PDMS), a polymeric substance. The advantage of using PDMS is that it can be easily moulded to the required shape and specific stiffness. Neuronal marker expression was analyzed by Immunofluorescence staining. H9, a human embryonic cell line, was cultured on patterned PDMS in the presence of N2 and B27 supplements for 8 days. Unpatterned PDMS served as the control. It was observed that 250 nm gratings were the most efficient in inducing the expression of Microtubule associated protein 2 (MAP2), a mature neuronal marker. MAP2 expression was 1.5 times higher on 250 nm gratings in comparison to the unpatterned control. The MAP2 positive dendrites were mostly aligned along the grating axis. Inspite of the effect of neuronal induction factors, pillars and wells showed higher number of undifferentiated human ESC colonies compared to gratings. The unpatterned surface showed a comparatively higher ratio of astrocyte to neuronal population and random arrangement of the astrocytes. Our results suggested that the nanotopographical cues from the extracellular substrate do play an important role in the efficient derivation of neurons from human ESCs. The use of topographical cues could potentially reduce the duration of neuronal differentiation from human ESCs.

Disclosures: S. Ankam: None. **K.S. Lim:** None. **A.A. Kywe Moe:** None. **J. Goh:** None. **E.K. Yim:** None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.5

Topic: A.03. Stem Cells

Support: Adelson Medical Research Program (AMRP)

Israel Science Foundation 158/07

Title: Generation of Schwann cells from human embryonic stem cells

Authors: L. ZIEGLER[1], *R. S. GOLDSTEIN[2];
[1]Mina and Everard Goodman Fac. of Life Sci., [2]Bar-Ilan Univ., Ramat-Gan, Israel

Abstract: Schwann cells (SC), the glial cells of peripheral nerves, play a pivotal role in peripheral axon regeneration. Following nerve injury, SC demyelinate, proliferate and de-differentiate, forming tubular scaffolds that provide a permissive environment and guide

regenerating axons to their targets. This process involves activation of specific receptors, intracellular signaling pathways and transcription factors, the mechanisms of which are focus of study. There are also many diseases involving SC or their precursors, including Charcot Marie Tooth and neurofibromatosis. Although it is possible to obtain human SC for study from nerve biopsies, they are difficult to expand and maintain in culture. Here we describe an in vitro system for directing the differentiation of human embryonic stem cells (hESC) into cells with the morphological and molecular characteristics of SC.
Neurospheres were generated from hESC using PA6 stromal cell induction and grown under conditions supportive of SC differentiation. After 8 weeks in culture, hESC-derived SC-like cells expressed (immunostaining) SC markers P75, HNK1, glial fibrillary acidic protein (GFAP), S100, myelin basic protein (MBP) and peripheral myelin protein (PMP)-22, and were observed in close association with processes of hESC-derived neurons in the cultures. Approximately 60% of the cells were double-immunostained for the Schwann cell markers GFAP/S100. FACS analysis using an antibody to p75 detected about ~10% positive cells. RT-PCR analysis confirmed the expression of GFAP, PMP-22 and MBP and demonstrated expression of additional SC markers: myelin protein zero (P0), CAD19, KROX20 and PLP. Expression of CAD19 was observed at 4 weeks cultures and then was downregulated, consistent with its known expression in SC precursor stages. In co-cultures of the hESC-derived SC with murine or chick dorsal root ganglion neurons tight association of the putative SC with the axons was observed. Apparent wrapping of the axons by SC was observed occasionally, suggestive of myelination. The results demonstrate that hESC-derived SC potentially constitute a potential source of human SC for studies of their role in nerve regeneration and modeling of SC disease.

Disclosures: L. Ziegler, None; **R.S. Goldstein**, None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.6

Topic: A.03. Stem Cells

Support: CIHR Grant MOP-102649

CIHR Grant IG1-94505

CIHR Doctoral Award CGD-96480

Title: Induction of pluripotent stem cells to model Rett syndrome

Authors: *N. FARRA[1,2], W. ZHANG[5], A. HOTTA[6], P. PASCERI[1], A. Y. L. CHEUNG[1,2], M. W. SALTER[3,4,5], J. ELLIS[1,2,7];
[1]Program in Developmental & Stem Cell Biol., Hosp. For Sick Children, Toronto, ON, Canada; [2]Dept. of Mol. Genet., [3]Dept. of Physiol., [4]Univ. of Toronto Ctr. for the Study of Pain, Univ. of Toronto, Toronto, ON, Canada; [5]Program in Neurosciences & Mental Hlth., Hosp. for Sick Children, Toronto, ON, Canada; [6]Basic Biol. Dept., Ctr. for iPS Cell Res. & Application, Kyoto Univ., Kyoto, Japan; [7]Ontario Human Induced Pluripotent Stem Cell Facility, Toronto, ON, Canada

Abstract: Induced pluripotent stem (iPS) cells hold great promise for making patient-specific cell culture disease models for central nervous system disorders. Rett Syndrome (RTT) is a neurodevelopmental autism spectrum disorder caused by mutations in the methyl CpG-binding protein 2 (*MECP2*) gene. Due to the inaccessibility of patient neurons, it is difficult to study RTT *in vitro* or perform drug screens. As a consequence, underlying phenotypes have been primarily described using mouse models. iPS cells provide a potential solution, whereby neuronal differentiation of RTT-specific iPS cells creates a limitless supply of defective neurons for *in vitro* disease study and functional correction experiments. However, it is still unclear whether iPS cells accurately model autism spectrum disorders. Here we describe the characterization of mouse RTT *Mecp2*^{308} iPS cell lines to validate the use of this technology for characterizing human neurons derived from RTT patient iPS cells. This mouse model expresses a truncated *Mecp2* allele and reproduces the defects in synaptic function, behaviour, and learning typical of RTT. These wild-type and heterozygous iPS cell lines express endogenous pluripotency markers, reactivate the X-chromosome, and differentiate into the three germ layers *in vitro* and *in vivo*. Via retinoic acid-mediated differentiation of embryoid bodies, the lines were directed to differentiate into active glutamatergic neurons that form functional synapses and produce action potentials captured by whole-cell patch clamp recordings. Glutamatergic synapses were examined by immunofluorescence for the presynaptic marker vesicular glutamate transporter 1 (VGLUT1) and the post-synaptic density 95 (PSD95) marker. In these preliminary studies, iPS cell-derived neurons generate action potentials and miniature excitatory postsynaptic currents (EPSCs). We anticipate that electrophysiology will reveal RTT iPS cell-derived glutamatergic neurons recapitulate defects previously reported in RTT mouse brain samples. Detailed studies of synaptic function of iPS cell-derived neuronal cells are currently underway and will allow investigation of phenotypes in comparison to normal and RTT cortical neurons to validate the iPS cell system.

Disclosures: N. Farra, None; **W. Zhang**, None; **A. Hotta**, None; **P. Pasceri**, None; **A.Y.L. Cheung**, None; **M.W. Salter**, None; **J. Ellis**, None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.7

Topic: A.03. Stem Cells

Support: NIHR Biomedical Research Centre, UK

Medical Research Council, UK

Title: Antidepressants modulate human hippocampal neurogenesis by activating the glucocorticoid receptor

Authors: *C. ANACKER, P. A. ZUNSZAIN, A. CATTANEO, L. A. CARVALHO, S. THURET, J. PRICE, C. M. PARIANTE;
Ctr. for the Cell. Basis of Behaviour, Inst. of Psychiatry, King's Col. London, London, United Kingdom

Abstract: Antidepressants have been shown to contribute to the resolution of depressive symptoms at least in part by increasing adult hippocampal neurogenesis in animals. However, studies in human cells are missing, and the molecular mechanisms by which antidepressants modulate neurogenesis remain elusive. Antidepressants are known to enhance the function of the glucocorticoid receptor (GR) in cells, rodents and humans. This effect is common for chemically unrelated antidepressants, and ultimately results in enhanced GR-mediated gene transcription. Using a novel *in vitro* assay in human hippocampal progenitor cells, we investigated whether antidepressants modulate human neurogenesis by activating the GR. Furthermore, we analyzed changes in gene expression of the GR-target genes $p27^{Kip1}$ and $p57^{Kip2}$, which have been implicated in neuronal development.
Human hippocampal progenitor cells (HPC03A/07, ReNeuron, UK) were incubated with the antidepressant sertraline (SERT) and the GR-antagonist RU486. To investigate changes in neuronal differentiation, cells were treated for 10 days, and immunocytochemistry for the neuronal marker micotubulin-associated protein 2 (MAP2) was applied to identify mature neurons. To investigate changes in progenitor cell proliferation, cells were treated for 72 hours, and the synthetic nucleotide 5-bromo-2-deoxyuridine (BrdU), which gets specifically incorporated into dividing cells, was added for 4 hours at the end of treatment. Dividing cells were detected by immunostaining for BrdU, and the fraction of MAP2-positive and BrdU-positive cells over total cells was determined by cell counting in an unbiased setup. Gene expression was analyzed by quantitative real-time PCR.
SERT increased the number of MAP2-positive cells by 28.4%±2.4% at a concentration of 1µM ($p=0.0021$). Co-treatment with SERT and the GR-antagonist RU486 (50 nM) abolished this effect. Moreover, SERT decreased the number of BrdU-positive, proliferating cells by 16.4%±1.8% ($p=0.0002$). Again, co-treatment with RU486 also abolished this effect. Furthermore, SERT induced GR-transactivation after 12h of treatment and subsequently increased the expression of the cell cycle inhibitors and neuronal differentiation genes $p27^{Kip1}$ (by 51%±9.7%; $p=0.036$) and $p57^{Kip2}$ (by 48.1%±10.8%; $p= 0.042$). This effect was again

abolished by RU486 co-treatment.
In conclusion, we demonstrate that sertraline induces neuronal differentiation and decreases proliferation of human hippocampal progenitor cells by activating the GR. Our results indicate that this effect is mediated by sertraline-induced expression of the GR-target genes $p27^{Kip1}$ and $p57^{Kip2}$.

Disclosures: C. Anacker: None. **P.A. Zunszain:** None. **A. Cattaneo:** None. **L.A. Carvalho:** None. **S. Thuret:** None. **J. Price:** Consultant/Advisory Board; Prof. Jack Price acted as a consultant and received payment from ReNeuron group within the last 2 years. **C.M. Pariante:** None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.8

Topic: A.02. Neurogenesis and Gliogenesis

Support: NINDS grant P01NS050315

NINDS grant R01NS39559

National Multiple Sclerosis Society

Title: Pleiotrophin regulation of β-catenin dependent signaling in PDGFRα+ fetal human glial progenitor cells

Authors: *C. R. MCCLAIN, F. J. SIM, S. A. GOLDMAN;
Dept Neurol., Univ. Rochester, ROCHESTER, NY

Abstract: Glial progenitor cells (GPCs) persist throughout the adult human central nervous system, wherein they are responsible for oligodendrocyte production and remyelination after demyelinating injury. However, remyelination often fails in the adult disease environment. This inhibition of myelination might be due to either the aborted mobilization of glial progenitors, or their differentiation into reactive astrocytes rather than oligodendrocytes. We propose that pleiotrophin, a secreted heparin-binding cytokine, suppresses the differentiation of human GPCs. On the basis of a whole genome screen of isolated adult human glial progenitor cells, we found that these cells express high levels of a constitutively active receptor tyrosine phosphatase, RPTPβ/ζ (PTPRZ1). Quantitative PCR analysis demonstrated similar enrichment of RPTPβ/ζ in

PDGFαR-sorted fetal human GPCs (n=6, 3.7-fold, q=0.034). Importantly, RPTPβ/ζ can dephosphorylate β-catenin, and by so doing can modulate β-catenin-dependent transcription, including that of canonical wnt signaling. RPTPβ/ζ has few known ligands, the most prominent is pleiotrophin, which serves as an endogenous inhibitor of RPTPβ/ζ phosphatase activity. In this study, we used isolates of PDGFRα^{+} GPCs freshly sorted from fetal human forebrain, to assess the roles of both pleiotrophin and RPTPβ/ζ on glial progenitor cell expansion and differentiation. We found that pleiotrophin increased the pool of activated β-catenin in fetal human GPCs, and did so to a similar extent as that accomplished by pharmacological inhibition of GSK3β, a kinase that targets β-catenin for degradation. We further noted that GSK3β inhibition increased TCF transcription (n=16, $p<0.01$), suggesting a role for β-catenin-dependent TCF transcriptional activation of wnt target genes in GPC maintenance. Moreover, lentiviral shRNAi knockdown of RPTPβ/ζ significantly augmented the GSK3β inhibition-induced increase in TCF-mediated transcription (n=5, $p<0.05$), and potentiated the sustained in vitro passage and expansion of GPCs. On that basis, we propose that pleiotrophin inhibition of PTPRZ1 might comprise a feasible strategy by which to potentiate the mobilization of glial progenitor cells while suppressing their astrocytosis. As such, strategies directed at the pleiotrophin/PTPRZ1 interaction may prove effective at enhancing the potential for remyelination by local glial progenitor cells. More broadly, these studies of signal control in human glial progenitor cells may provide us great insight into a broad category of neurological diseases that share reactive gliosis and aborted remyelination as key impediments to recovery.

Disclosures: C.R. McClain, None; **F.J. Sim**, None; **S.A. Goldman**, None.

Nanosymposium

118. Stem Cells and Neural Progenitors from Humans

Location: Room 25A

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 118.9

Topic: A.03. Stem Cells

Support: Grant-in-Aid for Scientific Research on Priority Areas (C) by JSPS

Title: Differences in neural differentiation propensity among cell lines of human pluripotent stem cells

Authors: *A. MORIZANE[1,2], D. DOI[1,2], T. KIKUCHI[1], J. TAKAHASHI[1,2];
[1]Inst. Frontier Med. Sci., Kyoto Univ., Kyoto, Japan; [2]Dept. of Cell Growth and Differentiation, Ctr. for iPS Cell Res. and Application, Kyoto Univ., Kyoto, Japan

Abstract: Introduction:
There are several reports on different propensities of human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells. Recently it has been reported that iPS cells have less efficiency and more variability on neural differentiation comparing to ES cells. Considering clinical application of ES cells and iPS cells, it is important to differentiate stem cells stably and efficiently to the cells needed. We compared several cell lines of human ES cells and iPS cells on neural differentiation with the efficient induction system that we had reported before.
Material and methods:
For induction of neurons from several lines of human ES cells and iPS cells, we used dual SMAD inhibition by small molecules combined with widely used neural differentiation protocols (both co-culture system and serum free embryoid body like structure system: SFEB). To evaluate the efficiency of induction, we analyzed the differentiated cells with early neural markers and pluripotent stem cell markers by immunocytology and RT-PCR.
Results:
As previously reported, there were some differences between cell lines in propensity for neural differentiation by the standard neural induction protocols. Although dual SMAD inhibition combined with SDIA method or SFEBq promoted neural induction efficiently, there remained differences between cell lines regarding propensities of early stages of neural induction as well as the subtypes of differentiated neurons.
At an early stage (1-2 week) of differentiation, some cell lines remained less differentiated than others. However, even the cell lines with poorest neural differentiation efficiency at the beginning mostly committed into neuronal cells after 4-6 weeks of differentiation.
The expression levels of midbrain markers varied greatly between different cell lines.
Discussion:
Considering the clinical application of ES cells or iPS cells for the treatment of neurological disorders, the usage as the donor cells for transplantation and as the material for drug screening might be the main candidate. For both cases, it is important to differentiate ES cells or iPS cells into the desired types of neurons. Ideally we need to develop efficient protocol for neural differentiation to overcome the variant propensities of the cell lines.
Conclusion
Dual SMAD inhibition combined with standard neural induction protocol namely SDIA and SFEBq promote neural commitment efficiently, but still harbored the different propensities of regional subtypes of differentiated neurons.

Disclosures: A. Morizane, None; **D. Doi**, None; **T. Kikuchi**, None; **J. Takahashi**, None.

Nanosymposium

119. Alzheimer's Disease: Cholesterol and APOE

Location: Room 31C

Time: Sunday, November 14, 2010, 8:00 am - 10:00 am

Program Number: 119.1

Topic: C.02. Alzheimer's disease and other dementias

Support: La Marató de TV3 Grant 054

European Comission Grant FP6-005045 NANOBIOMAPS

Stockholm County Council

SAF Grant 2006-13642

Alzheimer Fund

Palm Foundation

NARSAD

Title: Cholesterol accumulates in the vicinity of amyloid deposits in brain tissue

Authors: *S. SOLÉ DOMÈNECH[1], P. SJÖVALL[5], V. VUJOCEVIć[2], S. SALVE[3], A. CODITA[4], M. SCHALLING[3], F. M. LAFERLA[6], L. GIMÉNEZ-LLORT[7], P. NILSSON[8], L. TERENIUS[2], B. JOHANSSON[3];
[2]Dept. of Clin. Neurosci., [3]Dept. of Mol. Med., [4]Dept. of Neurobiology, Care Sci. and Society, [1]Karolinska Institutet, Stockholm, Sweden; [5]Dept. of Chem. and Materials Technol., SP Tech. Res. Inst. of Sweden, Borås, Sweden; [6]Dept. of Neurobio. and Behaviour, Univ. of California Irvine, Irvine, CA; [7]Dept. of Psychiatry and Forensic Med., Univ. Autònoma de Barcelona, Barcelona, Spain; [8]Dept. of Chem., Linköpings Universitet, Linköping, Sweden

Abstract: Amyloid beta (Aβ) aggregation plays a central role in the pathogenesis of Alzheimer's disease (AD), but increasing evidence suggests that altered cholesterol (Ch) homeostasis may also be implicated in the disease etiology[1]. The aim of our study is to clarify the role of Ch in the aggregation process of Aβ. In the present work we combined time-of-flight secondary ion mass spectrometry (ToF-SIMS) with confocal laser scanning microscopy (CLSM) to study Ch distribution around Aβ deposits in mouse (3xTgAD transgenic model) and human AD brain tissue. Aβ deposits were identified by p-FTAA, an amyloid-specific fluorescent probe and visualized by CLSM imaging. CLSM images were used as guiding templates for ToF-SIMS experiments. ToF-SIMS analyses were performed on the immediately adjacent, mirror-image section in order to evaluate the Ch/lipid profile. The sections subjected to ToF-SIMS were thereafter incubated with p-FTAA and imaged by CLSM. Colocalization between Aβ and Ch was studied in overlaid ToF-SIMS and CLSM images obtained from the same section.
In some cases, Ch accumulations could be observed in the vicinity of Aβ deposits, within a distance of 0 to 50 μm from the plaque core. As an example, images of a 3xTgAD mouse brain section (approx. 3.15 mm from bregma) are shown in figure 1. The brain hippocampus is visualized in figure 1**a** by DAPI staining of neuronal nuclei (blue). The region enriched with Aβ deposits, visualized by p-FTAA (yellow-green), is delineated by a white rectangle. A magnified

image of this area is shown in figure 1**b**. A smaller area containing amyloid deposits (area 1, delineated by the white rectangle in figure 1**b**) was analyzed with ToF-SIMS, as shown in figure 1**c**. The image shows Ch distribution (orange gradient) around the Aβ deposits (purple). Experiments in mouse and human brain tissue are still on-going. The combination of CLSM and ToF-SIMS has been shown to be a suitable approach to study Ch/lipid profile around Aβ deposits.

1. Puglielli, L., Tanzi, R.E. & Kovacs, D.M. Alzheimer's disease: the cholesterol connection. *Nat Neurosci* 6, 345-51 (2003).

$$graphic_80F32C66-13DC-4A19-8349-55EFED3C3847$$

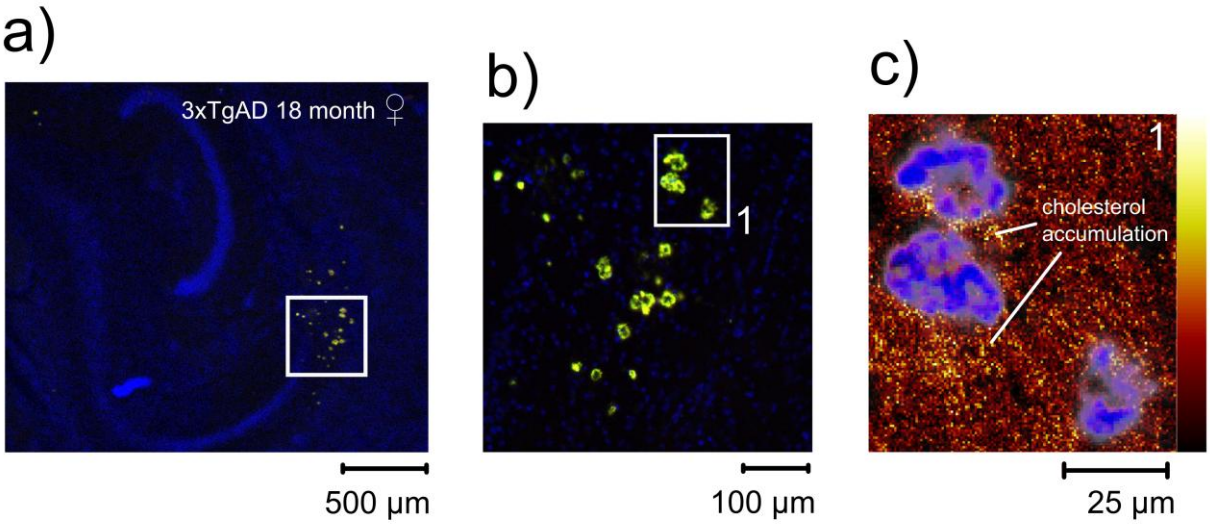

Figure 1

Disclosures: S. Solé Domènech, None; **P. Sjövall**, None; **V. Vujocević**, None; **S. Salve**, None; **A. Codita**, None; **M. Schalling**, None; **F.M. LaFerla**, None; **L. Giménez-Llort**, None; **P. Nilsson**, None; **L. Terenius**, None; **B. Johansson**, None.

Nanosymposium

119. Alzheimer's Disease: Cholesterol and APOE

Location: Room 31C

Time: Sunday, November 14, 2010, 8:00 am - 10:00 am

Program Number: 119.2

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant AG-23524

NIH Grant AG-18357

Hanna Bragard-Apfel Foundation

Department of Veterans Affairs

Title: Reducing cholesterol but not FPP levels activates SREBP-2 in neural cells

Authors: U. IGBAVBOA[1], G. P. ECKERT[3], G. P. HOOFF[3], W. E. MULLER[3], Y.-W. WANG[4], *W. G. WOOD[2];
[1]Pharmacol., Univ. of Minnesota, Minneapolis, MN; [2]Pharmacol., Univ. of Minnesota, MINNEAPOLIS, MN; [3]Pharmacol., Goethe Univ., Frankfurt, Germany; [4]Pharmacol., China Med. University/University of Minnesota, Taichung, Taiwan

Abstract: Numerous cell functions (e.g., synaptic plasticity, cell growth, cytoskeletal function, vesicle trafficking and gene expression) require activity of prenylated proteins. Those proteins can only work if they are prenylated by farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP). There is evidence that prenylated proteins play a role in Alzheimer disease (AD). We recently reported (Neurobiol. Dis. 2009, 35, 251-257) that levels of FPP and GGPP and gene expression of FPP synthase and GGPP synthase were significantly elevated in the frontal cortex of AD patients as compared with normal neurological controls; cholesterol levels and HMG-CoA reductase gene expression were unchanged. Stimulation of FPP synthase gene expression can occur with the binding of the transcription factor sterol regulatory element binding protein-2 (SREBP-2). Activation of the SREBP pathway is induced by low cholesterol levels and inhibition of that pathway occurs when cholesterol levels are normal or elevated. What is not known is if SREBP-2 can be activated by changes in FPP levels when cholesterol levels are unaltered as in the case of AD. In spite of the indispensable role that FPP and GGPP play in protein prenylation, knowledge has not been forthcoming on their regulation especially in brain. To begin to understand FPP and GGPP regulation in brain, effects of inhibition of FPP synthase by a direct inhibitor and inhibition of HMG-CoA reductase by a statin on nuclear SREBP-2 protein levels were examined in mouse primary neurons and SH-SY5Y neuroblastoma cells. Cells were treated with simvastatin or alendronate for 48 h and protein levels determined by Western analysis. Simvastatin-induced inhibition of HMG-CoA reductase significantly increased nuclear SREBP-2 abundance. In contrast, inhibition of FPP synthase which reduces FPP levels but not cholesterol did not alter nuclear SREBP-2 protein levels. SREBP-2 activation may not be driving the increase in FPP levels in AD brain suggesting the involvement of other transcription factors. A consequence of the increase in FPP and GGPP in AD patients could be an over-abundance of prenylated proteins which could certainly exacerbate loses in synaptic plasticity.

Disclosures: U. Igbavboa, None; **G.P. Eckert**, None; **G.P. Hooff**, None; **W.E. Muller**,

None; **Y. Wang**, None; **W.G. Wood**, None.

Nanosymposium

119. Alzheimer's Disease: Cholesterol and APOE

Location: Room 31C

Time: Sunday, November 14, 2010, 8:00 am - 10:00 am

Program Number: 119.3

Topic: C.02. Alzheimer's disease and other dementias

Support: AG023084

NS34467

Title: The role of apolipoprotein E genotype and cyclophilin A in Alzheimer's disease neurovascular uncoupling

Authors: *B. V. ZLOKOVIC[1], R. D. BELL[1], A. SAGARE[1], I. SINGH[1], B. C. BERK[2], D. M. HOLTZMAN[3], R. DEANE[1];
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Abstract: Apoliprotein E4 (apoE4) is associated with neurological disorders that share neurovascular dysfunction, including Alzheimer's disease. How apoE affects the neurovascular unit is largely unknown. Here, we report that apoE2 and apoE3, but not apoE4, effectively maintain the blood-brain barrier (BBB) integrity, brain vascular density and cerebral blood flow (CBF) in *Apoe*$^{-/-}$ mice. Lack of cyclophilin A (CypA), a proinflammatory cytokine which mediates extracellular matrix degradation and endothelial and neuronal apoptosis, and/or CypA inhibition with cyclosporine inhibits BBB disruption and CBF reductions and improves faulty synapto-dendritic connections in *Apoe*$^{-/-}$ and *APOE4*-expressing mice, respectively. Furthermore, we showed that CypA is directly cytotoxic to primary mouse brain endothelial cells, but not to primary mouse cortical neurons suggesting that the neuronal injury found in *Apoe*$^{-/-}$ and *APOE4*-expressing mice may be due to a primary vascular insult. We next showed that the microvasculature-derived pool of CypA is significantly increased in *Apoe*$^{-/-}$ and *APOE4*-expressing mice. Interestingly, ApoE2 and apoE3, but not apoE4, blocked CypA synthesis via low-density-lipoprotein receptor-related protein-1 *in vitro* in primary brain endothelial cells isolated from *Apoe*$^{-/-}$ cells. Thus, CypA may be an exciting new target for treating brain disorders affected by apoE4.

Disclosures: B.V. Zlokovic, None; **R.D. Bell**, None; **A. Sagare**, None; **I. Singh**, None; **B.C. Berk**, None; **D.M. Holtzman**, None; **R. Deane**, None.

Nanosymposium

119. Alzheimer's Disease: Cholesterol and APOE

Location: Room 31C

Time: Sunday, November 14, 2010, 8:00 am - 10:00 am

Program Number: 119.4

Topic: C.02. Alzheimer's disease and other dementias

Support: European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 202167. Food, Agriculture and Fisheries, and Biotechnology) KBBE-2007-2-2-02: Impact of diet on ageing

Title: Effects of specific DHA and -cholesterol containing diets on cognition, cerebral metabolism and hemodynamics, in APP/PS1 Alzheimer mice, and ApoE4 and ApoE ko mouse models of vascular factors in Alzheimer's Disease

Authors: *A. J. KILIAAN[1], V. ZERBI[1], C. I. F. JANSSEN[1], D. VAN ROOIJ[1], B. ZINNHARDT[1], L. M. BROERSEN[3], A. HEERSCHAP[2], D. JANSEN[1];
[1]Depts Anat. & CNS, [2]Radiology, Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands; [3]Danone Res. BV, Wageningen, Netherlands

Abstract: Alzheimer's disease (AD) is still incurable and nowadays preventive interventions come into focus. There is increasing evidence that a combination of lifestyle (e.g. diets and exercise), cerebral ischemia, and genetic background play important roles in cognitive impairment and neural degeneration during ageing. Especially docosahexaenoic acid (DHA), an omega-3 long chain poly-unsaturated fatty acid and component of neural membrane phospholipid layers, has been shown to positively influence cardiovascular disease and the course of AD.
We therefore studied the effect of diets on cognition, brain metabolism, cerebral hemodynamics and neurodegeneration in three distinct transgenic mouse models for AD. One transgenic strain resembles familial AD by carrying the Swedish family-specific amyloid precursor protein (APP) aberration and mutated presenilin 1 (PS1) and two transgenic strains resemble vascular factors in sporadic AD by carrying human Apolipoprotein 4 (ApoE4) alleles, and ApoE knockout (-/-) mice. The ApoE gene codes for a transporter protein involved in cholesterol metabolism, and in specific the ApoE4 allele is a risk factor for cardiovascular disease and AD. Our mice were fed a standard diet, a cholesterol-rich diet and a multi-nutrient diet containing precursors and cofactors

in brain membrane synthesis, such as DHA, phospholipids, uridine monophosphate, choline, B-vitamins and antioxidants (Fortasyn™ Connect). Experiments were carried out in 12 months old mice. Behavior was studied in the Open Field. The Morris Water Maze (MWM) and was used to assess spatial learning and memory. Brain metabolism was investigated in the hippocampus with proton magnetic resonance spectroscopy (^{1}H MRS), measuring metabolites such as *N*-acetyl aspartate, *myo*-inositol, glutamate, choline, and GABA. Relative Cerebral Blood Volume (rCBV) was determined with contrast enhanced MRI techniques enabling the extraction of measurements from both the macro- and microvasculature. With immunohistochemistry we also studied neurogenesis and amount of synapses in hippocampus. Our results are currently being processed and will be presented.Our diets are expected to influence spatial learning and cerebral metabolism due to effects on cerebral circulation and neural membrane composition

Disclosures: A.J. Kiliaan, None; **V. zerbi**, None; **C.I.F. Janssen**, None; **D. van Rooij**, None; **B. Zinnhardt**, None; **A. Heerschap**, None; **D. Jansen**, None; **L.M. Broersen**, None.

Nanosymposium

119. Alzheimer's Disease: Cholesterol and APOE

Location: Room 31C

Time: Sunday, November 14, 2010, 8:00 am - 10:00 am

Program Number: 119.5

Topic: C.02. Alzheimer's disease and other dementias

Support: European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 202167.

Title: Cognition, cerebral metabolism and hemodynamics, and neuropathology in APP/PS1 Alzheimer mice, and ApoE4 and ApoE ko mouse models of vascular factors in Alzheimer's disease

Authors: *D. JANSEN[1], V. ZERBI[1], C. I. F. JANSSEN[1], D. VAN ROOIJ[1], B. ZINNHARDT[1], A. HEERSCHAP[2], A. J. KILIAAN[1];
[1]Depts. Anat. & CNS, [2]Radiology, Radboud Univ. Nijmegen Med. Ctr., Nijmegen, Netherlands

Abstract: Alzheimer’s Disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in the elderly. Research into the development of AD provides increasing evidence that lifestyle, vascular and genetic factors together strongly influence the development of vascular disorders and neural degeneration. Results from trials and epidemiological studies suggest that docosahexaenoic acid (DHA) and cholesterol may affect the course of AD, possibly by influencing the cerebral circulation indirectly.

In this study we investigated explorative and anxiety-related behaviour, spatial learning and memory, brain metabolism, cerebral hemodynamics and neurodegeneration in three distinct transgenic mouse models for AD. Our mouse models consist of one transgenic strain resembling familial AD by carrying the Swedish family-specific amyloid precursor protein (APP) aberration and mutated presenilin 1 (PS1) and their wild type littermates (WT), and two transgenic strains resembling vascular factors in sporadic AD by carrying human Apolipoprotein 4 (ApoE4) alleles, and ApoE knockout (-/-) mice. The ApoE gene codes for a transporter protein involved in cholesterol metabolism, and in specific the ApoE4 allele is a risk factor for cardiovascular disease and AD. Experiments were carried out in 8 and 12 months old mice. Behaviour was studied in the Open Field. Both the Morris Water Maze (MWM) and the reverse MWM were used to assess spatial learning and memory. Brain metabolism was investigated in the hippocampus with proton magnetic resonance spectroscopy (^{1}H MRS), measuring metabolites such as *N*-acetyl aspartate, *myo*-inositol, glutamate, choline, and GABA. Cerebral hemodynamics were determined with contrast enhanced MRI techniques enabling the extraction of relative Cerebral Blood Volume (rCBV) measurements from both the macro- and microvasculature. Neuropathology was determined by visualizing synaptophysin-immunoreactive presynaptic boutons (SIPBs) in several hippocampal regions. Our results are currently being processed and will be presented. We expect to find the most severe AD pathology in the APP/PS1 mice, followed by the ApoE knockout, and the ApoE4 animals respectively. The overproduction of Abeta in the APP/PS1 mice at an early age can aggravate the effects of cerebrovascular hypoperfusion resulting in subsequent hypometabolic abnormalities, neuronal degeneration, and cognitive impairment. ApoE4 is a major risk factor for the development of atherosclerosis via hypercholesterolemia and may subsequently lead to hypoperfusion of specific brain regions, followed by increased Abeta production and neuronal degeneration.

Disclosures: D. Jansen, None; **A. Heerschap**, None; **V. Zerbi**, None; **A.J. Kiliaan**, None; **C.I.F. Janssen**, None; **D. van Rooij**, None; **B. Zinnhardt**, None.

Nanosymposium

119. Alzheimer's Disease: Cholesterol and APOE

Location: Room 31C

Time: Sunday, November 14, 2010, 8:00 am - 10:00 am

Program Number: 119.6

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH 5AG016570

Title: Does presenilin activity play a role in the development of late onset Alzheimer’s disease?

Authors: S. P. AROLD[1], C. A. MILLER[3], H. VINTERS[1], G. COLE[1,4], E. TENG[1,4], *K. GYLYS[2];
[1]UCLA, Los Angeles, CA; [2]UCLA, LOS ANGELES, CA; [3]USC, Los Angeles, CA; [4]Sepulveda VAMC GRECC, Los Angeles, CA

Abstract: Presenilin (PS) is one of four membrane bound proteins that make up the gamma secretase cluster of proteins that is responsible for the cleavage of membrane bound amyloid precursor protein (APP) to the toxic amyloid beta species implicated in Alzheimer's disease (AD). The mutation of the presenilin gene leads to certain development of familial Alzheimer disease which manifests early, usually before the age of 50, however there is little evidence at this time to suggest that PS alterations play a primary role in pathology during late onset AD. A number of other proteins, including the low density lipoprotein receptor (LDLR) family, are substrates for gamma secretase and recent results demonstrate that loss of gamma secretase function inhibits the internalization of the LDL receptor. This results in the accumulation of the LDLR receptor on the cell membrane and eliminates the internalization of apoliprotein E in tissue culture. Cellular down regulation of cholesterol synthesis was also shown to be a result of PS-1 knockout. In the present study, we isolated synaptosomes from post-mortem brain tissue of humans with late onset AD as well as those from a rat model of AD containing one PS-1 and two APP mutations and measured apoE, cholesterol and LDLR by flow cytometry and Western blot. In addition to upregulation of PS-1, Tg rats demonstrated decreased levels of full length LDLR in the synaptosome, increased levels of LDLR intracellular domain (ICD) fragment and increased apoE content. Additionally, we observed decreased free cholesterol in the synaptosome (2478±884 vs 3592±882 RFU $p<0.03$), a likely result of the downregulation of cholesterol synthesis. Interestingly, similar results were observed in synaptosomes from AD parietal cortex: elimination of full length LDLR, significant increase in LDLR ICD fragment and apoE, as well as decreased cholesterol in the synaptosome. Taken together, these data suggest a model in which increased synaptic PS1 and APP levels may contribute to a vicious cycle of amyloid beta generation/release within surviving synaptic terminals in AD.

Disclosures: S.P. Arold, None; **C.A. Miller**, None; **H. Vinters**, None; **G. Cole**, None; **E. Teng**, None; **K. Gylys**, None.

Nanosymposium

119. Alzheimer's Disease: Cholesterol and APOE

Location: Room 31C

Time: Sunday, November 14, 2010, 8:00 am - 10:00 am

Program Number: 119.7

Topic: C.02. Alzheimer's disease and other dementias

Support: AFI grant 08823

NIH grant AG18357

NIH grant AG-23524

Dept. Verterans Affairs

Title: Modulation of cholesterol, farnesyl- and geranylgeranylpyrophosphate in neuroblastoma SH-SY5Y-APP695 cells - Impact on amyloid beta-protein production

Authors: *G. P. ECKERT[1], G. P. HOOFF[1], I. PETERS[1], W. G. WOOD[2], W. E. MULLER[1]; [1]Goethe-University/Department of Pharmacol., Frankfurt, Germany; [2]VA Med. Ctr., Univ. of Minnesota, Minneapolis, MN

Abstract: The mevalonate-pathway is a crucial metabolic pathway for most eukaryotic cells. Cholesterol is a highly recognized product of this pathway but growing interest is being given to the synthesis and functions of isoprenoids. Isoprenoids are a complex class of biologically active lipids including for example, dolichol, ubiquinone, farnesylpyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Early work had shown that the long-chain isoprenoid dolichol is decreased, but that dolichyl-phosphate and ubiquinone are elevated in brains of Alzheimer´s diseased (AD) patients. There is keen interest in the role of the short-chain isoprenoids FPP and GGPP in protein prenylation and cell function in AD (BBA, DOI 10.1016/j.bbalip.2010.03.014). Until recently, levels of their biological active precursors FPP and GGPP were unknown. These short-chain isoprenoids are critical in the post-translational modification of certain small GTPases, such as Rho-proteins, which function as molecular switches in numerous, signaling pathways. We recently reported elevated FPP and GGPP brain levels and increased gene expression of FPP synthase (FPPS) and GGPP synthase (GGPPS) in the frontal cortex of AD patients. Cholesterol levels and gene expression of HMG-CoA reductase were similar in AD and control samples suggesting that homeostasis of FPP and GGPP but not cholesterol is specifically targeted in brain tissue of AD patients (Neurobiol. Disease 2009, 35: 251-7). In the present study, it was determined if cellular levels of FPP, GGPP and cholesterol affect Aβ abundance in SH-SY5Y cells, expressing human APP695. Cells were treated with different inhibitors of the mevalonate/isoprenoid/cholesterol pathway. FPP, GGPP, cholesterol and $A\beta_{1-40}$ levels were determined and activities of farnesyl- (FTase) and geranylgeranyltransferase I (GGTase I) were measured. Inhibitors of different branches of the mevalonate/isoprenoid/cholesterol pathway as expected reduced cholesterol and isoprenoid levels in neuronal cells. $A\beta_{1-40}$ levels were selectively reduced by cholesterol synthesis inhibitors but not by inhibitors of protein isoprenylation, indicating that changes in cholesterol levels per se and not endogenous isoprenoid levels account for the observed modifications in Aβ production.

Disclosures: G.P. Eckert, None; **G.P. Hooff**, None; **I. Peters**, None; **W.G. Wood**, None; **W.E. Muller**, None.

Nanosymposium

119. Alzheimer's Disease: Cholesterol and APOE

Location: Room 31C

Time: Sunday, November 14, 2010, 8:00 am - 10:00 am

Program Number: 119.8

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant 5R01-AG-031124-02

Title: Apolipoprotein E modulates cellular response to estrogen by interacting with estrogen receptor in neuronal cells

Authors: *Q. ZHAO, C. COLTON;
neurology, Duke Univ., Durham, NC

Abstract: Despite the notion that estrogen is a neuroprotective agent, conflicting outcomes have come from clinical trials of estrogen-based hormone replacement therapy. Whereas some clinical trials have shown protective effect of postmenopausal estrogen therapy, others show no benefit of estrogen replacement therapy. Growing evidence has suggested that these contradictory data could be due to the intervention of another important factor in neurodegenertive disease-Apolipoprotein E (apoE). It has been shown that women with apoE 3 or apoE 2 genotypes exhibit cognitive improvement from estrogen therapy whereas women with apoE 4 allele, which is a risk factor for Alzheimer's disease (AD), receive no benefit from estrogen therapy. Moreover, both anti-inflammatory activity and neurite growth-promoting effect of estrogen in microglia and neuronal cells, respectively, are compromised in cells expressing apoE 4 which explains the lost of neuroprotective effect of estrogen in APOE4 expressing individuals. However, the mechanism of how apoE modulates cellular response to estrogen is still unknown. Based on our *in vitro* data showing binding of APOE to estrogen receptor, we hypothesized that apoE interacts with estrogen receptor at a molecular level and thereby modulates estrogen function. Co-Immunoprecipitation studies indicate that apoE protein binds to estrogen receptor beta (ERβ) in a central nervous system-derived CAD cell line in both cytoplasmic and nuclear fractions and this interaction is independent of estrogen. However, apoE protein does not seem to interact with estrogen receptor alpha (ERα) in mouse neuroblastoma cells (N2A). To determine the effect of the apoE3 or apoE4 allele on estrogen response element dependent transactivation, we performed SEAP reporter assays in both N2A cells (express only ERα) and CAD cells (express only ERβ). In N2A cells, there is a strong induction of ERα transcriptional activity in response to estrogen treatment and addition of apoE leads to a significant increase of ERα transcriptional activity. However, there is no difference in transcriptional activation between cells that express apoE3 and apoE4. In CAD cells, we didn't observe a detectable ERβ transcriptional activation after estrogen treatment and both apoE3 and apoE4 failed to activate

ERβ transactivation in response to estrogen. Based on our result, we speculate that apoE may modulatewith estrogen function by directly interacting with ERβ and in turn modulating the transactivation of ERα downstream pathways involved in neuroprotection. Study of the effect of apoE allele on estrogen function in cells contain both estrogen receptors is underway.

Disclosures: Q. Zhao, None; **C. Colton**, None.

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.1

Topic: C.02. Alzheimer's disease and other dementias

Support: Partners for Cures

VA MERIT grant

Title: Increasing CNS noradrenaline levels reduces pathology in TgAPP mice

Authors: *D. L. FEINSTEIN[1,2], P. E. POLAK[1], S. KALININ[1];
[1]Anesthesiol., Univ. of Illinois Chicago, Chicago, IL; [2]Jesee Brown VA Med. Ctr., Chicago, IL

Abstract: The Locus coeruleus (LC) is the major source of noradrenaline (NA) in the CNS. The number of LC neurons are significantly decreased in Alzheimer's disease (AD) and LC damage occurs in transgenic mouse models of AD. We tested the hypothesis that raising CNS NA levels would provide benefit in 5xFAD mice which have 3 mutations in amyloid precursor protein and 2 in presenilin-1. The 4.5 month old 5xFAD mice were treated 3 x per week with the NA precursor L-threo-3,4-dihydroxyphenylserine (L-DOPS) which is converted to NA via decarboxylation by the ubiquitous enzyme L-aromatic amino acid decarboxylase. Peripheral conversion was blocked by administering L-benserazide which does not cross the blood brain barrier. CNS levels were further increased by use of the NA selective reuptake inhibitor atomoxetine. After 4 weeks behavior was assessed by Morris water maze. Mice in the treatment group showed improved learning during training sessions, and better recall in a retest done one week later. NA levels in the frontal cortex were significantly increased by L-DOPS, and there was a strong correlation between improved behavior and increased NA levels. Soluble beta-amyloid levels were significantly reduced by treatment, and thioflavin-S stained plaques were reduced and inversely correlated to NA levels. L-DOPS reduced GFAP and Iba1 staining throughout the brain. Quantitative PCR analysis revealed changes in several mRNAs in the

frontal cortex including increased levels of synaptophysin, BDNF, NGF; and the amyloid degrading enzyme IDE. Gene profiling of mRNA isolated from the LC showed approximately 900 mRNAs whose levels were altered in 5xFAD mice compared to controls. Together these data indicate that raising CNS NA levels can provide cognitive benefit and reduce pathology in a robust model of AD.

Disclosures: D.L. Feinstein, None; **S. Kalinin**, None; **P.E. Polak**, None.

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.2

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH AG02219 (Project 2)

Title: Synaptosomal versus non-synaptosomal mitochondrial bioenergetics in the Tg2576 mouse model of Alzheimer’s disease: Differential regulation by age and beta-amyloid peptide content in the brain

Authors: *M. VARGHESE[1], W. ZHAO[1], J. WANG[1], A. CHENG[1], G. M. PASINETTI[1,2]; [1]Neurol., The Mount Sinai Sch. of Med., New York, NY; [2]Geriatric Res. and Clin. Ctr., James J. Peters Veterans Affairs Med. Ctr., Bronx, NY

Abstract: Background: Impairment of mitochondrial energy metabolism has been implicated in the neurodegeneration seen in Alzheimer’s disease (AD). Analysis of post-mortem brain has indicated a decline in the mitochondrial respiratory pathway in AD as compared to the normal brain.
Objective: We investigated whether the mitochondrial dysfunction seen in AD patients is replicated in the AD transgenic mouse Tg2576 and correlates with age and amyloid load in the brain.
Methods: We analyzed respiration in synaptosomal and non-synaptosomal mitochondria in the cerebral cortex and cerebellum of young (6-8 month) and old (12-14 month) female Tg2576 mice and wild type controls using the Seahorse XF24 extracellular flux analyzer. We also measured mitochondrial superoxide generation in these preparations by spectrofluorimetric analysis using the MitoSOX Red superoxide indicator. The brain beta-amyloid (Aβ) peptides were quantified by enzyme-linked immunosorbent assay.
Results: Our results indicate that the basal oxygen consumption rate in cortical synaptosomes

declines in both young and old Tg2576 mice as compared to age-matched wild type controls, while it remains unchanged in the cerebellum. Interestingly, in the non-synaptic mitochondria, while there was no change in the basal respiration in either group, the ADP-stimulated state 3 respiration was decreased in the young Tg2576 mice but increased in the old transgenics as compared to the wild type controls. This trend was reversed in both groups in the maximal respiration at both complex I and IV. Interestingly, we found that the cortical Aβ content was inversely associated with maximal oxygen consumption rate in the non-synaptic mitochondria in young versus old Tg2576 mice.
Conclusion: These ongoing studies indicate that while the content of Aβ peptides increases primarily as a function of age and causally attenuate synaptosomal mitochondrial functions in the Tg2576 mouse, the effect of amyloidogenic Aβ peptides on non-synaptic mitochondrial function with respect to age is more complex. In particular our studies on mitochondrial function in the brain of AD or wild type mice as a function of age suggest that careful design is required for therapeutic intervention at the level of mitochondrial bioenergetics in AD.

Disclosures: M. Varghese, None; **W. Zhao**, None; **G.M. Pasinetti**, None; **A. Cheng**, None; **J. Wang**, None.

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.3

Topic: C.02. Alzheimer's disease and other dementias

Support: FIS PI 071057

ciberned

Title: Beta amyloid (abeta) induces dendritic spine loss and neuritic changes through gsk3 activation

Authors: *B. DA ROCHA, JR[1], T. SCOTTON[2], M. COMA[2], M. SIAO[2], I. BARROETA[3], E. HUDRY[4], T. HASIMOTO[4], M. ARBEL[4], P. SANCHEZ[4], O. DOLS[4], B. HYMAN[4], T. GOMEZ[4];
[1]Massgeneral Inst. For Neurodegenerative Disease, Harvard Med. Sch., CHARLESTOWN, MA; [2]Massgeneral Inst. For Neurodegenerative Disease, Harvard Med. Sch., Charlestown, MA; [3]Hosp. Santa Cruz y San Pablo, Univ. Autónoma de Barcelona, Barcelona, Spain; [4]MassGeneral Inst. for Neurodegenerative Disease, Harvard medical School,, Charlestown, MA

Abstract: Background: It has been proposed that glycogen synthase kinase 3 (GSK3), a kinase with crucial roles in major signaling processes in the brain, might be a key downstream effector of Aβ in Alzheimer's disease (AD). A remarkable amount of data establish striking connections between GSK3, especially GSK3β isoform, and Aβ and tau. However, fundamental questions remain unknown: is GSK3β activity increased in the human AD brain? Is there a unique molecular species of Aβ that is responsible for GSK3β overactivity in AD? Is GSK3β a critical mediator of Aβ induced synaptic compromise? **Objectives**: To measure GSK3β levels and activity in human AD brains in comparison to controls and in primary neuronal cultures exposed to oligomeric Aβ (oAβ). To investigate whether the oAβ induced neurodegenerative phenotype of dendritic spine loss and neuritic changes can be blocked by GSK3β inhibition. **Methods**: GSK3β activity was measured in total brain homogenates and nuclear extracts from temporal cortex of human AD and control brains (N=12 per group). Similar assessments where done in mouse primary neuronal cultures after 24 hour exposure to wild type or Tg2576 conditioned media enriched in oAβ species, and to oAβ isolated by size exclusion chromatography from human AD brains. Immunodepletion experiments were used to ensure that the active component in the media is Aβ. Spine densities were determined in GFP labelled mature neurons in each experimental condition (N=8 dishes per condition) and after treatment with a GSK3 inhibitor (TDZ-8). **Results**: GSK3β levels were decreased by about 35% in AD brains in comparison to controls, as expected due to neuronal cell loss in AD. However, the ratio GSK3β/PSer9GSK3β was increased by 50%, indicating that GSK-3β is more active in AD brains. GSK3β activity was also significantly increased in primary cultures after exposure to oAβ, especially in the nuclear compartment, and resulted in a robust reduction of dendritic spine density. This phenotype was fully rescued by pharmacological inhibition of GSK3. **Conclusion**: We find evidence for GSK3β activation due to Aβ in human AD brains and neurons in culture. Aβ exposure in those two settings is associated with synaptic derangement. The oAβ induced dendritic spine loss in neurons in culture can be prevented by pharmacological blockade of GSK3. These results agree with our findings that neuritic changes in the vicinity of Aβ deposits in an APP/tau mouse model are ameliorated by GSK-3 inhibition. Previous data had implicated calcineurin and MAPK alterations in the setting of oAβ; the current data suggest that oAβ may also activate GSK3, which also contributes to neuronal and synaptic degeneration in AD.

Disclosures: B. Da rocha: Employment; MassGeneral Institute for Neurodegenerative Disease, Harvard medical School. Research Grant; FIS PI 071057, ciberned. **T. Scotton:** Employment; MassGeneral Institute for Neurodegenerative Disease. Research Grant; FIS PI 071057, ciberned. **M. Coma:** Employment; IRB. Research Grant; FIS PI 071057, ciberned. **M. Siao:** Employment; MassGeneral Institute for Neurodegenerative Disease, Harvard medical School,. **I. Barroeta:** Hospital Santa Cruz y San Pablo, Universidad Autónoma de Barcelona. **E. Hudry:** None. **T. hasimoto:** None. **M. Arbel:** None. **P. Sanchez:** None. **O. Dols:** None. **B. Hyman:** None. **T. Gomez:** MassGeneral Institute for Neurodegenerative Disease, Harvard medical School,. Research Grant; FIS PI 071057, Ciberned.

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.4

Topic: C.02. Alzheimer's disease and other dementias

Support: P01: 2-AG17216

P01: NS048447

Title: Spread of pathology and functional impairment in mice with entorhinal cortex restricted expression of human Tau or APP

Authors: *K. E. DUFF[1], L. LIU[2], A. BRETTEVILLE[2], H. MORENO[2,3], S. SMALL[2]; [1]Taub Inst. at Columbia Univ/ NYSPI, NEW YORK, NY; [2]Taub Inst. at Columbia Univ., NEW YORK, NY; [3]Dept of Neurology/Physiology, SUNY Downstate Med.Ctr., Brooklyn, NY

Abstract: The occurrence of pathological lesions (amyloid plaques and neurofibrillary tangles) in the brain of patients with Alzheimer's Disease is a defining feature of the disease. Pathology develops in a stereotypic manner, with the earliest manifestations occurring in the hippocampal formation - the entorhinal cortex (EC) especially. It is likely that both types of lesion ultimately contribute to brain dysfunction, and that the progressive spread of pathology out of the hippocampal formation correlates with worsening disease. How pathology spreads is unknown - it is thought to follow discrete, synaptically connected circuits, but the path taken, and the mechanism by which pathology spreads, is not well known due to limitations of post-mortem human tissue, and the prevalence of mouse models using heterologous promoters to drive transgene expression. To examine whether Abeta and tau pathology spreads from the EC via a transynaptic route, and the functional consequence of pathology build up in the EC on remote regions, we have generated inducible mice with regionally-restricted expression of mutant APP and tau transgenes driven by the Neuropsin promoter (Yasuda and Mayford, 2006). Human APP (antibody 6E10) was restricted to cell bodies of the medial entorhinal cortex and parasubiculum, cortical layer II cell bodies and hippocampal pyramidal cell layers in CA1, 2 and 3. Abeta peptide (including intracellular Abeta) could not be identified on the brain sections at ages studied (up to 6 months). Interestingly, and different to mice such as Tg2576 or J20 that use a more generally expressed promoter, the dentate gyrus was completely spared in the Neuro/APP mice. APP fragments showed a different distribution suggesting that fragments are either processed in different locations, or transported differently. Tau mice showed human tau staining in the parenchyma of the medial entorhinal cortex but also in terminals of the performant pathway, in keeping with the diffuse distribution of soluble tau. Phospho tau (either mouse or human) showed a different distribution to total human tau with cell bodies in the hippocampal fields being immunoreactive (CP13 antibody) by 4 months of age. Functional assessments including fMRI and electrophysiological analysis are currently being undertaken. Data will be

presented on the accumulation of APP, Abeta and other APP intermediates in the Neuro/APP mice, and tau and potential pathological forms (phospho-tau and conformationally abnormal forms) in the Neuro/Tau mice, with aging, and correlated to functional outcomes.

Disclosures: K.E. Duff, None; **L. Liu**, None; **A. Bretteville**, None; **H. Moreno**, None; **S. Small**, None.

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.5

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH K08 DC04807

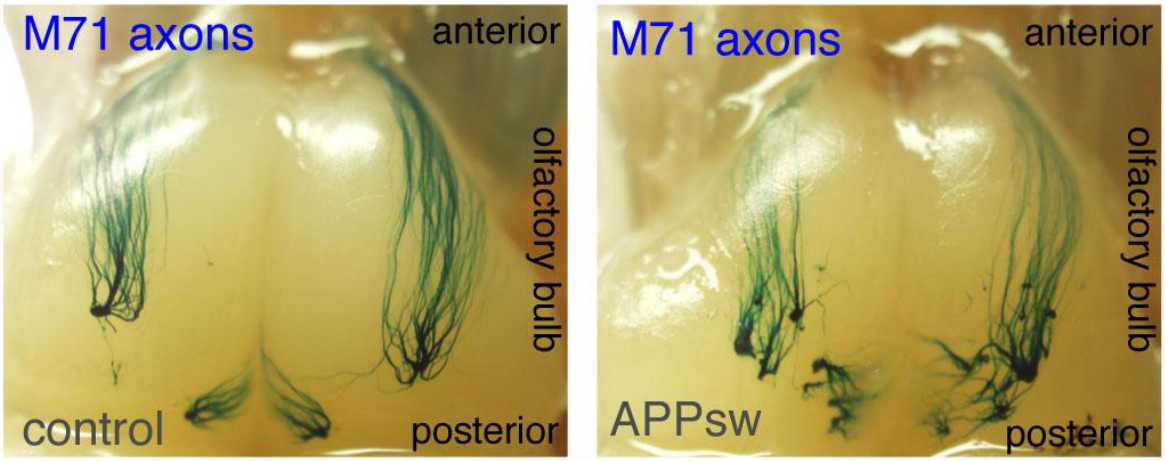
NIH DP2 OD006662

Title: Sparse expression of pathogenic human APP induces altered connectivity and accelerated cell death in mouse olfactory neurons

Authors: *M. ALBERS, L. CAO, S. RODRIGUEZ, G. T. RICKENBACHER, E. G. BENZ; Massachusetts Gen. Hosp., BOSTON, MA

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder that manifests with cognitive deficits arising from neural circuit dysfunction. Accumulating evidence indicates that the pathogenic process is present years before the onset of symptoms. The amyloid precursor protein (APP) and its cleavage product, the Aß peptide, are thought to trigger a series of events that result in neuronal loss. Efforts to recapitulate accelerated neuronal loss in mice by overexpressing pathogenic isoforms of APP have not been successful, unless a human tau allele is also present. Thus, the relationship between amyloid plaque formation, tangle formation, and neuronal cell loss in the preclinical stage of the disease is poorly understood. Here, we report that mouse lines overexpressing the Swedish mutation of human APP (hAPPsw) exhibit altered connectivity of their olfactory sensory neurons (OSNs) and accelerated OSN death prior to the formation of plaques. In mouse lines we generated that express hAPPsw exclusively in all mouse OSNs or in a small, stochastic fraction of OSNs (< 1%), we found OSN axon mistargeting (see image), increased expression of activated caspase 3, and increased TUNEL staining in mouse OSNs. Moreover, genetically-defined subpopulations of OSNs were dramatically diminished in lines expressing hAPPsw relative to littermate controls. Sparse expression of hAPPsw alters the

structure of the peripheral olfactory neural circuit and causes accelerated neuronal cell death in the absence of amyloid plaques. These actions of hAPPsw may provide insight into the early pathogenic steps of Alzheimer's disease that precede the formation of amyloid plaques and the onset of clinical symptoms.



Disclosures: M. Albers, None; **L. Cao**, None; **S. Rodriguez**, None; **G.T. Rickenbacher**, None; **E.G. Benz**, None.

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.6

Topic: C.02. Alzheimer's disease and other dementias

Title: Calcium/calcineurin/NFAT signaling in Alzheimer's disease

Authors: *I. GRAEF;
Pathology, Stanford Med. Sch., Stanford, CA

Abstract: Alzheimer's disease (AD) is defined by the deposition of amyloid plaques, neurofibrillary tangles, and progressive neuronal loss. While genetic studies have clearly confirmed the importance of amyloid-beta (Ab) accumulation and aggregation in the CNS, the molecular mechanisms that lead to neuronal dysfunction and death are still largely undefined. The hypothesis that a dysregulation of intracellular Ca2+ contributes to the pathogenesis of AD has been put forward more than 20 years ago. However, even today the role of Ca2+ homeostasis

and Ca2+ signaling in AD is unclear and the question whether the modulation of Ca2+-regulated signaling pathways is beneficial or detrimental is still unanswered. One Ca2+ regulated signaling molecule that has been implicated in AD pathogenesis by multiple studies is the Ca2+/calmodulin activated phosphatase, calcineurin (CaN). However, the results regarding the putative of role of Ca2+/CaN signaling in AD have been contradictory and the involvement of CaN in AD has not been validated in genetic models of the CaN signaling pathway. CaN is a three-subunit enzyme made up of a catalytic subunit encoded by three genes, a regulatory subunit that binds Ca2+, encoded by two genes and calmodulin. Overexpression studies using a constitutively active form of the catalytic subunit of CaN and studies with the purified enzyme suggested that CaN would dephosphorylate many substrates. One of the in vivo validated substrates of CaN is the NFATc family of transcription factors (NFATc1-c4) and mice carrying mutations of NFATc genes phenocopy most of the developmental defects observed in CaN mutant mice. CaN binds directly to NFATc proteins and dephosphorylates NFATc proteins, which results in their rapid cytoplasmic-to-nuclear translocation. The direct interaction between CaN and NFATc might explain why much of CaN becomes dedicated to NFATc proteins during embryonic neuronal development when NFATc proteins are highly expressed. In the adult brain CaN is expressed at very high levels and hence cannot be dedicated to the much less abundant NFATc1-c4. The relative change in abundance of the NFATc proteins to CaN during neural development probably accounts for a shift in CaN function from NFAT-dependent transcriptional control in embryos to other aspects of neural function in the adult nervous system. To investigate the role of this signaling pathway in the pathogenesis of AD we have used transgenic mice carrying gain-of-function and loss-of-function alleles of CaN and NFATc genes and examined whether changes in CaN activity and/or NFATc-dependent transcription alter the development of AD like pathology in mouse models of human AD.

Disclosures:

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.7

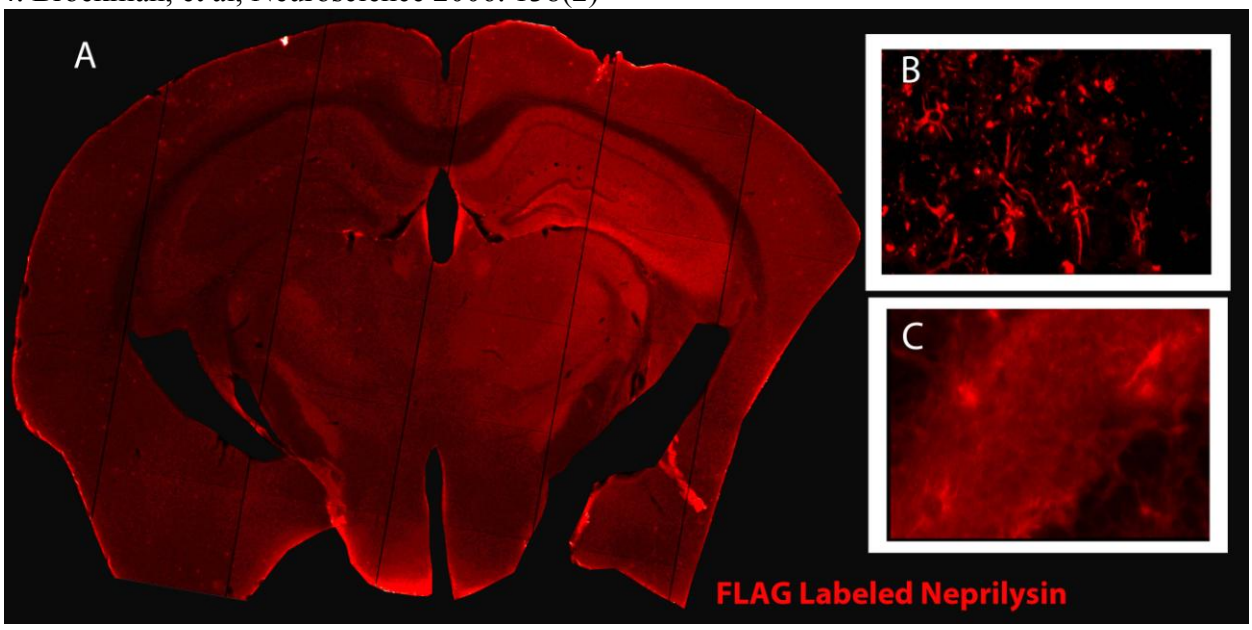
Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant A10485

Title: Adeno-associated virus type 8 overexpression of a FLAG labeled neprilysin is cleared by astrocytes

Authors: *A. C. HIRKO[1], L. ZHANG[2], M. A. KING[2];
[1]Pharmacol., Univ. of Florida, GAINESVILLE, FL; [2]Pharmacol., Univ. of Florida, Gainesville, FL

Abstract: Alzheimer's disease (AD) is the leading cause of dementia. Accumulation of amyloid beta (AB) peptide, resulting from increased production or decreased clearance, may underlie cognitive and synaptic deficits observed in AD. A potential treatment strategy is to increase clearance by enzymes involved in AB degradation. Neprilysin (NEP) has been identified as a key enzyme responsible for AB degradation at synapses. Gene therapy strategies aimed at increasing the expression of NEP have been successful in transgenic models of AD [1-3]. Based on the observation that adeno-associated virus (AAV) type 8 results in more efficient and widespread transgene delivery in the mouse brain as compared to other vectors [4], we hypothesized that AAV8 could be a more effective vector for NEP gene therapy for AD. To test this we developed a FLAG-labeled NEP vector driven by the hybrid CMV/enhanced CBA promoter and packaged it into AAV8 capsids. APPSWE/PS1dE9 transgenic mice were unilaterally injected into hippocampus with AAV8-FLAG-NEP or AAV8-GFP. Two months after injections animals were sacrificed and analyzed. Immunostaining for the FLAG epitope revealed diffuse labeling throughout the injected side hippocampus, cortex and thalamus (panel A in figure, 4x). At higher magnification astrocytic labeling of FLAG was found in the contralateral hippocampus and cortex (panel B, 10x). Astrocytic labeling was also observed on the injected side hippocampus panel C, 20x) but obscured by neuronal immunoreactivity. Because GFP is rarely found in astrocytes, astrocytic NEP suggests that astrocytes participate in the clearance of FLAG-NEP gene product secreted from transduced neurons. Thiazine red staining of amyloid deposits revealed significant reductions in the FLAG-NEP group. Our results support the hypothesis that AAV8 is a suitable gene delivery agent targeting AB.
1. Marr, et al, J Neurosci 2003. 23(6)
2. Iwata, et al, J Neurosci 2004. 24(4)
3. Spencer, et al, BMC Neurosci 2008. 9
4. Broekman, et al, Neuroscience 2006. 138(2)



Disclosures: A.C. Hirko, None; **L. Zhang**, None; **M.A. King**, None.

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.8

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH grant AG023084

NIH grant AG029481

ISOA

ADDF/Elan

Title: Tertiary amide reduced BACE1 activity and Aβ production in a mouse model of AD

Authors: *R. DEANE[1], I. SINGH[1], A. SAGARE[1], R. BELL[1], N. ROSS[2], B. MILLER[2], B. ZLOKOVIC[1];
[1]Dept Neurosurg, [2]Dept. of Dermatol., Univ. Rochester, ROCHESTER, NY

Abstract: Receptor for advanced glycation endproducts (RAGE), a multiligand receptor, binds neurotoxic Alzheimer's amyloid β-peptide (Aβ) and this is associated with neurovascular stress. In Alzheimer's disease (AD) and in transgenic (Tg) mouse models of AD, increased RAGE expression is associated with higher brain Aβ levels and neurovascular stress. Thus compounds that block Aβ/RAGE interaction may reduce Aβ levels in brain and Aβ-related neurovascular stress, which should have beneficial therapeutic effects in AD. Using primary screening on RAGE-transfected Chinese hamster ovary (CHO) cells, a tertiary amide (FPS2) was identified to block RAGE-Aβ interaction with high affinity, and reduce oxidative stress. A second generation tertiary amide, FPS2-BM, was then produced, with greater blood-brain barrier delivery (53-fold) and affinity (2-fold) to block Aβ/RAGE interaction than FPS2. Since RAGE increases NF-κB activation, and Aβ production via BACE1, we reasoned that FPS2-BM will have a greater inhibitory effect on RAGE-induced Aβ production than FPS2. $APPsw^{+/-}$ mice treated with FPS2-BM (intraperitoneally) daily for two months, starting at 8 months old, had a 4-fold reduction in brain Aβ pathology, compared to FPS2. In these mice BACE1 mRNA expression levels, protein levels and activity were reduced with FPS2-BM by 3-fold in the cortex and hippocampus compared to FPS2. The levels of nuclear p65 (NF-κB) in these brain regions were reduced with

FPS2 (1.3-fold) and FPS2-BM (2.6-fold) while sAPPβ levels reflected BACE1 activity. We then used a neuronal cell line (SH-SY5Y), which expresses RAGE, and showed that levels of Aβ-induced BACE1 and nuclear p65 were reduced with FPS2 and FPS2BM by 1.5- and 3-fold, respectively**.** Furthermore, a mutant form of IκB-α (S32, 36A), which acts as a suppressor of NF-κB gene expression, inhibited the Aβ-induced increased levels of BACE1.These compounds or their chemical related entities could lead to the discovery of new brain Aβ lowering agent(s) that can inhibit Aβ/RAGE-mediated Aβ production via BACE1.

Disclosures: R. Deane: None. **I. Singh:** None. **A. Sagare:** None. **R. Bell:** None. **N. Ross:** None. **B. Miller:** None. **B. Zlokovic:** Other; BVZ is the scientific founder of Socratech L.L.C., a startup biotechnology company with a mission to develop new therapeutic approaches for stroke and Alzheimer's disease..

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.9

Topic: C.02. Alzheimer's disease and other dementias

Support: Sigrid Juselius Foundation

Academy of Finland

Title: Effect of genetically and dietary induced insulin resistance on amyloid-beta metabolism and neurological symptoms in APP/PS1 transgenic mouse model of Alzheimer's disease

Authors: *H. TANILA[1,2], V. KHANDELWAL[3], T. TIILIKAINEN[4], M. TUSA[1], H. KOIVISTO[1], M. KRZISCH[1], S. VEPSÄLÄINEN[4], P. MÄKINEN[4], A. HAAPASALO[4], S. KEMPPAINEN[1], P. MIETTINEN[1], H. SOININEN[4,2], M. LAAKSO[4,5], M. HILTUNEN[4]; [1]A. I. Virtanen Institute, Univ. Eastern Finland, Kuopio, Finland; [2]Dept. Neurology, Kuopio Univ. Hosp., Kuopio, Finland; [3]Dept. Pharmacy, Univ. Eastern Finland, Kuopio, Finland; [4]Dept. Clin. Medicine, Univ. Eastern Finland, Kuopio, Finland; [5]Dept. Medicine, Kuopio Univ. Hosp., Kuopio, Finland

Abstract: Background: Epidemiological studies indicate that type 2 diabetes is a risk factor for Alzheimer's disease (AD). Amyloid-beta metabolism is coupled with insulin signaling pathway. To investigate this relationship *in vivo*, we induced hyperglycemia in amyloid producing APPswe/PS1dE9 (APdE9) mice either genetically by cross-breeding these mice with insulin-like

growth factor 2 (IGF2) overexpressing mice or by feeding them with high-fat diet. IGF2 overexpression results in hypertrophy of pancreatic beta-islet cells, hyperinsulinemia, and hyperglycemia upon aging.
Results: Glucose and insulin tolerance tests revealed significant hyperglycemia in all mice expressing IGF2 genotype, which was exacerbated by high-fat diet. However, sustained hyperinsulinemia and insulin resistance could be observed only in mice co-expressing IGF2 and APdE9 transgenes. In neurological tests at 8 or 12 months of age, APdE9 genotype was associated with poor spatial learning, and the combination of IGF2 genotype and high-fat diet further impaired learning. Neither high-fat diet nor IGF2 genotype increased amyloid plaque burden in the brain. In males, however, IGF2 genotype decreased microglial activation around the plaques and increased amyloid-beta 42/40 ratio. IGF2 genotype increased the Ser473 phosphorylation of Akt in males. In contrast, the Ser21 phosphorylation of GSK3alpha was increased only in APdE9 and IGF2 transgenic females on standard diet, while female mice on high-fat diet with the same genotype did not differ from controls. IDE levels were not affected by genetic or dietary manipulations.
Conclusions:These findings indicate that amyloid-beta production, glucose metabolism and insulin signaling are coupled through several regulatory loops, which may further differ between the sexes.

Disclosures: H. Tanila, None; **V. Khandelwal**, None; **T. Tiilikainen**, None; **M. Tusa**, None; **H. Koivisto**, None; **M. Krzisch**, None; **S. Vepsäläinen**, None; **P. Mäkinen**, None; **A. Haapasalo**, None; **S. Kemppainen**, None; **P. Miettinen**, None; **H. Soininen**, None; **M. Laakso**, None; **M. Hiltunen**, None.

Nanosymposium

12. Animal Models of Alzheimer's and Neurodegeneration

Location: Room 23A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 12.10

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant AG031845

NIH Grant RC1ES018221

Title: Mice expressing low levels of inducible nitric oxide synthase (iNOS) represent a more complete model for Alzheimer's disease

Authors: *M. D. HOOS, D. M. WILCOCK, J. G. WILSON, A. EVERHART, C. A. COLTON;

Medicine/Neurology, Duke Univ., Durham, NC

Abstract: Alzheimer's disease is characterized by the formation of amyloid plaques consisting of amyloid-β peptide (Aβ) in the parenchyma and cerebral vasculature, as well as the formation of neurofibrillary tangles (NFTs) consisting of aggregates of hyperphosphorylated tau within neurons. These features are thought to lead to neuronal loss that promotes the cognitive decline associated with AD. To date several animal models have been developed to replicate the pathology of Alzheimer's disease (AD), however most have failed to produce Aβ deposition, NFTs, and neuronal loss all in one model. As well as these major pathological features in human patients there is also observed to be chronic brain inflammation, which has features of both classical and alternate immune activation. One consequence of this is a down regulation of the gene nitric oxide synthase 2 (NOS2) and its product inducible NOS (iNOS). This enzyme produces nitric oxide (NO), an important regulator of inflammation in the brain as well as an important inhibitor of caspases. Our studies demonstrate that mice expressing mutated human Aβ precursor protein (AβPP) on a NOS2 knockout background (AβPP/NOS2$^{-/-}$) display amyloid deposition accompanied by NFTs, significant loss of neurons, marked behavioral changes, neuroinflammation, and neurovascular unit involvement. These changes were in contrast to mutant AβPP animals, which showed only severe Aβ deposition. Preliminary studies with trigenic mice expressing a human NOS2 transgene under regulation of the human NOS2 promoter (AβPP/huNOS2/NOS2$^{-/-}$) demonstrate similar pathologies compared to the bigenic AβPP/NOS2$^{-/-}$. These animals mimic the low levels of NO found during chronic inflammation in human patients by utilizing the human NOS2 promoter and provide direct evidence that the lower iNOS activity, as compared to mouse iNOS, may explain why other models of AD fail to show complete AD pathology. These new models represent a more complete platform for studying the pathology of AD and has lead us to hypothesize that low NO levels in the brain brought about by chronic inflammation may be an important factor in the pathogenesis of AD.

Disclosures: M.D. Hoos, None; **C.A. Colton**, None; **D.M. Wilcock**, None; **J.G. Wilson**, None; **A. Everhart**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.1

Topic: C.06. Developmental Disorders

Support: HHMI, Simons Foundation, IRSF

NIH Grant HD053862

NIH Grant HD024064

NIH Grant 29709

NIH Grant F31MH078678

Autism Speaks

BRASS and McNair Fellowships

Title: MeCP2 deficiency in GABAergic neurons recapitulates Rett syndrome phenotypes

Authors: *H.-T. CHAO[1], H. CHEN[2], R. C. SAMACO[2], M. XUE[4], M. CHAHROUR[5], J. YOO[2], J. L. NEUL[3], S. GONG[6], N. HEINTZ[6,7], M. EKKER[9], J. L. R. RUBENSTEIN[10], J. L. NOEBELS[2], C. ROSENMUND[2,11], H. Y. ZOGHBI[2,8];
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Abstract: Loss of function mutations in the gene *MECP2* encoding the transcriptional regulator methyl-CpG-binding protein 2 (MeCP2) causes the neurodevelopmental disorder Rett syndrome (RTT). RTT is characterized by neurological regression, cognitive impairments, impaired motor function, respiratory dysfunction, repetitive stereotypies, and altered social behavior. Of these features, altered social behavior and repetitive stereotypies are also important hallmarks of autism spectrum disorders (ASD). However, the mechanisms contributing to these neurobehavioral alterations are poorly understood. We show that deletion of *Mecp2* from global inhibitory γ-amino-butyric-acid-(GABA)-ergic neurons in mice recapitulates nearly all major RTT features, including repetitive stereotypies. Furthermore, this selective MeCP2 deficiency in GABAergic neurons results in altered social behavior. The critical role for GABAergic neurons in RTT pathogenesis is further illustrated by findings that deletion of *Mecp2* from ~50-60% of forebrain (telencephalon and diencephalon) GABAergic neurons recapitulates selective deficits in motor function, repetitive stereotypy, and social behavior. Electrophysiological measurements reveal alterations in the physiological function of GABAergic neurons, providing insight into MeCP2's endogenous role in regulating GABAergic signaling. These findings demonstrate that dysfunction of GABAergic neurons is a crucial causative factor in RTT pathogenesis.

Disclosures: H. Chao, None; **H. Chen**, None; **R.C. Samaco**, None; **M. Xue**, None; **M. Chahrour**, None; **J. Yoo**, None; **J.L. Neul**, None; **S. Gong**, None; **N. Heintz**, None; **M. Ekker**, None; **J.L.R. Rubenstein**, None; **J.L. Noebels**, None; **C. Rosenmund**, None; **H.Y. Zoghbi**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.2

Topic: C.06. Developmental Disorders

Support: NIMH Grant 5R01MH079079

ALSAC

Title: Age-dependent dysregulation of presynaptic calcium, synaptic plasticity, and spatial memory in a mouse model of 22q11 deletion syndrome

Authors: *L. R. EARLS[1], G. FRICKE[1], I. BAYAZITOV[1], R. BERRY[1], E. ILLINGWORTH[2], G. MITTLEMAN[3], S. S. ZAKHARENKO[4];
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Abstract: 22q11 deletion syndrome (22q11DS) is caused by the hemizygous deletion of a 1.5- to 3-megabase region of DNA on chromosome 22 and occurs in approximately 1 in every 4,000 live births. The resulting syndrome is characterized by physical and cognitive deficits in children. Cognitive abnormalities progress during adolescence and adulthood, and approximately 30% of patients develop schizophrenia in adulthood. The molecular mechanisms of age-related neuronal dysfunction in 22q11DS are poorly understood. Studies of a mouse model of 22q11DS, the *Df(16)1/+* mouse carrying a hemizygous deletion of the 22q11DS-relevant region , revealed substantially enhanced short- and long-term synaptic plasticity in the hippocampus, a brain region important for memory. These plasticity alterations coincided with deficits in hippocampus-dependent spatial memory. Behavioral and synaptic changes become evident in mature but not young animals, consistent with the progression of cognitive deficits in 22q11DS patients. Electrophysiological, two-photon imaging, two-photon glutamate uncaging, and structural studies in acute hippocampal slices showed that these changes were caused by enhanced neurotransmitter release from presynaptic neurons, but not by altered postsynaptic function or structure. Enhanced neurotransmitter release in *Df(16)1/+* mice coincided with altered Ca^{2+} kinetics in presynaptic terminals and upregulated sarco(endo)plasmic reticulum Ca^{2+}-ATPase type 2 (SERCA2). SERCA inhibitors rescued all tested synaptic phenotypes of *Df(16)1/+* mice. These results suggest that presynaptic SERCA2 upregulation is a pathogenic event contributing to the cognitive symptoms of 22q11DS. A screen for the 22q11DS disease-

critical genes responsible for these phenotypes is currently underway. So far, this screen has revealed that a complex interaction between genes produces the ultimate synaptic plasticity imbalance observed in the *Df(16)1/+* mouse. The identification of the causal genes important for cognitive symptoms of disease, as well as downstream targets, such as SERCA2, will provide us with potential targets for treatment of this disease.

Disclosures: L.R. Earls, None; **G. Fricke**, None; **I. Bayazitov**, None; **R. Berry**, None; **E. Illingworth**, None; **G. Mittleman**, None; **S.S. Zakharenko**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.3

Topic: C.06. Developmental Disorders

Support: Inserm Avenir program

ANR-08-MNP-013

Fondation Bettencourt Schueller

Swiss National Science Foundation SNSF - SPUM - 33CM30-124089

Fondation Gianni Biaggi de Blasys

Title: Characterization of a new microtubule-associated protein critical for cortical development

Authors: *F. FRANCIS[1], F. PHAN DINH TUY[1], M. KIELAR[2], K. BOUTOURLINSKY[1], R. OLASO[3], A. BOLAND[3], W. CARPENTIER[4], E. WELKER[2], A. CROQUELOIS[2];
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Abstract: The proliferation, migration and differentiation of neurons are processes critical for cortical development. A number of cytoskeletal proteins (e.g. DCX, LIS1, TUBA1A) have been found to be mutated in severe cortical malformations, such as type 1 lissencephaly, associated with mental retardation and epilepsy. In addition to studying these proteins, we have been searching for novel genes playing an important role in cortical development. Here, we used

genetic linkage and transcriptome studies to identify a novel mutant gene in the HeCo mouse model. HeCo mice, which arose spontaneously, exhibit a heterotopic band of neurons in the isocortex, suggestive of arrested neuronal migration during development, and present a susceptibility to epilepsy, thus strongly resembling the subcortical band heterotopia phenotype in human. Transcriptome studies successfully revealed the perturbed expression of a gene, mapping to the candidate genomic region identified by genetic linkage. This gene, for which we are now performing rescue experiments in HeCo mice, codes for a little-studied microtubule-associated protein (MAP) not previously known to be important for neuronal migration. A retrotransposon insertion in intron 21 disrupts the full length version of this gene and creates aberrant chimeric transcripts. We are now studying the normal expression pattern of this MAP in the developing and adult brain and performing biochemical and cell biological experiments to better characterize the function of the protein. These studies should help us understand the role of this MAP in immature neurons and the primary pathophysiological mechanisms leading to perturbed cortical development in the HeCo mouse model.

Disclosures: F. Phan Dinh Tuy, None; **F. Francis**, None; **M. Kielar**, None; **K. Boutourlinsky**, None; **R. Olaso**, None; **A. Boland**, None; **W. Carpentier**, None; **E. Welker**, None; **A. Croquelois**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.4

Topic: C.06. Developmental Disorders

Support: National Institute of Dental and Craniofacial Research

Title: Development of the pituitary in healthy children and children with isolated cleft lip and/or palate

Authors: *E. VAN DER PLAS[1], C. J. CASPELL[2], A. M. AERTS[1], J. CANADY[3], L. RICHMAN[4], E. TSALIKIAN[4], J. D. DAWSON[2], P. NOPOULOS[1];
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Abstract: Growth problems are common in children with isolated cleft lip and/or palate (ICLP); however the causes of these problems are unclear. The pituitary is of primary importance in physical growth as it produces and releases Growth Hormone. Magnetic Resonance Imaging

(MRI) studies on healthy populations demonstrate that the pituitary increases linearly with age up to young adulthood. In view of the embryologic relations of the lip, palate and the pituitary, it was hypothesized that subjects with ICLP would have abnormalities in structure (volume) and growth of pituitary volume over time.
The objective of this study was compare physical stature (height) and volume of the pituitary in ICLP subjects compared to healthy controls. In addition, both height and pituitary volume were evaluated as a function of age to assess the developmental trajectory of these measures. MRI scans were obtained from 39 boys with ICLP and 57 healthy boys in the age range of 7 - 17, and the pituitary was manually traced.
Results showed that boys with ICLP were on average shorter than age matched peers and this pattern was consistent across the entire age range. The mean volume of the pituitary was not different between the two groups. We replicated findings of a positive relationship between age and pituitary volume in our sample of healthy boys, $r = .379$. However, in boys with clefts there was no relationship between age and pituitary volume, $r = .057$.
These results suggest that development of the pituitary may be abnormal in children with ICLP. We are currently collecting data on girls with orofacial clefts, and we are obtaining data on Growth Hormone release in individuals with ICLP and healthy comparison subjects.

Disclosures: E. Van der Plas, None; **C.J. Caspell**, None; **A.M. Aerts**, None; **J. Canady**, None; **L. Richman**, None; **E. Tsalikian**, None; **J.D. Dawson**, None; **P. Nopoulos**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant K08NS52550

Peripheral Neuropathy Society

The Deater Foundation

Title: L-serine supplementation: A new treatment for hereditary sensory neuropathy type 1

Authors: *B. P. SCHMIDT[1], K. GAROFALO[1], T. HORNEMANN[2], C. WIDDICOMBE[1], O. GIANNIKOPOULOS[1], A. PENNO[2], R. BROWN[3], F. EICHLER[1];
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Abstract: Hereditary sensory and autonomic neuropathy type I (HSAN1) is caused by missense mutations in the SPTLC1 gene encoding a subunit of the enzyme serine palmitoyltransferase (SPT). We recently identified two novel deoxysphingoid bases (DSB), deoxysphinganine (DoxSA) and deoxymethylsphinganine (DoxMeSA), that accumulate in plasma of HSAN1 patients as well as of mice bearing a transgene expressing mutant SPTLC1. We hypothesize that the mutant enzyme has a dramatically reduced reactivity with its normal preferred substrate L-serine and a promiscuously increased reactivity with L-alanine and glycine, causing the mutant animals to form DSB. In support of this hypothesis, mutant mice on a 10% L-alanine enriched diet had increased levels of DoxSA. Further, the mice developed a severe peripheral neuropathy within 2-4 months. After 6 months of L-alanine supplementation, ultrastructural analysis of distal sciatic nerves showed a dropout in larger myelinated axons and pronounced hypermyelination. The L-alanine supplemented mice had lower G ratios compared to untreated mutant mice. In contrast, mice on a 10% L-serine enriched diet showed reduced DoxSA levels by 5-fold within 2 to 4 days. Once on the diet, these mice were dramatically protected from neurodegeneration on both measures of sensory (mechanical sensitivity) and motor performance (rotorod). These mice retained their neurological function up to 15 months of age, unlike their untreated peers who by that age had developed a severe neuropathy. Similarly, during a pilot study of 14 human HSAN1 patients, we again observed dramatic effects of L-serine supplementation. Patients on a low dose regimen (200mg/kg/d) had 2-fold lower DoxSA levels within a month; while on a high dose regimen (400mg/kg/d) levels were decreased 4-fold in the same time period. Therefore, our observations suggest that a key to the pathophysiology of HSAN1 is the altered substrate selectivity of the enzyme. Thus we propose a new paradigm that altered substrate reactivity can lead to neurodegeneration and, moreover, give rise to new treatment opportunities. Lending support to the latter notion, we demonstrate rescue of the HSAN1 phenotype by mass action; that is, by flooding the enzyme environment in both mice and humans with L-serine, greatly in excess of L-alanine and glycine. L-serine supplementation has so far been used to treat serine deficiency disorders but not disorders of functional utilization, such as HSAN1.

Disclosures: B.P. Schmidt, None; **K. Garofalo**, None; **T. Hornemann**, None; **C. Widdicombe**, None; **O. Giannikopoulos**, None; **A. Penno**, None; **R. Brown**, None; **F. Eichler**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: W.M. Keck Foundation.

Title: Identification of client proteins involved in cysteine string protein alpha (cspα)-mediated neuroprotection

Authors: Y. ZHANG[1], M. HENDERSON[1], T. WU[2], C. COANGELO[2], *S. S. CHANDRA[1]; [1]Yale Univ., NEW HAVEN, CT; [2]Keck Yale/NIDA Neuroproteomic Ctr., New Haven, CT

Abstract: Cysteine String Protein alpha (CSPα) is a synaptic vesicle associated protein involved in neuroprotection. Deletion of CSPα causes severe synaptic loss, neurodegeneration and early lethality. CSPα, a Hsp40 type co-chaperone, keeps its client proteins in their proper conformation, thereby allowing for their normal synaptic functions. To elucidate the molecular mechanism of CSPα-mediated synaptic maintenance and neuroprotection, we set out to identify its specific client proteins. We used two quantitative mass spectrometric methods --DIGE and iTRAQ-- to compare the synaptic proteome of wild type and CSPα knockout mice brains. We identified a few proteins whose levels were selectively decreased in CSPα knockout mice, suggesting that they are potential clients for CSPα. The reduction in protein amounts was confirmed by quantitative immunoblotting. To demonstrate direct binding of potential clients to CSPα, GST pulldown was performed using both brain extracts and overexpression in HEK293 cells. Three proteins involved in the synaptic vesicle cycle were identified as binding partners for CSPα in vitro. Coimmunoprecipitation experiments revealed that they also interacted with CSPα in vivo, implying a physiological significance. A difference in protein aggregation, ubiquitination and expression pattern for these clients was observed between wild type and CSPα knockout brains, indicating that CSPα plays a role in preserving conformation of these proteins. Altogether, our data strongly suggest that CSPα fulfills its protective role in synaptic maintenance by acting on client proteins that regulate steps in the synaptic vesicle cycle.

Disclosures: Y. Zhang, None; **T. Wu**, None; **C. Coangelo**, None; **S.S. Chandra**, None; **M. Henderson**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: UL1DE019583-03

RL1NS062412

TL1DA024854

Title: A comprehensive assessment of structural connectivity in young male premutation carriers

Authors: *J. WANG[1], D. HESSL[2], F. TASSONE[2], A. SCHNEIDER[2], C. IWAHASHI[3], P. HAGERMAN[3], S. RIVERA[1];
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Abstract: A significant proportion of carriers of premutation alleles of fragile X mental retardation-1 (FMR1) gene show deficits in cognition and problems in emotion and social interaction. In addition, they may develop fragile X-associated tremor/ataxia syndrome (FXTAS) late in life. Neuroimaging studies of premutation carriers over 50 years old without FXTAS have reported significantly decreased brain stem and increased ventricular volumes compared to controls. It remains unknown how brain structural connectivity is affected by the fragile X premutation in young, asymptomatic carriers and whether the strength of structural connectivity is correlated with FMR1 gene expression and cognitive functions. The current study aimed to provide a comprehensive assessment of 11 white matter fiber tracts in young premutation males and evaluate the molecular and cognitive correlates of the connections. We conducted genetic and cognitive testing and acquired diffusion tensor imaging from 16 male premutation carriers (mean age 33.5, SD 8.12, range 20-42) and 19 age-matched controls (mean age 28.6, SD 7.1, range 19-42). Structural connectivity assessment was performed on 38 fiber regions of 11 fiber tracts. Linear regression was employed to investigate group, age, and genetic effects on tractography data. Partial least square (PLS) regression was used to determine the association between tractography data and carriers' standardized test scores of executive control, memory, and attention. To account for multiple comparisons, the alpha was set to 0.005. Mean length (ML) and fractional anisotropy (FA) of right extreme capsule fibers to posterior temporal lobe showed main effects of group and age, and a group × age interaction. Group and age explained ML of fornix body and FA of posterior body of the corpus callosum. Scatter plots of these measurements reveal age-related declines of connectivity strength in premutation carriers, but not in controls. Using age as a covariate, significant correlation was observed between CGG repeat size and fiber volume (FV) of brain stem fibers to superior frontal lobe, FMR1 mRNA and FV of left arcuate fasciculus (AFL), and FMR1 protein expression and AFL FA. PLS regression for cognitive performance did not survive cross-validation. This comprehensive structural connectivity analysis revealed cross-sectional age-related deficiency in young male premutation carriers in areas that are important for self awareness and memory, and associations between some fiber integrity measures and FMR1 gene expression. The associations between tractography data and cognitive functions were relatively weak and may require more statistical power to detect.

Disclosures: J. Wang, None; **D. Hessl**, None; **S. Rivera**, None; **F. Tassone**, None; **A. Schneider**, None; **C. Iwahashi**, None; **P. Hagerman**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS RO1 NS050199

Hereditary Disease Foundation

Title: The autophagy-linked FYVE protein (Alfy) is required for survival and implicates a role for selective macroautophagy in brain development

Authors: *J. M. DRAGICH, J. BOSCO, A. YAMAMOTO;
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Abstract: The goals of this study are to better understand the role of selective macroautophagy in the brain and the mechanisms by which neurons can degrade aggregated proteins. Selective macroautophagy involves the targeting of a substrate, such as a protein aggregate, to the autophagosome, ultimately leading to degradation by the lysosome. We are currently investigating a novel protein called Autophagy-linked FYVE protein (Alfy), which we have previously shown to be essential for the selective elimination of aggregated proteins by macroautophagy in cell-based assays. Here we present the first characterization of Alfy-deficient mice. Notably, in the absence of Alfy, neonatal pups fail to successfully suckle, are rejected by their dams, and die 12 to18 hrs after birth. Perinatal lethality is not due to an inability to acutely respond to starvation, since basal macroautophagy was unaffected by the absence of Alfy as determined by various methods including visualization of the formation of autophagosomes, measurements of long-lived protein degradation, and the conversion of LC3 to its lipidated form (LC3-II). While the examination of liver, lung, intestine and heart by H&E staining revealed no gross alterations, the Alfy-deficient mice did exhibit brain malformations including enlarged ventricles throughout the forebrain, malformed hippocampi and apparent loss of fiber tracts. These results signify a potential role of Alfy and selective macroautophagy in brain development

Disclosures: J.M. Dragich, None; **A. Yamamoto**, None; **J. Bosco**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Research Project Grant (R01)

Title: Modeling adrenoleukodystrophy in Drosophila

Authors: *S. JOHRI[1], A. SIVACHENKO[2], B. HOWARD[3], A. LETSOU[3];
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Abstract: Adrenoleukodystrophy (ALD) is a rare and fatal progressive neurodegenerative disease. The most common form of the disease is X-linked (X-ALD); it occurs equally in all ethnic groups with an incidence of 1:17,000. X-ALD is a clinically heterogeneous disorder, with incomplete penetrance and variable expressivity, possibly due to modifier loci in the genome. The most severe form of X-ALD is cerebral ALD and is characterized by demyelination in the central nervous system. Affected children present with visual and hearing loss, cognitive impairment and the neurological symptoms rapidly progress to paralysis and death. A less severe form of the disease Adrenomyeloneuropathy [AMN] occurs in 35% of X-ALD patients and is characterized by pathology of the peripheral nervous system.
The gene responsible for X-ALD encodes a peroxisomal ATP-binding-cassette transporter (ABCD1). Investigators have sought to resolve the clinical heterogeneity of ALD in animal models with limited success. Mouse models of X-ALD experience a normal life span and exhibit no signs of degeneration in the CNS. Thus, the need for a better animal model for ALD still remains in order to understand disease mechanisms and treatment possibilities. Acyl-CoA synthetases (ACS's), which are mis-expressed in ALD patients and function upstream of ABC transporters might also be central to ALD disease pathology. Mouse models for these have thus far been unsuccessful as well.
The objective of our research is to assess the Drosophila bubblegum/double bubble double mutant as a model for X-ALD. Both bubblegum (bgm) and double bubble (dbb) code for VLCFA (very long chain fatty acid) acyl-CoA synthetases. To assess the potential value of the bgm dbb mutant fly as a model for ALD, we have evaluated characteristics of neurodegeneration and ALD in bgm dbb mutant flies.
First, we have shown that single and double amorphic mutations in the bgm and dbb-encoded ACSVLs are causative of neurodegenerative phenotypes affecting the optic lobe and retina in Drosophila. Second, we have shown that amorphic mutations in bgm and dbb lead to VLCFA accumulation and to neuronal. Third, we have shown that the double knockout in the fly

recapitulates essential features of human ALD thereby providing the best evidence to date of a powerful animal model of fatty acid induced neurodegeneration. Fourth, our data suggest that VLCFA accumulation in ALD affects both glial and neuronal cell types, and similarly excess fatty acid accumulation leads to widespread cell losses in the fly brain. Finally, the bgm dbb fly is expected to provide an effective genetic tool for identification of drugs resolving the symptoms of ALD and VLCFA induced neurodegeneration.

Disclosures: S. Johri, None; **A. Sivachenko**, None; **B. Howard**, None; **A. Letsou**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: American Heart/Stroke Association

National Eye Institute

Stanford Medical Scholars Research Grant

Title: Local axonal protection by wlds as revealed by conditional regulation of protein stabilization

Authors: *J. T. WANG, Z. A. MEDRESS, B. A. BARRES;
Dept. of Neurobio., Stanford Univ. Sch. of Med., STANFORD, CA

Abstract: Axons of the Wallerian degeneration slow (Wlds) mice survive several weeks after nerve transection, and this protection has been shown to be cell autonomous in neurons. The mutant Wlds protein, which is a chimeric fusion protein consisting of the N70 amino acids of UBE4B and the full functional sequence of NMNAT1, is localized primarily in the nucleus, although low levels of the protein is also detected in the axoplasm. Wlds protein has previously been shown to regulate the expression of neuronal genes, although others have also shown that increasing the cytoplasmic concentration of Wlds protein potentiated axon protection. Thus, it remains unclear the mechanistic site of Wlds activity that leads to axon protection, and whether the protection requires new gene transcription. To address this, we tested whether reversibly "turning on" or "turning off" Wlds expression and activity affected axon protection by regulating the post-translational stability of Wlds protein. We found that conditional expression of Wlds

protein at the time of axotomy or shortly afterwards was sufficient to provide distal axon protection, whereas shutting off Wlds activity after axotomy by destabilizing the protein abolished axon protection in Wlds expressing neurons. As the injury prevents communication between the cell body and the axon, the finding that conditional expression of Wlds after nerve transection still resulted in axon protection indicates that the protection is mediated through a local event in the axon that is independent of new gene transcription. Moreover, the finding also suggests that axons destined for degeneration can be rescued within a time window after injury. As exogenous addition of NAD+, a metabolite by the NMNAT1 enzyme and a key bioenergetic cofactor in the mitochondria, resulted in axon protection, we further tested whether mitochondrial activity is required for Wlds protection. By using genetic approaches to block the entry of mitochondria into the axon, we showed that Wlds protected axotomized nerves even in the absence of mitochondria in the axon, indicating that the mitochondria is not required for Wlds protection. Together, the study showed that Wlds protects axons through a local axonal event independent of gene transcription, and that this process is not mediated through bioenergetic changes in the axonal mitochondria. These findings provide insight into the molecular site of action of Wlds axon protection, and may help in identify novel therapeutic targets to prevent axon degeneration in CNS diseases and injuries.

Disclosures: J.T. Wang, None; **Z.A. Medress**, None; **B.A. Barres**, None.

Nanosymposium

120. Neurodevelopmental Disorders II

Location: Room 10

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 120.11

Topic: C.06. Developmental Disorders

Support: Epilepsy Foundation Postdoctoral Fellowship

DoD TS093067

Title: Single-cell knockout of Tsc1 in utero generates cortical tuber-like lesions and heterotopic nodules with cytomegalic neurons

Authors: *D. M. FELICIANO, T. SU, A. BORDEY;
Neurosurg., Yale Univ., NEW HAVEN, CT

Abstract: Tuberous Sclerosis Complex (TSC) is a multisystem genetic disorder characterized by mutations in Tsc1 or Tsc2 leading to mammalian target of rapamycin (mTOR) hyperactivity. 80-

90% of TSC individuals suffer from intractable seizures resulting from cortical malformations (called tubers) which form during embryonic life. Understanding how these lesions form and lead to hyper-excitability has been limited by the absence of an animal model exhibiting tubers. To address this limitation, we used in utero electroporation of Cre recombinase-containing vector in transgenic mice carrying a floxed and a mutant Tsc1 allele for knocking out Tsc1 in selected neuronal populations at a precise developmental time-point. Single-cell knockout of Tsc1 led to increased mTOR activity and soma size of Tsc1null neurons. This approach generated heterotopic nodules above or in the white matter and discrete cortical tuber-like lesions displaying a mosaic of cell size and phospho-S6 immunoreactivity. The electroporation time-point determined the severity of the malformations with late-born cortical structures being the most affected. Tuber-like lesions display ectopic neurons resulting in loss of cortical architecture, cytomegalic and multinucleated neurons with abnormal dendritic trees resembling giant cells, (and patches of demyelination). No gliosis was visible and phospho-pS6 immunoreactivity was surprisingly not up-regulated in Tsc1null astrocytes despite a lower seizure threshold. These data suggest that a double-hit strategy to eliminate Tsc1 in discrete neuronal populations generates TSC-associated cortical lesions providing a model to uncover the mechanisms of lesion formation and hyper-excitability. In addition, the absence of gliosis raises questions regarding the contribution of astrocytes to TSC lesion-associated hyper-excitability.

Disclosures: D.M. Feliciano, None; **T. Su**, None; **A. Bordey**, None.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.1

Topic: C.07. Epilepsy

Support: NIH R21 NS061111

NIH K25 AG02778

NIH K24 NS058386

NIH P30 NS045839

NIH T32 EB000814

Title: Interhemispheric activation asymmetry within hippocampal subfields in unilateral

temporal lobe epilepsy

Authors: *S. DAS[1], D. MECHANIC-HAMILTON[2], M. KORCZYKOWSKI[2], J. PLUTA[2], J. DETRE[2], P. YUSHKEVICH[2];
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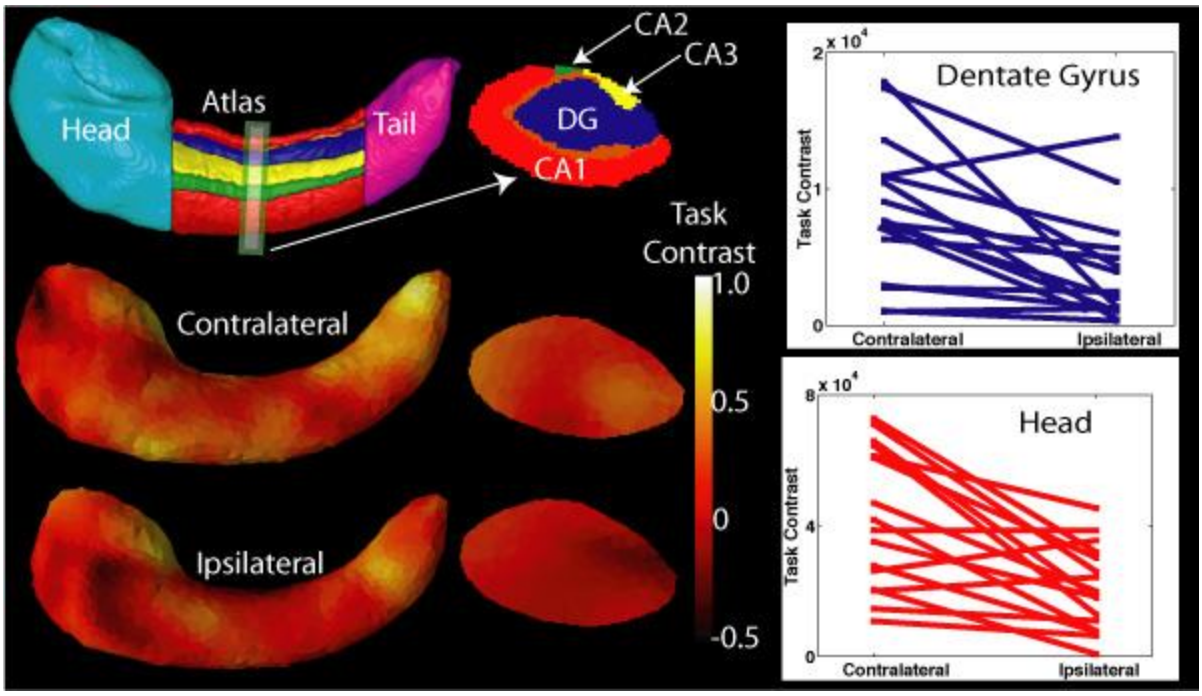
Abstract: *We present, for the first time, an analysis of activation asymmetry in refractory, unilateral temporal lobe epilepsy (TLE) patients across different hippocampal subfields.*
Lateralization of memory function is important in the presurgical evaluation of these patients. Since TLE presents with differential involvement of hippocampal subfields, lateralization measures based on hippocampal subfields may be more powerful than those using larger, inhomogeneous ROIs. These methods can also be applied to functional studies of episodic memory.
Eighteen subjects with TLE were imaged in a 3 Tesla Siemens Trio scanner. T1-weighted structural MRI scans (0.9375x0.9375x1mm) and BOLD fMRI scans (3x3x3mm) were acquired during a blocked design experiment consisting of a visual scene encoding encoding task.
A general linear model was used to generate activation maps. The contrast images were sampled within 6 different subfield ROIs (Head, CA1, CA2, CA3, Dentate Gyrus (DG), Tail) labeled in the anatomical image by using shape-based normalization of the subject's whole hippocampus to a postmortem hippocampus atlas (Neuroimage 44(2): 385-398) containing subfield labels. Task contrast was integrated over each subfield and pairwise t-tests were conducted between activations in the epileptogenic (ipsilateral to seizure focus) and the non-epileptogenic (contralateral) sides, yielding measurements of subfield-level activation asymmetry.
Mean local activation within the contralateral hippocampi is generally greater than that in the ipsilateral hippocampi as expected (Figure 1). Activation within each hippocampal subfield except CA2 is significantly greater in the contralateral side. The strongest effects are in the hippocampus head ($p=0.0002$) and in the DG ($p=0.0008$), consistent with imaging (Epilepsia 50(6):1476-1483) as well as histological findings.
Using subfield level measurements may provide better localization and lateralization of memory function in TLE. This study demonstrates the utility of atlas-based methods in functional studies of the hippocampus.



Disclosures: S. das, None; **D. Mechanic-Hamilton**, None; **M. Korczykowski**, None; **J. Pluta**, None; **J. Detre**, None; **P. Yushkevich**, None.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.2

Topic: C.07. Epilepsy

Support: NIH R01-NS047605

Title: EEG-fMRI seeded connectivity as a predictor of the surgical outcome of epilepsy

Authors: M. NEGISHI[1], R. MARTUZZI[1], D. D. SPENCER[2], *R. T. CONSTABLE[1,2];
[1]Dept Diagnos. Radiol, [2]Neurosurg., Yale Univ., New Haven, CT

Abstract: The success rate of epilepsy surgery is quite high, 60-80% of patients who underwent medial temporal lobe epilepsy surgery attaining seizure free post surgery. However, epilepsy surgery incurs risk of infection and declining cognitive abilities. Therefore, it is important to

assess the possible success of surgery. To this end, we developed and tested a hypothesis that patients whose functional brain connectivity spreads extensively to the opposite side of the affected area have lower chances of becoming seizure-free.
Nine intractable epilepsy patients (age: 15-50, mean 36, 5 males) underwent simultaneous EEG-fMRI recording before surgery. Functional MRI (fMRI) images were acquired in four to eight, six-minute scans from each patient using a 3T scanner. EEG (32 channel, 1 kHz sampling) was recorded during the fMRI runs, using carbon fiber electrodes and an in-house built anti-polarization EEG amplifier to ensure MRI transparency and the subject comfort. The patient was deemed seizure-free if he / she did not seize at least for 6 months. Fourteen healthy control subjects (age: 22-34, mean 26, 7 males) also participated in the study.
Two different seeds were used for computing the functional connectivity. (1) EEG-fMRI seed: based on interictal spike-correlated fMRI activation (2) resection area seed: based on the difference image between the pre- and post- surgical anatomical images. When the spike correlated fMRI analysis resulted in multiple clusters, the connectivity map that overlapped most with the planned resection area (described using Yale Brodmann atlas) was chosen by a computer program. Same seeds were used for the controls. From the obtained connectivity maps, laterality indices were computed by L=(Ni-Nc)/(Ni+Nc) where Ni (Nc) is the number of supra-threshold voxels in the ipsi- (contra-) lateral side of the surgery. Corresponding laterality indecesindices from the controls were subtracted and the resultant value was Fisher-transformed to yield control-subtracted laterality indices. Finally the control-subtracted laterality indecesindices were compared between non seizure-free and seizure-free groups.
The laterality indieces computed from the EEG-fMRI seed differed significantly between the two groups ($F=7.12$, $p<0.05$). The average laterality index was smaller in the nom non seizure-free group. The laterality indecesindices computed from the resection seed did not differ significantly between the two groups ($F=3.59$, $p>0.1$).
We conclude that the low laterality of the functional connectivity computed from the spike-correlated fMRI seed is a predictor of unsuccessful surgery.

Disclosures: M. Negishi, None; **R. Martuzzi**, None; **D.D. Spencer**, None; **R.T. Constable**, None.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.3

Topic: C.07. Epilepsy

Support: NIH Grant R01NS063039

NIH Grant U24NS063930

Title: Detection of multiple, single unit activity from continuous, long-duration, high-frequency recordings in patients with epilepsy

Authors: *M. R. BOWER, M. STEAD, G. WORRELL;
Mayo Clin., ROCHESTER, MN

Abstract: Wide bandwidth recordings (>100 Hz) have opened new opportunities for understanding the mechanisms underlying epilepsy. In addition to prior observations of pathological, high-frequency oscillations and microseizures in humans, pathological unit activity in animal models of epilepsy has been associated with seizure onset and holds promise for aiding seizure prediction. Recent advances in both acquisition and storage technology now make it feasible to record continuously at sampling frequencies (>20 kHz) sufficient to isolate the action potentials of single neurons ("unit spikes") from multiple electrodes for days at a time. These new techniques, however, create new challenges for unit spike detection compared to current methods based on windowed, millisecond snippets of data obtained at the time of data acquisition. Artifact removal is aided by continuous recordings, but still requires visual inspection of recording sessions that may last longer than a day. Unit action potentials can be identified by multiple techniques after the data have already been acquired (e.g., thresholding, template matching), which raises new questions concerning the effects of processing on final properties of isolated units (e.g., the effect of raising and lowering thresholds on observed firing rates). Such questions are complicated by the fact that over multiple hours of recording, background noise levels are observed to change, suggesting that the appropriate detection parameters may change for a given electrode within the same recording session (e.g., changes due to the sleep/wake cycle). For these reasons, unit detection thresholds were altered based on the local RMS activity for each microelectrode, after filtering activity between 600-6,000 Hz. Annotations regarding artifact time windows, seizure times and unit activity detections were stored automatically to a relational database system for future retrieval. Data were collected from 8 MTLE patients implanted with hybrid depth electrodes, which contained a total of 298 microwires in addition to standard clinical contacts. Over a total of 88.1 hours of recording, a total of 24 spontaneous seizures were recorded. The ratio of the number of detections observed on an electrode compared to the average RMS of that same electrode was used to separate bad (i.e., "noisy") electrodes from more suitable candidates for the detection of unit activity. Multiple, single units were isolated from these data and measured against seizure onset times determined by visual review from low frequency data obtained from clinical macroelectrodes.

Disclosures: M.R. Bower, None; **M. Stead**, None; **G. Worrell**, None.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.4

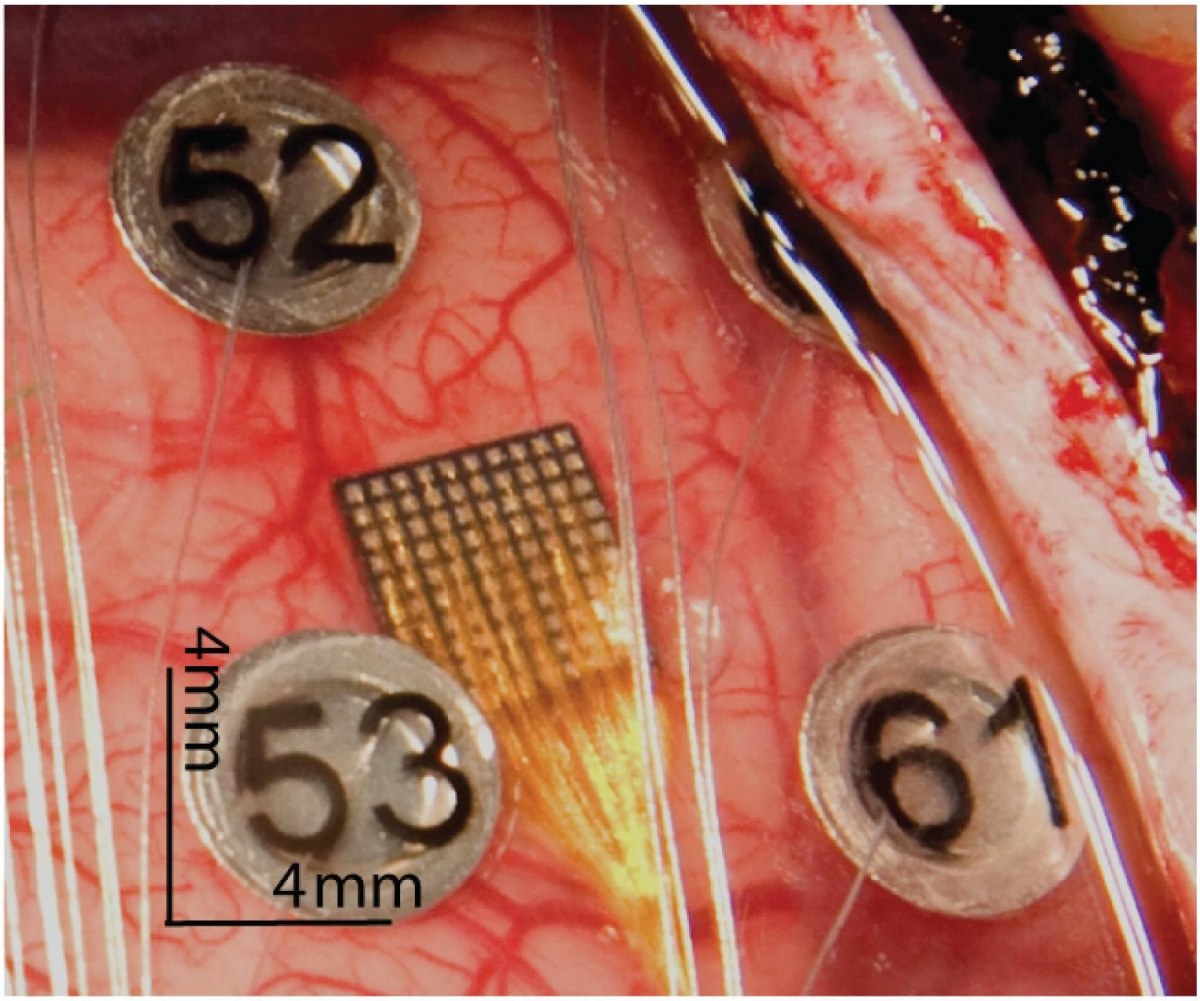
Topic: C.07. Epilepsy

Support: R01EY019363

Title: Inactivated action potentials during interictal spikes in human epileptic neocortex: implications for high-frequency oscillations and for epileptogenesis

Authors: *B. GREGER[1], K. THOMSON[2], P. HOUSE[3], C. SCHEVON[5], L.-R. SHAO[4], R. EMERSON[5], G. MCKHANN[6], R. GOODMAN[6], E. DUDEK[4];
[1]Bioengineering, Univ. of Utah, Salt Lake City, UT; [2]Bioengineering, Univ. of Utah, Salt lake cty, UT; [3]Neurosurg., [4]Physiololgy, Univ. of Utah, Salt Lake City, UT; [5]Neurol., [6]Neurosurg., Columbia Univ., New York, NY

Abstract: Interictal spikes have long been used as a diagnostic feature of epileptic cortex, but how they function in the pathophysiology and pathogenesis of epilepsy remains controversial. Interictal spikes and action potentials were simultaneously recorded in four patients with intractable epilepsy using an array of 96 penetrating microelectrodes. These in vivo recordings from populations of individual neurons revealed bursts of action potentials during interictal spikes. Neocortical slices were taken from adjacent to the array in one patient, and paroxysmal depolarizing shifts and intracellular action potentials were recorded simultaneously. The in vivo and in vitro recordings together led to the conclusion that the profound depolarization during interictal spikes in vivo prolonged the duration of the action potential waveforms. Therefore, the increased power in the 250-600 Hz band observed during interictal spikes with electrocorticography can be generated by bursts of depolarization-inactivated action potentials rather than other types of oscillations in local field potentials (e.g., synaptically generated rhythms). We further examined these findings using Hodgkin-Huxley modeling of the effects of an interictal spike on intracellular and extracellular recordings. Using this model we were able to replicate the inactivated action potentials and the increased 250-600 Hz power observed during interictal spikes in the electrophysiological recordings. The in vivo extracellular recordings also reveal propagation of the interictal spikes through the neocortex. Taken together, these data support the hypothesis that propagating interictal spikes recruit neocortical neurons to form aberrant neural networks through Hebbian plasticity.



Disclosures: B. Greger: None. **K. Thomson:** None. **P. House:** None. **C. Schevon:** None. **L. Shao:** None. **R. Emerson:** None. **G. McKhann:** None. **R. Goodman:** None. **E. Dudek:** None.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.5

Topic: C.07. Epilepsy

Support: NINDS RO1-NS041811-04

NINDS R01 NS 48598-04

The Klingenstein Foundation

The Epilepsy Therapy Project

• National Security Science and Engineering Faculty Fellowship

• U.S. Department of Energy, Division of Materials Sciences Award no. DEFG02-91ER45439

• The Frederick Seitz Materials Research Laboratory and Center for Microanalysis of Materials at the University of Illinois at Urbana-Champaign

Title: Multiplexed, high-density active electrodes using flexible silicon electronics

Authors: J. VIVENTI[1], L. VIGELAND[2], D.-H. KIM[3], V. R. TIRUVADI[1], J. A. ROGERS[3], D. CONTRERAS[2], *B. LITT[1];
[1]Bioengineering, [2]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [3]Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Current brain-machine interface devices for clinical and research applications utilize electrodes that must be individually wired to separate control systems. The number and density of the electrodes in these devices quickly reaches a limit due to the large volume of the associated wiring. Active circuitry to reduce this wiring burden is currently limited by the mismatch between the rigid, planar nature of conventional silicon electronics and the irregular brain surface. Flexible electronics capable of intimate, non-invasive integration with the soft, curvilinear surfaces of the brain can solve this mismatch and enable improved capabilities for clinical diagnostic and therapeutic brain-computer interfaces. Here, we report new dense arrays of multiplexed electrodes using flexible electronics that can enable an unprecedented level of spatial and temporal electrocorticographic (ECoG) resolution over large areas of cortex. The extreme flexibility of the devices can further enable simultaneous sampling of gyral and intrasulcal ECoG from regions of the brain that were previously inaccessible or difficult to reach. We demonstrate this new technology platform in a sensor system composed of 720 silicon nanomembrane transistors configured to record electrical activity directly from the in vivo feline brain. Sampling with simultaneous sub-millimeter and sub-millisecond resolution through 360 amplified and multiplexed channels, the device requires a mere 39 external connection wires. The design can be scaled up to span large areas of tissue without dramatically increasing the number of external connection lines. The associated data acquisition hardware and software interfaces with a wide variety of electrode configurations, displaying in real-time the demultiplexed and filtered signals in customizable software frontend. We use this system to map visual and stimulation evoked potentials at high resolution on the surface of primary visual cortex. This demonstration is one of many possible uses for the technology in a new generation of minimally invasive clinical and research devices, including brain-computer interfaces, cardiac electrophysiology and cortical mapping.

Disclosures: J. Viventi: None. **L. Vigeland:** None. **D. Kim:** None. **V.R. Tiruvadi:** None. **J.A. Rogers:** Ownership Interest; MC10. **D. Contreras:** None. **B. Litt:** Consultant/Advisory Board; MC10, Startup company that has licensed our patents.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.6

Topic: C.07. Epilepsy

Support: NIH K08 NS48871

Title: Spike-triggered traveling waves in human temporal neocortex

Authors: *C. A. SCHEVON[1], A. TREVELYAN[2], R. R. GOODMAN[3], G. MCKHANN[3], R. G. EMERSON[3], M. CARANDINI[4];
[1]Columbia Univ., NEW YORK, NY; [2]Univ. of Newcastle, Newcastle upon Tyne, United Kingdom; [3]Columbia Unversity, New York, NY; [4]UCL Inst. of Opthalmology, Univ. Col. London, London, United Kingdom

Abstract: Lateral connectivity on a local scale is thought to play an important role in the neocortex. In the visual cortex of anesthetized animals, spiking activity at multiple sites triggers traveling waves of postsynaptic activity. These waves have been demonstrated as a significant contributor to local field potentials (Nauhaus et al, Nature Neuroscience 2009 12:70-76). We asked if similar traveling waves are present in humans, and specifically if they can be detected in epilepsy patients in cortical areas not involved in primary sensory processing.
We simultaneously recorded multiunit activity and local field potentials (LFP) during light sleep from a 10 x 10 multielectrode array (Neuroport Neural Monitoring System, Blackrock Microsystems Inc, Salt Lake City, UT) implanted in lateral temporal neocortex in three patients with medically refractory partial epilepsy. Electrodes were 1 mm long with 400 micron orthogonal spacing. Location of the recording tips in cortical layers IV and V was histologically confirmed. We computed spike-triggered LFP averages across the entire array, and analyzed the result to determine the amplitude of the negative peaks and their time delay relative to the generating spike. At multiple sites, we found examples of spike-triggered propagating traveling waves that appeared to be generated locally, with amplitudes decreasing proportionally to the inverse exponential of the Euclidean cortical distance. The apparent propagation speed of the waves was on the order of 0.2 - 0.3 m/s, similar to the values observed in experimental animals.

Our findings suggest that traveling waves contribute significantly to local field potentials in areas outside of primary sensory regions and that are commonly involved in neocortical partial epilepsy syndromes; indeed, they may represent a fundamental property of neocortex. We hypothesize that this mechanism may be differentially affected in regions that are prone to epileptiform activity. Further investigation may yield insights into the neural network mechanisms that contribute directly to seizure generation.

Disclosures: C.A. Schevon, None; **G. McKhann**, None; **R.R. Goodman**, None; **R.G. Emerson**, None; **A. Trevelyan**, None; **M. Carandini**, None.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.7

Topic: C.07. Epilepsy

Support: NIH R01 NS063360

Wilder Center of Excellence for Epilepsy Research

Children's Miracle Network

University of Florida Alumni Fellowship

Medical Guild Research Incentive Award

Title: Gene therapy for epilepsy - Dissecting the role of somatostatin as a neuroprotective agent

Authors: *R. ZAFAR[1], M. A. KING[2], C. J. FRAZIER[3], P. R. CARNEY[4];
[1]Univ. Florida, GAINESVILLE, FL; [2]Pharmacol. and Therapeut., [3]Pharmacodynamics, [4]Pediatric Neurol., Univ. Florida, Gainesville, FL

Abstract: Background: Gene therapy provides a promising alternative treatment for intractable epilepsy. Preliminary results in our lab show an anti-epileptogenic effect of somatostatin (SST) in rats over-expressing the AAV-delivered neuropeptide. These animals are also protected against co-morbidities associated with epilepsy, and the current study attempts to elucidate these mechanisms both in vivo and in vitro.
Methods: The efficacy of gene delivered SST was assessed in electrically kindled adult male

Sprague-Dawley rats by monitoring behavior and EEG dynamics in three groups of rats. Group 1 comprised of rats with bilateral bipolar stainless steel electrodes implanted in the amygdala for electrical stimulation. The kindling paradigm continued until the rats were fully kindled (i.e. attained 3 consecutive grade 5 seizures on the Racine scale). Group 2 consisted of identically prepared animals with the addition of injections of 8 ul AAV5-SST into the dentate gyrus and CA1 of the hippocampi bilaterally prior to electrode implantation. Group 3 was composed of sham-injected rats with AAV5 expressing GFP instead of SST. Brain extraction and histology was performed for detecting SST and GFP as well as any micro- and astro-gliosis. The remaining animals were used for electrophysiological experiments to record the frequency of sIPSCs and sEPSCs from granule cells to get a first estimate of the mechanism of SST action.
Results: Robust transduction of GFP and SST was observed within the hippocampal formation using AAV-5. 73% of AAV-SST injected animals (n=11) did not experience a single grade 5 seizure, while the remaining 27% had a delayed rate of kindling as compared to control (n=10) and sham-injected (n=6) animals. There was no destructive pathology associated with kindling, and no immune response was observed across any animal group. An initial assessment of sIPSCs and sEPSCs recorded from dentate granule cells showed no change in frequency or amplitude of events.
Conclusions: Our results suggest that AAV-delivered somatostatin in the dentate gyrus and CA1 hippocampal regions modulates seizure threshold and protects against the development of generalized seizures. The treatment has no adverse effects by way of immune response and neurodegeneration. We did not find any significant changes in frequency and amplitude of spontaneous events and are now beginning to tease apart the in vitro network more in detail.

Disclosures: R. Zafar, None; **M.A. King**, None; **C.J. Frazier**, None; **P.R. Carney**, None.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.8

Topic: C.07. Epilepsy

Support: NIH grant R01 EB004752

NIH grant R01 EB007082

Wilder Epilepsy Research Center

Children's Miracle Network

Title: Brain structural changes in a rat model of temporal lobe epilepsy observed using enhanced MRI

Authors: *M. B. PAREKH[1], W. TRIPLETT[2], P. R. CARNEY[3], T. H. MARECI[4];
[1]Neurosci, Univ. Florida, GAINESVILLE, FL; [2]Mathematics, [3]Pediatric Neurol., [4]Biochem. and Mol. Biol., Univ. Florida, Gainesville, FL

Abstract: Objective: Higher angular resolution diffusion imaging (HARDI) was used in a rat model of temporal lobe epilepsy (TLE) to identify and evaluate white matter changes in and around the hippocampus.
Background: TLE is one of the most common types of intractable epilepsies. Damage to the hippocampus and parahippocampal gyrus is commonly observed in both patients and animal models of TLE. However, the temporal evolution and the role of these structures and their interconnections in the onset of spontaneous seizures remain unclear.
Methods: Adult rats were stereotactically implanted with electrodes in the right ventral hippocampus and electrically stimulated to induce status epilepticus (SE). Seizures were assessed behaviorally and longitudinal in vivo T2 and HARDI MRI scans were obtained pre- and post-implantation and at 1, 3, 5, 7, 10, 20, 40 and 60 days post-SE at 11.1 Tesla. Excised brains were imaged at 17.6 Tesla for days 1 (n=3), 3 (n=3), 10 (n=3), and 60 (n=11) post-SE. HARDI data for excised brains from naïve rats was collected with isotropic resolution to trace the perforant pathway. For fiber tracking, the diffusion orientation distribution function (dODF) at each voxel was estimated using the mixture of Wisharts (MOW) approach. This post-processing method allows for the resolution of complex fiber structures such as crossing and kissing fibers within a voxel. Tractography was performed by seeding all voxels above a fractional anisotropy (FA) value of 0.01 with the following thresholding parameters: 64 seeds per voxel, a step size of 0.5 of the voxel length, and a turning angle threshold of 50° at each step. Regions of interest were drawn in the dentate gyrus and the entorhinal cortex in order to filter out the perforant pathway fibers.
Results and Conclusion: Early time-point histology shows extensive neurodegeneration, bilaterally in the hippocampus, the dorsal thalami and in the contralateral parahippocampal gyrus. This damage correlates with changes in average diffusivity and T2 observed with both in vivo and excised MRI. Only rats that developed spontaneous seizures showed mossy fiber sprouting at 60 days post-SE. Preliminary results show that isotropic acquisition of HARDI data allows for fiber tracking the perforant pathway.

Disclosures: M.B. Parekh, None; **W. Triplett**, None; **P.R. Carney**, None; **T.H. Mareci**, None.

Nanosymposium

121. Epilepsy: Human Studies I

Location: Room 6B

Time: Sunday, November 14, 2010, 8:00 am - 10:15 am

Program Number: 121.9

Topic: C.07. Epilepsy

Support: NIH R01 NS063360

NIH R01 EB007082-02

Wilder Center of Excellence for Epilepsy Research

University of Florida Alumni Graduate Fellowship

University of Florida Grinter Graduate Fellowship

Children's Miracle Network

Title: Status epilepticus affects hippocampal structure and infusate distribution profiles

Authors: *S. KANTOROVICH[1], G. W. ASTARY[2], M. PAREKH[1], T. H. MARECI[3], M. SARNTINORANONT[4], P. R. CARNEY[5];
[1]Neurosci., [2]Biomed. Engin., [3]Biochem. and Mol. Biol., [4]Mechanical and Aerospace Engin., [5]Pediatric Neurol., Univ. of Florida, Gainesville, FL

Abstract: Rationale: Convection-enhanced delivery (CED) has emerged as a promising method of targeted drug delivery for treating central nervous system (CNS) disorders, but the influence of brain structure on infusate distribution is unclear. Our previous reports have shown distributions within the hippocampus are influenced by its underlying structure. In CNS disorders that alter the structure of the hippocampus, it is reasonable to expect variability of infusate distribution. When structural changes in hippocampi render other treatment options ineffective, targeted delivery of therapeutics via CED may provide an appropriate method for delivery. Therefore, we have used this approach to study extracellular transport of a contrast agent in the hippocampus of rats that have undergone electrically-induced status epilepticus (SE), an unremitting seizure known to cause neuronal damage and edema in limbic structures.
Methods: Male Sprague-Dawley rats were implanted with a cannula guide into the dorsal hippocampus and with electrodes into the ventral hippocampus, then stimulated to undergo SE. Twenty-four hours later, MR imaging was performed *in vivo* in an 11.1T magnet system. High resolution T1, T2, and diffusion-weighted (DWI) images were acquired prior to infusion to generate baseline contrast enhancement images (T1-weighted) and visualize morphological changes (T2-weighted, DWI). Animals were then infused at a rate of 0.3 l/min with 5l of MR contrast agent DTPA chelated gadolinium-labeled albumin (Gd-albumin), tagged with Evans blue. Immediately following infusion, high resolution T1-weighted imaging was repeated to visualize distribution profiles of the contrast agent. Perfusion-fixation was performed for histological assessments. Fluorojade C, Cresyl violet, and Black-Gold staining was used to visualize neuronal degeneration, cell swelling, and myelin abnormalities resulting from SE.

Results: Our preliminary results show an episode of SE resulted in structural changes within the hippocampus, as evidenced by T2 and diffusion-weighted images and verified in histological preparations. Infusions of Gd-albumin into injured hippocampi resulted in distribution profiles of different shape and volume as compared to controls.
Conclusions: Our results demonstrate that electrical injury may result in acute changes in hippocampal cytoarchitecture and porosity that in turn are major influencing factors of distribution volume and shape. These changes may be accounted for by edema, ventricle enlargement, axonal sprouting, or other anatomical modifications that impact distribution profiles.

Disclosures: S. Kantorovich, None; **G.W. Astary**, None; **M. Parekh**, None; **T.H. Mareci**, None; **M. Sarntinoranont**, None; **P.R. Carney**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.1

Topic: C.08. Ischemia

Title: Study of hyperthermia on the change of ATP and IL-1β in the ischemically injured rat brain

Authors: *C. C. PEGG[1], J. KULACZ[1], C. HE[1], K. KATTNER[2], A. STROINK[2], C. WANG[1,2]; [1]Illinois State Univ., NORMAL, IL; [2]Central Illinois Neurosci. Fndn., Normal, IL

Abstract: Hyperthermia has been correlated with worsened stroke outcome in many studies. In the present study, we have devised a method to investigate this problem. First, we have created a new methodology for cerebrospinal fluid (CSF) collection in which we create a viewing window of the cistern magna, enabling blood-free sampling. Via cresyl violet staining, we have shown this method is not associated with neuronal degeneration *per se*. Further, we have shown this method is a functionally reliable. That is, we have used this method to investigate ATP concentrations in the CSF and found levels comparable to that of previously published literature. In order the investigate the deleterious effects of hyperthermia, we employed this technique to collect CSF from northermic, hyperhermic, and sham rats after middle cerebral artery occlusion (MCAo). ATP and IL-1β release in the cerebrospinal fluid (CSF) were evaluated via luminetric and ELISA assays, respectively. ATP concentrations are significantly higher in hyperthemric rats as compared with northermic or sham controls. No trend is observed with IL-1β concentrations. Currently, we are investigating changes in IL-1 concentration in homogenized brain tissue from

all groups. Preliminary results from western blot analysis are shown.

Disclosures: C.C. Pegg: None. **J. Kulacz:** None. **C. He:** None. **K. Kattner:** None. **A. Stroink:** None. **C. Wang:** None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.2

Topic: C.08. Ischemia

Support: NIH grant NR003521 (PDH)

AHA grant 09POST2190040 (SED)

Title: Dihydrotestosterone reduces infarct size and is immunosuppressive after experimental stroke

Authors: *S. E. DZIENNIS[1], K. AKIYOSHI[1], S. SUBRAMANIAN[3], H. OFFNER[3,1,2], P. D. HURN[1,2];
[1]Dept Anesthesiol & Peri Oper Med., [2]Dept. of Neurol., Oregon Hlth. & Sci. Univ., Portland, OR; [3]Neuroimmunology Res., Portland Veterans Affairs Med. Ctr., Portland, OR

Abstract: Increased risk of stroke in adult males occurs when endogenous levels of testosterone are in decline. Dihydrotestrosterone (DHT), the most potent metabolite of testosterone, is a known neuroprotectant and immunomodulator. In intact male mice, experimental stroke induces immunosuppression of the peripheral immune system after 96 h, characterized by a reduction in spleen cell numbers, decreased proliferative response to mitogens and an increase in the percentage of CD4+CD25+FoxP3+ regulatory T cells. The reduced splenocyte number is partly due to their migration into brain where they contribute to the developing infarct. The effect of DHT on peripheral immunosuppression of spleen prior to and after experimental stroke has not been investigated. Peripheral and central nervous system (CNS) immune responses were examined in castrated mice with or without sustained, controlled levels of DHT (0.5 mg) administered by subcutaneous implant before and 96 h after 90 min middle cerebral artery occlusion (MCAO) induced by reversible intraluminal filament method. Prior to MCAO, DHT-replacement reduced spleen cell numbers, suggesting fewer available cells to migrate to and exacerbate damage in brain once stroke occurs. After MCAO, the reduced infarct volume in DHT-replaced animals corresponded with decreased splenocyte proliferation in response to

stimulation with mitogenic anti-CD3/CD28 antibodies and increased percentage of immunosuppressive CD4+CD25+FoxP3+ regulatory T cells relative to castrates. However, post-MCAO splenocyte numbers were not further reduced in DHT-replaced animals relative to castrates. Moreover, DHT-replacement did not reduce the number or activation of immune cells in brain after MCAO. These data taken together suggest DHT induces characteristics of peripheral immunosuppression relative to hormone deficient males, but may reduce infarct size by mechanisms independent of peripheral immunosuppression.

Disclosures: S.E. Dziennis, None; **K. Akiyoshi**, None; **S. Subramanian**, None; **H. Offner**, None; **P.D. Hurn**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.3

Topic: C.08. Ischemia

Title: APT102 protects the ischemically injured brain in rats

Authors: *C. WANG[1,2], A. P. SHABANZADEH[1], C. HE[1], C. C. PEGG[1];
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Abstract: Extracellular ATP has been implicated as a mediator of microglial activation and inflammatory reactions in the brain. In the present study we examined the protective effects of APT102, a novel apyrase, in ischemic brain injury in rats. Focal cerebral ischemia was induced by embolizing a preformed clot into the middle cerebral artery (MCA). Infarct volume and brain edema were measured in the 2,3,5-triphenyltetrazolium chloride (TTC) stained brain sections at 24 hours after MCA occlusion. Neurological deficits were determined using a modified Bederson's scoring system at 4, 12 and 24 hours after MCA occlusion, respectively. Hemoglobin levels in the brain were quantified spectrophotometrically. Animals were randomly assigned into one of four groups (n=10/group) described here: 1) Control, gastrically injected with saline; 2) Aspirin, gastrically injected with 1.25 mg/kg aspirin once a day starting 3 days prior middle cerebral artery occlusion and continuing until the end of the experiment; 3) APT102, intravenously injected with 0.5 mg/kg APT102 two hours after MCA occlusion; 4). Combination, aspirin + APT102 applied as described in 2) and 3). Infarct volume was larger [30 ± 3.0% (mean ± sem)] in the control group. Compared with the control group, APT102 and aspirin alone group reduced infarct volume by 40% ($p<0.005$) and 31.3% ($p<0.05$), respectively. Moreover, the combination group, APT102 plus aspirin, reduced infarct volume by 61.6%

($p<0.001$). Brain edema and neurological deficits were also reduced significantly following these treatments. Also, both APT102 and aspirin treatments reduced hemoglobin content significantly compared with the controls. Although aspirin plus APT102 treatment reduced hemoglobin significantly compared to the control group, this combination treatment was not superior to either treatment alone. This study thus suggests that APT102 and aspirin are protective agents. Further, combination treatment with APT102 and aspirin produced a synergistic effect for the protective actions. Thus, ATP102 either alone or in combination with aspirin can be a potential drug candidate for the treatment of ischemic brain injury.
Acknowledgement: Authors thank APT Therapeutics for providing APT102.

Disclosures: C. Wang, None; **A.P. Shabanzadeh**, None; **C. He**, None; **C.C. Pegg**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.4

Topic: C.08. Ischemia

Support: P20 RR15636

Title: Matrix metalloproteinase-2/9 mediate severe blood brain barrier damage at the superacute phase of cerebral ischemia in rats

Authors: *X. JIN[1], K. J. LIU[1,2], J. LIU[1], R. PAN[1], Y. YANG[2], W. LIU[1];
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Abstract: Disruption of the blood brain barrier (BBB) is an antecedent event to intracerebral hemorrhage (ICH) in ischemic stroke. Interestingly, our previous studies showed that tPA-induced ICH invariably occurred in subcortical areas and piriform cortex, where we also observed BBB disruption at a much earlier stroke stage. In the present study, we sought to determine whether severe BBB damage occurs at an early stroke stage relevant to acute thrombolytic therapy and whether matrix metalloproteinase (MMP)-2 and 9 contribute to this BBB damage. Rats were subjected to 1, 2 or 3 hrs filament occlusion of the middle cerebral artery (MCAO), followed by 10 min reperfusion. Successful MCAO was confirmed by 2,3,5-triphenyltetrazolium chloride (TTC) staining.Fluorescent tracers fluorescein isothiocyanate-dextran (FITC-dextran) (2 MDa) was injected to observe severe BBB damage, and MMP-2/9 were measured by in situ and gelatin gel zymography. Extravasation of high molecular weight

FITC-dextran was seen in brain sections after 2 hrs MCAO, with the tracer leakage limited to subcortical regions and the piriform cortex, indicating severe BBB damage in these brain regions. Interestingly, TTC staining showed that this severe BBB damage did not necessarily occurr in brain regions with tissue infarction. With the extension of ischemia duration to 3 hrs, FITC-dextran leakage was spread to the whole known MCAO territory, as marked by tissue infarction on TTC-stained brain sections while there is not any FITC-dextran leakage when rats were subjected to 1-hr MCAO. Paralleling to BBB damage, MMP-2/9 were significantly increased in the ischemic brain after 2 and 3 hrs, but not 1 hr, of MCAO. Moreover, in situ zymography demonstrated the colocalization of increased MMP-2/9 activities with FITC-dextran extravasation. Finally, pretreatment of rats with MMP inhibitor GM6001 (30 μg/kg body weight, intraartery, 10 min before MCAO) drastically reduced FITC-dextran leakage, but did not reduce tissue infarction. Taken together, severe BBB damage occurs at early stroke stages relevant to acute thrombolytic therapy, and MMP-2/9 critically contributes to this early ischemia-induced BBB disruption. MMP inhibition may be an important strategy to preserve BBB integrity at acute phase of stroke, though it alone may not interrupt the evolution of tissue infarction.

Disclosures: X. Jin, None; **K.J. Liu**, None; **J. Liu**, None; **R. Pan**, None; **Y. Yang**, None; **W. Liu**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.5

Topic: C.08. Ischemia

Support: funded by the EU (LSHB-CT-2006-018936)

Title: Attenuated inflammatory immune response in aged mice following cerebral ischemic infarct

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Abstract: Background The inflammatory response after an acute CNS injury influences the spatial and temporal expansion of the lesion as well as the regeneration progress considerably. Adult healthy organisms display a well balanced immune response. Several studies indicate that this balance is disturbed with age and age is one of the major determinants of clinical outcome

following stroke. Here we characterized the inflammatory response after an experimental induction of stroke by transient middle cerebral artery occlusion (MCAO) of adult (2month) and aged (24month) mice. The cerebral expression pattern of TNF, IL-1α, IL-1β, IL-6, TGFβ1, IL-10, Mip-1α, MCP-1 and RANTES were analyzed 6h, 12h, 24h, 2 and 7 days after ischemia. **Results** Almost all tested inflammatory mediators showed increased expression levels with age. First of all, the pro-inflammatory cytokines, IL-1α, IL-1β and notably TNF are increased. Following stroke the early post ischemic phase is characterized by a burst of TNF, IL-1α, IL-1β, IL-6, MCP-1 and Mip-1α. The expression of TGFβ1 and RANTES is delayed after ischemic injury. Our results clearly demonstrate that the strong inflammatory response following an acute ischemic brain injury attenuates with age considerably. All pro-inflammatory cytokines (TNF, IL-1α, and IL-1β) and particularly the inflammatory cytokine IL-6 as well as the chemokines Mip-1α and MCP-1 displayed reduced peak expression levels after ischemia in aged brains. However, also the anti-inflammatory capacity seems to be attenuated with age, mostly apparent in the later post ischemic phase. **Conclusion** Our study revealed an elevated inflammatory status within the CNS during ageing *per se*. Furthermore we demonstrated an attenuated inflammatory response after an acute ischemic injury with age. Our results underline the relevance to study age associated diseases like stroke in aged animals.

Disclosures: M.W. Sieber, None; **C. Frahm**, None; **R.A. Claus**, None; **O.W. Witte**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.6

Topic: C.08. Ischemia

Support: Department of Neurosurgery

Title: Erythropoietin increases expression of microRNAs in cerebrocortical neurons

Authors: *L. FLETCHER[1], M. ZIU[2], S. SPRAGUE[2], D. F. JIMENEZ[2], M. DIGICAYLIOGLU[2];
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Abstract: Erythropoietin (EPO) has emerged as a major player in experimental models of neuropathological diseases contributing to the preservation of cultured neurons and brain tissue. EPO has been identified as a neurotrophic and neuroprotective agent in many different animal models of brain injury and stroke. EPO exerts its protective effects by activating anti-apoptotic

cellular signaling pathways in neurons and suppressing the activation of apoptosis that results in cell death. Despite a significant number of studies performed using cultured neurons and animal models, the neuroprotective signaling induced by EPO is not completely understood. MicroRNAs are short regulatory RNAs that modulate gene expression at the post-transcriptional level by inhibiting translation of target mRNAs. Multiple studies suggest that microRNAs regulate several biological processes, including cell differentiation, proliferation, metabolism, tumorigenesis and cell death. In addition, microRNA's are crucial for neural development and brain physiology. Recently, it was shown that EPO-induced expression of microRNAs is necessary for the survival of erythroid progenitor cells. However, so far the contribution of miRNA in neuronal EPO signaling has not been elucidated. The ultimate goal of this project is to identify EPO regulated expression of microRNA's that are likely to be involved in adaptation of intracellular signaling to improve cell viability under ischemic conditions. In this study we investigated whether microRNA's known to be involved in cell survival are expressed in neuronal cells and induced by EPO incubation. We found that EPO (10 U/ml) increased the expression of mir-210 in cerebrocortical cultures in a time-dependent manner. This effect was abolished when EPO blocking antibodies were added to the cultures. In addition, miR-210 expression was elevated in cerebrocortical cultures subjected to 1 hour of oxygen-glucose deprivation. Our study indicates that specific microRNAs are upregulated in neurons following ischemia and that EPO can regulate microRNA expression in neurons.

Disclosures: L. Fletcher, None; **M. Ziu**, None; **S. Sprague**, None; **D.F. Jimenez**, None; **M. Digicaylioglu**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.7

Topic: C.08. Ischemia

Support: NIH 2R01 NS045727

March of Dimes

American Heart Association

Title: The PGE2 EP4 receptor is protective in a model of cerebral ischemia

Authors: *K. I. ANDREASSON, X. LIANG, L. LIN, H. TANIGUCHI, C. ANACKER, Q.

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Abstract: The inducible cyclooxygenase COX-2 contributes significantly to brain injury in models of cerebral ischemia, and recent studies indicate an important role for the downstream prostaglandin E_2 EP1 signaling in mediating this toxicity. However, sustained COX-2 inhibition exerts deleterious cerebrovascular side effects, indicating that certain prostaglandin signaling pathways may function protectively in brain. Prostaglandin E_2 (PGE_2) is a lipid messenger derived from cyclooxygenase metabolism of arachidonic acid and signals through a class of four distinct G-protein coupled receptors, the EP1-4 receptors. In this study, we investigated the function of the PGE_2 EP4 receptor in models of cerebral ischemia. EP4 receptor is expressed in forebrain in neurons and is markedly induced in endothelial cells after transient focal cerebral ischemia. Pharmacologic activation of the EP4 receptor resulted in a significant rescue of cerebral tissue in the mouse middle cerebral artery occlusion-reperfusion (MCAO-RP) model when given after MCAO. Similarly, EP4 receptor activation in the rat hypoxic-ischemic encephalopathy model of peri-natal ischemia also resulted in significant cerebroprotection. In vivo, cell specific conditional knockout of EP4 receptor in neurons or endothelial cells resulted in significant increase in infarct size. These findings demonstrate dual cell-specific EP4 receptor signaling pathways that mediate cerebroprotection of EP4 in cerebral ischemia, and suggest a bi-modal therapeutic effect of EP4 agonism in stroke.

Disclosures: K.I. Andreasson, None; **X. Liang**, None; **L. Lin**, None; **H. Taniguchi**, None; **C. Anacker**, None; **Q. Wang**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.8

Topic: C.08. Ischemia

Title: Imaging the innate immune cells after experimental stroke

Authors: *J. NEUMANN[1], M. G. RIEK-BURCHARDT[2], M. G. GUNZER[3], K. G. REYMANN[2];
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Abstract: Cerebral ischemia is accompanied by an acute inflammation, involving the activation

of microglia and the infiltration of neutrophil granulocytes and monocytes into the brain. How these different immune cell types contribute to the neuronal outcome after cerebral ischemia is still under discussion. Various significant actions of immune cells are just revealable by imaging them either ex vivo or in vivo. We developed a postischemic ex vivo model of immune cell (fluorescence labeled) application on hippocampal slices with eYFP expression in neurons. We observed two significant mechanisms how microglia protect neurons after ischemia. On the one hand microglia were found in close proximity or in physical cell-cell contact to neurons and on the other hand microglia eliminated infiltrating neutrophil granulocytes very fast and efficient. Blocking both properties yield in an exacerbation of neuronal damage. To test our hypothesis in vivo we generated a mouse transgenic for neutrophils (Lys-EGFP) and microglia (CX3CR1-EGFP). For experimental cerebral ischemia we used a model of permanent middle cerebral artery occlusion combined with an occlusion of the common carotid arteries for 20 min. With intracranial two-photon microscopy (TPM) we are able to image these cells to a depth of 300 µm in vivo after ischemic lesions. To date we observed a rapid infiltration of neutrophils and a very fast response of microglia to damaged vessels after ischemia by surrounding them with their processes. This approach is suitable to answer a wide range of questions how immune cells respond to cerebral ischemic events and might contribute to gain intelligence for developing suitable therapeutic strategies.

Disclosures: J. Neumann, None; **M.G. Riek-Burchardt**, None; **M.G. Gunzer**, None; **K.G. Reymann**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.9

Topic: C.08. Ischemia

Support: European Stroke Network Grant No 201024

Swedish Research Council Grant No 2008-3134

Swedish Brain Fund

The Pia Ståhl Foundation

The Royal Physiographic Society in Lund

The Segerfalk Foundation

Greta och Johan Kocks Stiftelse

Title: Enriched environment improves functional recovery by attenuation of inflammation and inhibition of the stromal-derived-factor 1/CXC receptor 4 pathway after stroke

Authors: *K. RUSCHER[1], E. KURIC[1], Y. LIU[2], A. LOURENÇO INÁCIO[1], S. ISSAZADEH-NAVIKAS[2], T. WIELOCH[1];
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Abstract: After stroke, inflammation hampers beneficial mechanisms important for tissue reorganization in the ischemic hemisphere. The aim of the present study was to investigate if enriched environment (EE) effects improved functional recovery specifically by attenuation of inflammation in the ischemic hemisphere. Spontaneous hypertensive rats were subjected to permanent occlusion of the middle cerebral artery (pMCAO). Two days after stroke, rats with a significant neurological deficit were randomly allocated and kept in standard or enriched housing cages for 3 consecutive days. Analysis of the ischemic core and the peri-infarct area revealed that EE profoundly attenuated the level of the pro-inflammatory cytokines interferon-γ, TNFα, IL-1β, IL-4, and IL-5. Importantly, cytokine levels in the cerebro-spinal fluid and serum were not altered in respective animals. Along with changes of pro-inflammtory cytokines we found a significant reduction of the otherwise upregulated chemokine receptor CXCR4 and its natural ligand stromal-derived-factor 1 (SDF-1) in rats housed in EE after pMCAO. To study the effect of SDF-1/CXCR4 inhibition on functional recovery after transient MCAO (tMCAO), the specific CXCR4 antagonist 1,1-[1,4-Phenylenebis(methylene)]bis [1,4,8,11-tetraazacyclotetradecane] octohydrobromide dihydrate (AMD3100) was injected for 3 consecutive days (ip, 0.5mg/kg every 12h) starting 2 days after tMCAO. AMD3100 treated rats (n=8) showed an improved recovery compared with saline treated rats (n=8) at day 5 after tMCAO. Accompanied we found a reduction of infiltrating immune cells, in particular CD4(+) T-cells were inhibited to invade in the ischemic hemisphere. Moreover, AMD3100 treatment prevented spleen atrophy which however was observed in saline treated animals after tMCAO. Preliminary results also suggest antibacterial properties of AMD3100 by suppression of poststroke bacteriemia. Importantly, infarct size was unaffected by the treatment. We conclude that attenuation of poststroke inflammation obtained by housing rats in an enriched environment together with specific inhibition of the SDF-1/CXCR4 pathway significantly improves functional recovery and prevents detrimental secondary systemic effects in rats subjected to experimental stroke.

Disclosures: K. Ruscher, None; **E. Kuric**, None; **Y. Liu**, None; **A. Lourenço Inácio**, None; **S. Issazadeh-Navikas**, None; **T. Wieloch**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.10

Topic: C.08. Ischemia

Support: USDA CRIS 1235-51000-054-00D

Title: Purified type A polyphenols from cinnamon protect glial cells from ischemic injury by attenuating mitochondrial dysfunction and regulating intracellular calcium level

Authors: *K. S. PANICKAR[1], M. M. POLANSKY[1], J. F. URBAN, Jr[1], D. J. GRAVES[2], R. A. ANDERSON[1];
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Abstract: Dietary polyphenols, naturally present in fruits and vegetables, exert neuroprotective effects in ischemic injury. We evaluated components of cinnamon polyphenol extract (CPE) on cell swelling and mitochondrial dysfunction in glial cultures following ischemic injury. Protective effects of purified polyphenol fractions from CPE were observed on key features of ischemic injury including cell swelling, increased free radical production, increased intracellular calcium ($[Ca^{2+}]_i$), mitochondrial dysfunction, and reduction in glutamate uptake. Astrocyte (glial) swelling is a major component of cytotoxic brain edema in ischemia and, along with vasogenic edema, may contribute to increased intracranial pressure, brain herniation, and additional ischemic injuries. C6 glial cultures were exposed to oxygen-glucose deprivation (OGD) for 5 hr and cell swelling was determined 90 min after the end of OGD. OGD-induced increases in glial swelling were significantly blocked by a type A polyphenol trimer, MW 864, and a tetramer, MW 1152, but not by the non-polyphenol fractions of CPE including cinnamaldehyde and coumarin. Increased free radical production, a contributing factor in cell swelling following ischemic injury, was also significantly reduced by the type A trimer as well as by the combinations of the trimer with cyclosporin A or insulin. Mitochondrial dysfunction, another key feature of ischemic injury, is hypothesized to contribute to glial swelling. Depolarization of the inner mitochondrial membrane potential ($\Delta\Psi_m$) was assessed using a fluorescent dye (TMRE), and was significantly attenuated by the trimer. The OGD-induced increase in $[Ca^{2+}]_i$ was also attenuated by the trimer. Further, nifedipne, a blocker of L-type calcium channel, as well as dantrolene, a blocker of ryanodine receptor-mediated calcium release, similarly blocked cell swelling. An important feature of glial cells is maintenance of extracellular glutamate levels. Reduced glutamate uptake after ischemia can lead to excitotoxic damage. The type A trimer significantly attenuated OGD-induced decreases in glutamate uptake. In addition, cyclosporin A, a blocker of the mitochondrial permeability transition (mPT) pore, but not FK506 (that does not block the mPT), attenuated the decline in glutamate uptake after OGD. Our results indicate that the type A trimer may attenuate cell swelling by regulating intracellular $[Ca^{2+}]_i$. Effects of type A polymers in attenuating the reduction in glutamate uptake

are likely mediated through their action on the mitochondria.

Disclosures: K.S. Panickar, None; **M.M. Polansky**, None; **J.F. Urban**, None; **R.A. Anderson**, Integrity Nutraceuticals, Other Research Support; **D.J. Graves**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.11

Topic: C.08. Ischemia

Support: University of Wisconsin

Title: D609 protection through cell cycle regulation after stroke

Authors: *R. M. ADIBHATLA, J. F. HATCHER;
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Abstract: Expressions of cell cycle regulating proteins are altered after stroke. Post-mitotic neurons enter an aberrant cell cycle after stroke, resuting in cell death. Cell cycle inhibition has shown dramatic reduction in infarction after stroke. Sphingomyelin (SM) synthase (SMS) transfers the phosphocholine group from phosphatidylcholine (PC) to ceramide to form sphingomyelin and release DAG. PC-phospholipase C (PC-PLC) inhibitor D609 also inhibits SMS and increases ceramide. Ceramide can induce cell cycle arrest by up-regulation of Cdk inhibitors p21 and p27 through activation of protein phosphatases 1 and 2A. D609 reduced the infarct volume after stroke and may provide benefit through inhibition of SMS and increased ceramide levels. Ceramide may then induce cell cycle arrest by up-regulating p21 and causing hypo-phosphorylation of retinoblastoma (Rb) (through Cdk inhibition and/or increased protein phosphatase activity). D609 also reduced oxidized phosphatidylcholine (OxPC) protein adducts. This suggests that D609 may be affecting microglia/macrophages, a major source of ROS after stroke. However, it is unclear how D609 affects individual neural cell proliferation. Primary mouse neuronal cultures subjected to OGD/reoxygenation showed increased expression of Cdk4, evidence of entry into the cell cycle. D609 reduced the neuronal death following OGD/reoxygenation.
D609 may prevent mature neurons from entering the cell cycle. Others showed that D609 inhibited bFGF-stimulated astrocyte proliferation by increasing ceramide through SMS inhibition. Microglia/macrophages are the source for pro-inflammatory cytokines (TNFα, IL-1ß) as well neurotrophic factors (BDNF, IGF etc.,). To understand the role of D609 in cell cycle, in

vitro studies were conducted using primary mouse astrocyte cultures, microglia (N9 and BV-2) and macrophage (RAW 264.7) cell lines. The effects of D609 on expression of p21, p27, cyclin D1, phospho-Rb, PC-PLC and SMS were examined. Cultures exposed to D609 (0-200 uM, up to 24 hrs) exhibited concentration-dependent inhibition of cell proliferation (determined by cell counting) without affecting cell viability (determined by trypan blue exclusion); this was further confirmed by BrdU incorporation. D609 treatment also increased p21 and decreased phospho-Rb expressions. Low levels of ceramide may stimulate proliferation, whereas intermediate levels may cause cell cycle arrest and high levels induce apoptosis. High doses and longer exposure of D609, which may cause excessive ceramide accumulation through SMS inhibition, caused over-expression of p21, hypo-phosphorylation of pRb and apoptotic cell death.

Disclosures: **R.M. Adibhatla**, University of Wisconsin, Employment; **J.F. Hatcher**, University of Wisconsin, Employment.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.12

Topic: C.08. Ischemia

Support: AHA Grant 05553413YL

AHA Grant 0535201YC

AHA Grant 0625532XZ

NIH Grant NIH/NINDS

NIH Grant R01NS5051383YL

NIH Grant NIH/NIA R01AG033282YL

Title: Treatment of stroke by disrupting DAPK1-NMDA receptor NR2B interaction

Authors: *X. XU[1], W. TU[3], W. ZHANG[2], N. JIA[2], Y. LU[2];
[1]LSU Hlth. Sci. Ctr., NEW ORLEANS, LA; [2]LSU Hlth. Sci. Ctr., New orleans, LA; [3]Univ. of Central Florida, Orlando, FL

Abstract: Excessive stimulation of NMDA is considered to be the main factor responsible for brain damage in stroke. However, the essential physiological action of these receptors in synaptic transmission means that blocking them totally is not a feasible therapeutic option. An idea approach for the treatment of stroke should inhibit the specific NMDA receptor "cell death signals" whereby the pathological effects of the receptors is selectively blocked, leaving the physiological action unaffected. Recently, we have used proteomics assays combined with gene-targeting studies to explore NMDA receptor-associated cell death signals. We have demonstrated that DAPK1 physically and functionally interacts with glutamate NMDA receptor NR2B subunit at extra-synaptic site and its interaction acts as a central mediator in stroke damage. The findings lead us to hypothesize that the treatment of stroke can be achieved by selectively blocking the DAPK1-NR2B interactions. To test this hypothesis, we identified that DAPK1 directly binds with NMDA receptor NR2B C-terminal tail consisting of amino acid 1297-1304 (NR2Bi). In order to increase the bioavailability of NR2Bi, we created D-stereoisomer NR2Bi (D-NR2Bi), which was resistant to the cellular proteases. We synthesized a cell-permeable D-NR2Bi by fusing it with the HIV-1 Tat protein (Tat-D-NR2Bi). A scrambled D-NR2Bi (sD-NR2Bi) was used as a control. We administered intravenously (*i.v.*) Tat-D-NR2Bi at a dose of 0.5, 1, or 5 mg/kg. 30 min after administration, animals were operated with MCAO for 60 min. We found that D-NR2Bi at a dose of 1 mg/kg (*i.v.*) is able to completely eliminate the association of an activated DAPK1 with NMDA receptor NR2B subunit *in vivo* without affecting the catalytic activity of DAPK1. The cerebral infarction was then measured 24 hr after reperfusion and the total infarction volume in mice treated with 1 mg/kg Tat-D-NR2Bi was reduced to 10.9 ± 1.1 mm^3, compared to 37.3 ± 3.2 mm^3 in control, in which mice are injected with Tat-sD-NR2Bi (mean ± SEM, $n = 9$ mice per group, *ANOVA*, $F = 18.91$, $p < 0.001$). Thus, we conclude that DAPK1 physically and functionally interacts with NMDA receptor NR2B subunit at extra-synaptic sites and this interaction acts as a central mediator for stroke damage.

Disclosures: X. Xu, None; **W. Tu**, None; **W. Zhang**, None; **N. Jia**, None; **Y. Lu**, None.

Nanosymposium

122. Ischemia: Inflammation and Molecular Mechanisms

Location: Room 32B

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 122.13

Topic: C.08. Ischemia

Support: NIH Grant NS067071

CURE Foundation

Title: Role of microRNA-29c in ischemic brain damage

Authors: G. PANDI[1], V. P. NAKKA[1], A. DHARAP[1,2], W. B. POTTER[3,2], A. ROOPRA[3,2], *R. VEMUGANTI[1,2];
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Abstract: The microRNAs (miRNAs) are ~22 nt long, evolutionarily conserved non-coding RNAs which are known to control protein translation by binding to the 3'UTRs of mRNAs. We recently reported that stroke (focal cerebral ischemia induced by transient middle cerebral artery occlusion) significantly alters cerebral miRNAome in adult rat brain. The miRNA mir-29c was observed to be expressed at a high level in the normal rat brain and down-regulated by ~8 fold after focal ischemia in adult rats as well as in PC12 cells when exposed to oxygen-glucose deprivation (OGD). We presently evaluated the functional significance of mir-29c to post-ischemic brain damage. Bioinformatics indicated that DNMT3a mRNA is a major target of mir-29c and cotransfecting with premir-29c prevented the expression of DNMT3a 3'-UTR vector in PC12 cells. DNMT3a mRNA and protein expression also increased following in vivo or in vitro ischemia. Recovering mir-29c levels by treating with a premir-29c or inhibiting DNMT3a with a specific siRNA prevented OGD-induced cell death by ~70%. On the other hand, treating normal PC12 cells with an antagomir-29c killed ~55% cells within 24h. The mir-29c gene promoter showed several binding sites for the transcription factor REST/NRSF and cotransfecting with a REST plasmid prevented the mir-29c promoter vector expression in PC12 cells. Furthermore, treating PC12 cells with REST siRNA prevented the post-OGD down-regulation of mir-29c and cell death by 70% to 80%. Intracerebroventricular infusion of premir-29c to adult rats decreased the post-ischemic infarction (by ~45%) and neurological deficits compared to a control-mir treatment. These studies indicate that mir-29c down-regulation after focal ischemia leading to increased expression of its down-stream target DNMT3a might be a putative mediator of ischemic cell death and REST is an upstream transcriptional controller of mir-29c. Furthermore, curtailing REST induction or mir-29c down-regulation or DNMT3a induction can induce neuroprotection after ischemia.

Disclosures: G. Pandi, None; **V.P. Nakka**, None; **A. Dharap**, None; **W.B. Potter**, None; **A. Roopra**, None; **R. Vemuganti**, None.

Nanosymposium

123. Mitochondria in Health and Disease

Location: Room 23A

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 123.1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH 1R01NS057433

National MS Society CA 1055-A-3

NIH–NIND Training Program in Neuronal Signaling

Title: Permeability transition pore modulates mitochondrial Ca2+ and ATP levels in adult neurons under oxidative stress

Authors: *A. G. BARSUKOVA[1], D. BOURDETTE[2], M. FORTE[3];
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Abstract: Reactive oxygen species (ROS) are strongly implicated in a number of prevalent neuropathologies. Mitochondrial dysfunction driven by oxidative stress is considered a key player in neuronal death. Remarkable neuroprotection in animal models of neurodegeneration accompanied by oxidative stress was achieved by inhibiting the mitochondrial permeability transition pore (PTP). The PTP is a voltage-dependent, cyclosporin A (CsA)-sensitive, high-conductance channel of the inner mitochondrial membrane. Exogenous ROS and Ca2+ overload have been shown to act as the key activators of the PTP. While transient PTP activation acts as an additional Ca2+ efflux pathway, persistent activation of the PTP leads to respiratory inhibition, ensuing ATP depletion and the release of apoptotic activators, ultimately resulting in cell death. Although the molecular nature of the PTP is unknown, manipulation of the PTP was achieved through the analysis of isolated mitochondria, cells and mice devoid of cyclophilin D (CyPD-KO). CyPD is a key regulator of PTP activity and the target of cyclosporin A (CsA) action. Postnatal neurons devoid of CyPD are resistant to the pathological effects of reactive oxygen and nitrogen challenges, suggesting that de-activation of the PTP is neuroprotective. Moreover, CyPD-KO mice show neuroprotection in ischemia, Alzheimer's disease and multiple sclerosis models. However, the molecular mechanism underlying the CyPD-dependent neuroprotection remains unclear.
We hypothesized that CyPD inactivation modulates mitochondrial Ca2+ dynamics and ATP levels in adult neurons under oxidative stress, which serve as key neuroprotective effects. Here we evaluated for the first time mitochondrial Ca2+ dynamics, ATP production and their regulation via the CyPD-dependent PTP under hydrogen peroxide (H2O2) treatment in individual adult cortical neurons using real-time fluorescent imaging, combined with the use of an adult neuronal culture model and genetic constructs. We also evaluated the interplay between cytoplasmic and mitochondrial Ca2+ dynamics, mitochondrial membrane potential, pH and the role of the mitochondrial Ca2+ uniporter (MCU) under H2O2 in adult cortical neurons. Our results demonstrate novel aspects of mitochondrial Ca2+ dynamics under oxidative stress in adult neurons. The study shows that the CyPD-dependent neuroprotection is associated with the modulation of the PTP activation, stabilization of the mitochondrial Ca2+ and membrane potential to their homeostatic levels, lower cytosolic Ca2+ levels and higher mitochondrial ATP levels.

Disclosures: A.G. Barsukova, None; **D. Bourdette**, None; **M. Forte**, None.

Nanosymposium

123. Mitochondria in Health and Disease

Location: Room 23A

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 123.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 NS67078

NIH Grant R37 NS34179

Title: The mitochondrial protein prohibitin (PHB) reduces superoxide production and protects brain cells from different injury modalities

Authors: *P. ZHOU, L. QIAN, H. KURINAMI, G. MANFREDI, C. IADECOLA;
Dept Neurol Neurosci, Weill Med. Coll Cornell Univ., NEW YORK, NY

Abstract: PHB is an evolutionary conserved protein involved in multiple cellular functions, but its role in brain is not well understood (Trends Mol Med 11:192, 2005). In rodent models of cerebral ischemic tolerance, in which a non-lethal injurious stimulus confers protection from a subsequent lethal ischemic insult, PHB is upregulated in brain mitochondria (Abs# 354.10, SFN 2009). This observation raises the possibility that PHB contributes to the profound reduction in brain injury afforded by preconditioning. Therefore, we investigated whether PHB is neuroprotective and, if so, we sought to gain insight into its mechanisms of action. Primary mouse cortical neuronal cultures were cotransfected with PHB cDNA and a GFP plasmid, and cell viability was assessed by nuclear morphology**.** Transfected neurons were challenged with staurosporine (STS; 0.25μM) or with oxidative stress produced by xanthine (150μM)-xanthine oxidase (15 mU). STS induced significant cell death at 24 hrs, which was greater in neurons transfected with control vector (viable cells: 48±3%), than with PHB (70±4%; $p<0.001$ from control vector; n=4)**.** PHB-transfected neurons had improved viability also following exposure to oxidative stress (Control: 43±2 %; PHB: 71±4%; $p<0.001$; n=4). Similarly, mouse hippocampal slice cultures, in which PHB expression was increased by viral gene transfer, were relatively protected from the damage produced by oxygen-glucose deprivation (viability: Control: 55±5%; PHB: 78±4%; $p<0.05$; n=20). We then investigated the effect of downregulation of PHB expression with RNA interference (PHB-siRNA) on glutamate excitotoxicity. In neuronal cultures transfected with control siRNAs, glutamate (20μM) increased mitochondrial superoxide production, assessed using Mitosox as an indicator (1,143±28 fluorescence units; n=3).

However, the superoxide production was greater in neuronal cultures in which PHB was downregulated (2,194±27; $p<0.05$ from control siRNAs; n=3). The increase in superoxide production was associated with reduced viability to glutamate-induced neuronal death (Control siRNA: 72±7%; PHB-siRNA: 45±5%, $p<0.01$; n=3). These data demonstrate that PHB renders neurons more resistant to different injury modalities. In glutamate excitotoxicity, the protective effect of PHB is associated with a suppression of mitochondrial superoxide production. Although the in vivo significance of these observations remains to be determined, treatment strategies to upregulate endogenous PHB may be beneficial in cerebral ischemic damage and other forms of brain injury as well.

Disclosures: P. Zhou, None; **L. Qian**, None; **H. Kurinami**, None; **G. Manfredi**, None; **C. Iadecola**, None.

Nanosymposium

123. Mitochondria in Health and Disease

Location: Room 23A

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 123.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: ETF

ESTBIOREG

Title: Principles of mitochondrial fusion and fission cycle in neurons

Authors: *A. KAASIK[1], M. CAGALINEC[1], D. SAFIULINA[2], M. LIIV[2], J. LIIV[2], V. CHOUBEY[2];
[1]Dept. of Pharmacol., [2]Univ. of Tartu, Tartu, Estonia

Abstract: Mitochondrial fission and fusion are often viewed as a finely tuned balance within cells, yet an integrated and quantitative understanding of how these processes interact with each other and with other mitochondrial motility and morhology is not well formulated.
Here we therefore performed thorough analysis of mitochondrial fusion and fission events in primary cortical neurons using mitochondrial targeted photoactivatable fluorescent protein Kikume Green-Red 1. The fate of activated mitochondria were followed during the next 2h during which all fusion and fission events, lenght and motility was recorded.
First, our results demonstrate that fusions and fissions are not independent, randomly occurring events but mostly sequential events (with 85% regularity) following each other. Fusions were in

86,4% of cases followed by fission and only in 13,6% by second fusion. Fissions in turn were followed in 83,5% by fusion and 16,5% by second fission.
Second, our results demonstrate that mitochondria stay around 23% from cycle time between fusion and fission event and 77% between fission and next fusion. Time interval between fusion and fission was 280 ± 34 s was significantly shorter than between fission and next fusion 919 ± 119 s ($p < 0.0001$, cycle duration together 1200 s).
Third, our results demonstrate that fusion rate of independent mitochondria are regulated mainly by mitochondrial motility. Two mitochondria could fuse only when being in contact with each other. In our experiments axonal mitochondria made as an average 0.45±0.02 contacts/ mitochondria/min from which 7.0±0.4 percent ended with fusion. Contact rate in turn is mainly determined by motility of mitochondria. We observed clear correlation between contact rate and motility. We performed "twin analysis" of families were at least one of the mitochondria fused and analysed the motility there. Results demonstrate that daughter with higher motility fused before it's sister.
Fourth, our results demonstrate that fission rate of independent mitochondria depend on it's length. We observed that longer mitochondria tended to fissed immediately after the fusion event while shorter one not. We therefore grouped the mitochondria according their lengths and calculated fission rates. Results demonstrates that in cortical neurons the fission rate increases with mitochondrial length ($p<0.0001$). It is relevant to note that in both cases the fusion rate showed no length dependency.
Altogether our results provide clear-cut explanation how fusion and fission are interrelated with mitochondrial length and motility

Disclosures: A. Kaasik, None; **M. Cagalinec**, None; **D. Safiulina**, None; **M. Liiv**, None; **J. Liiv**, None; **V. Choubey**, None.

Nanosymposium

123. Mitochondria in Health and Disease

Location: Room 23A

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 123.4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS04748

NIH Grant F31NS061438

Title: Brain mitochondria remove hydrogen peroxide in a respiration-dependent manner via the thioredoxin/peroxiredoxin system

Authors: D. A. DRECHSEL, *L. LIANG, M. PATEL, Ph.D;
Pharmaceut. Sci., Univ. of Colorado Denver, Aurora, CO

Abstract: Mitochondrial reactive oxygen species (ROS) play an important role in physiological cell signaling processes and are implicated in a multitude of disease states including neurodegenerative disorders such as Parkinson's disease. While mitochondria are considered the major cellular source of ROS, their role in the removal of ROS remains largely unknown. The goal of this study was to determine the mechanisms underlying ROS removal in brain mitochondria. Using a polarographic method for real-time detection of steady-state H2O2, isolated rat brain mitochondria showed significant rates of H2O2 removal (9-12 nmol/min x mg prot) in the presence of substrates (malate + glutamate), indicating a respiration-dependent process. A pharmacological approach was used to assess the contributions of potential enzymatic systems involved in H2O2 removal by brain mitochondria. The glutathione system showed only minimal contributions (25% decrease by glutathione reductase inhibition and no effect by glutathione peroxidase inhibition). In contrast, inhibitors of thioredoxin reductase, auranofin and 1-chloro-2,4-dinitrobenzene, attenuated H2O2 removal rates in mitochondria by over 70%. Furthermore, oxidation of thioredoxin-2 via arsenic resulted in a significant decrease in H2O2 removal whereas copper-induced glutathione oxidation showed minimal effects. Interestingly, H2O2 removal from mitochondria of other rat organs, such as liver, did not show similar characteristics. Inhibition of the thioredoxin system in neuronal cell culture models also exacerbated ROS production and cell death in response to model PD toxicants, such as paraquat. These data suggest that the thioredoxin/peroxiredoxin system is the major contributor to respiration-dependent H2O2 removal, which is unique to brain mitochondria. Additionally, mitochondria pre-incubated with paraquat showed severely compromised H2O2 removal rates (up to 80% decrease) and inhibition of thioredoxin reductase activity in a concentration-dependent manner, suggesting dysfunction of the thioredoxin system in response to environmental neurotoxicants. These findings have significant implications in the understanding of the role for mitochondria and the thioredoxin/peroxidredoxin system in both physiological processes mediated by ROS and neurodegenerative disease.

Disclosures: D.A. Drechsel, None; **L. Liang**, None; **M. Patel**, None.

Nanosymposium

123. Mitochondria in Health and Disease

Location: Room 23A

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 123.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 NS062766 from NINDS

VA Merit Review Award

Title: Neuroprotective effects of anthocyanins on neuronal death induced by mitochondrial oxidative stress

Authors: *D. A. LINSEMAN, W. HULICK, N. KELSEY;
Biol. Sci., Univ. of Denver, DENVER, CO

Abstract: Neurodegenerative diseases such as Parkinson's and amyotrophic lateral sclerosis have devastating consequences to the afflicted patients. A major cellular pathophysiology underlying these diseases is mitochondrial oxidative stress (MOS) leading to neuronal death. Here, we investigated the neuroprotective effects of a novel class of nutraceuticals, anthocyanins, against MOS-induced death in primary cultures of rat cerebellar granule neurons (CGNs). Anthocyanins are natural antioxidants whose neuroprotective potential has yet to be examined. Kuromanin and callistephin are anthocyanins derived from black rice and strawberries, respectively. Glutathione (GSH)-sensitive MOS and intrinsic apoptosis were induced in CGNs by the Bcl-2 inhibitor, HA14-1. Callistephin and kuromanin each demonstrated equivalent neuroprotection from this MOS-induced death to that of the green tea polyphenol, epigallocatechin 3-gallate; however, neither anthocyanin was as effective as GSH at rescuing CGNs. Incubation with HA14-1 alone resulted in nearly 90% apoptosis of CGNs and either callistephin or kuromanin reduced this effect to approximately 20% cell death. Treatment with HA14-1 caused a marked depletion of mitochondrial GSH in CGNs to approximately 40% of the control level. Callistephin and kuromanin essentially prevented the reduction in this critical pool of endogenous antioxidant. These data indicate that callistephin and kuromanin represent a new class of neuroprotective compounds that warrant further study as possible therapeutic agents for the treatment of neurodegenerative diseases caused by MOS.

Disclosures: D.A. Linseman, None; **W. Hulick**, None; **N. Kelsey**, None.

Nanosymposium

123. Mitochondria in Health and Disease

Location: Room 23A

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 123.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR

Title: BH3-only transcriptional activation in Bax-mediated apoptosis

Authors: K. K. AMBACHER, *R. RYLETT, K. B. PITZUL, S. P. CREGAN;
Mol. Brain Res. Group, Univ. Western Ontario, London, ON, Canada

Abstract: Apoptosis has been implicated in many acute and chronic neurodegenerative conditions such as Huntington's disease, Alzheimer's disease, stroke and Parkinson's disease. In neurons, Bax, a protein belonging to the Bcl-2 family, has been shown to be important in inducing apoptosis following a variety of different types of cell stress. It is therefore important to identify the mechanisms that regulate Bax activation in neurons in order to develop therapeutic treatment for these neurodegenerative conditions. A large body of evidence suggests the involvement of BH3-only Bcl2-family member proteins in Bax activation however the key players are not yet known. Certain kinase pathways, namely the JNK and GSK3β pathways, have also been implicated as key pro-apoptotic pathways and most likely function through a Bax-dependent pathway of programmed cell death. Therefore, in the present study we investigated whether these signaling pathways promoted Bax activation (and subsequent apoptosis) by regulating the transcriptional induction of BH3-only family members. Bax-dependent apoptosis was induced in cerebellar granule neurons (CGNs) by potassium withdrawal. In this model we found that mRNA levels of the BH3-only proteins Bim, Puma and Hrk were significantly increased during apoptosis. However, only Puma-deletion inhibited Bax-mediated cell death processes. In contrast to wildtype neurons, Puma-deficient neurons retained mitochondrial cytochrome-c and membrane potential and did not exhibit an apoptotic nuclear morphology following potassium withdrawal. Inhibition of the JNK and GSK3β signaling pathways, using pharmacological inhibitors SP600 and SB415 respectively, attenuated Puma mRNA induction and protected neurons against potassium withdrawal induced apoptosis. Our results demonstrate that transcriptional induction of the BH3-only family member Puma plays a key role in regulating Bax-mediated neuronal apoptosis. Furthermore, we have determined that the pro-apoptotic JNK and GSK3β signaling pathways affect Bax-induced neuronal apoptosis by regulating the transcriptional induction of Puma.

Disclosures: K.K. Ambacher, None; **K.B. Pitzul**, None; **R. Rylett**, None; **S.P. Cregan**, None.

Nanosymposium

123. Mitochondria in Health and Disease

Location: Room 23A

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 123.7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: The Wallerian degeneration slow (Wlds) gene provides neuroprotective effects against the impairment of mitochondrial electron transport chain in primary cultured mouse cortical neurons

Authors: *S. TOKUNAGA[1,2], T. ARAKI[1,2];
[1]Fac. of Sci. and Engin., Waseda Univ., Shinjuku-ku, Japan; [2]Dept. of Peripheral Nervous Syst. Res., Natl. Inst. of Neuroscience, NCNP, Kodaira-shi, Japan

Abstract: Axonal degeneration occurs in many neurological disorders including diabetic neuropathies, demyelinating diseases, and neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease. The active nature of axonal degeneration was typically shown by the discovery of wldS mice, in which Wallerian degeneration is dramatically slowed by a spontaneous mutation that results in the expression of a mutant protein called WldS protein. Interestingly, some groups have reported that WldS protein prevents not only axonal degeneration but also neuronal cell death against some disease models including ischemia and glaucoma. But so far it is not clear when WldS protein can prevent cell death as well as axonal degeneration or whether the WldS protein in mitochondrial matrix has an important role for neuronal protection against cell death. To investigate against what types of cellular stress WldS protein can protect neural cells, we gave different stress stimuli to primary cultured cortical neurons from wild type and wldS mouse brains. We found that cortical neurons expressing WldS protein showed resistance to hypoxia-reoxygenation. Subsequently, to characterize the role of WldS protein for mitochondrial function, we investigated survival of cells treated by inhibitors of mitochondrial complexes. We found that WldS expressing neurons were protected against inhibitors for Complex I (rotenone), III (antimycin) and IV (potassium cyanide). These results suggest that WldS expression may enhance neuronal survival in neurodegenerative disorders involving mitochondrial electron transport chain defect.

Disclosures: S. Tokunaga, None; **T. Araki**, None.

Nanosymposium

124. Neural Correlates of Olfactory Behavior

Location: Room 1B

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 124.1

Topic: D.01. Chemical Senses

Support: PHS Grant GM07257

Title: Going with the Flow: A microfluidic approach to the behavioral analysis of chemotaxis in C. elegans

Authors: *K. E. MCCORMICK, M. SOTTILE, S. LOCKERY;
Biol., Univ. of Oregon, Eugene, OR

Abstract: C. elegans orients to chemosensory gradients using two main strategies. In klinokinesis (also known as the pirouette strategy) the worm randomly chooses a new heading upon sensing a decrease in concentration of a favored chemical. In klinotaxis (also known as the weathervane strategy) the worm directs the course of its forward movement such that its path curves toward increasing concentrations of a favored chemical. Whereas klinokinesis has been a subject of experimental study for some years, klinotaxis has only recently been characterized. In an initial report, Iino & Yoshida find that the curving rate of the worm increases as a linear function of the gradient steepness in the direction perpendicular to the worm's heading (hereafter, the transverse direction) [1]. This relationship suggests that the head swings of the worm's undulatory locomotion may be involved in sensing the gradient and executing gradual curves during klinotaxis.
We have developed a microfluidic device that has two key advantages over studying klinotaxis in freely moving worms. First, the concentration difference during head swings is known precisely because it is under the control of the experimenter rather than the animal. This should make it possible to measure behavioral thresholds more easily. Second, the concentration gradient in the transverse direction can be manipulated independently of the concentration gradient in the longitudinal direction. This allows us attribute changes in head sweep behavior specifically to the transverse gradient. In the microfluidic chip, we find that worms bias their mean head swing angle toward higher attractant concentrations. Furthermore, head-swing bias is proportional to the difference in concentration between the dorsal and ventral sides of the worm. These findings are consistent with the hypothesis that path curvature towards chemosensory peaks is implemented through bias to the head swings of undulatory locomotion.
We are currently addressing the contribution of candidate sensory and motor neurons to klinotaxis behavior in the microfluidic chip using cell ablation, calcium imaging, and photoactivation experiments.
1. Iino, Y. and K. Yoshida, Parallel use of two behavioral mechanisms for chemotaxis in Caenorhabditis elegans. Journal of Neuroscience, 2009.

Disclosures: K.E. McCormick, None; **M. Sottile**, None; **S. Lockery**, None.

Nanosymposium

124. Neural Correlates of Olfactory Behavior

Location: Room 1B

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 124.2

Topic: D.01. Chemical Senses

Support: NIH-NIDCD

FAPESP

Title: The vomeronasal organ mediates interspecies defensive behaviors through detection of protein pheromone homologs

Authors: *F. PAPES[1,2], D. W. LOGAN[2], L. STOWERS[2];
[1]Depto. Genetica e Evolucao, State Univ. of Campinas, Campinas, Brazil; [2]The Scripps Res. Inst., La Jolla, CA

Abstract: Potential predators emit uncharacterized chemosignals that warn receiving species of danger. Until now, neurons that sense these stimuli have not been identified. In this work, we show that detection and processing of fear-evoking odors emitted from cat, rat, and snake requires the function of sensory neurons in the vomeronasal organ. To investigate the molecular nature of the sensory cues emitted by predators, we isolated the salient ligands from two species using a combination of innate behavioral assays in naïve receiving animals, calcium imaging, and cFos induction. Surprisingly, the defensive behavior-promoting activity released by other animals is encoded by species-specific ligands belonging to the major urinary protein (Mup) family, homologs of aggression-promoting mouse pheromones. We show that recombinant proteins are sufficient to activate sensory neurons and initiate defensive behavior similar to native odors. This co-option of existing sensory mechanisms provides a molecular solution to the difficult problem of evolving a variety of species-specific molecular detectors.

Disclosures: F. Papes, None; **D.W. Logan**, None; **L. Stowers**, None.

Nanosymposium

124. Neural Correlates of Olfactory Behavior

Location: Room 1B

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 124.3

Topic: D.01. Chemical Senses

Support: NIH Fellowship F32DC009352

NIH Grant R01DC010381

G. Harold & Leila Y. Mathers Foundation

Title: A functional glomerular map in the mouse accessory olfactory bulb

Authors: *J. P. MEEKS, G. F. HAMMEN, D. TURAGA, T. E. HOLY;
Anat. and Neurobio., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: The mouse accessory olfactory system processes information about nonvolatile chemical cues, including social odors and pheromones. In the accessory olfactory system, vomeronasal sensory neurons (VSNs) make glutamatergic connections in the accessory olfactory bulb (AOB) at neuropil structures called glomeruli. These glomeruli are thought to contain synaptic terminals from many VSNs expressing the same odor-sensing vomeronasal receptor. Previous studies identified general patterns of AOB innervation based on the selective labeling of VSNs expressing certain vomeronasal receptors, but we currently lack understanding about how these general innervation patterns relate to sensory function.
We studied the patterns of AOB sensory innervation in *ex vivo* preparations from mice expressing the Ca^{2+}-sensitive fluorescent protein GCaMP2 in VSNs and their synaptic terminals. We delivered twelve molecularly distinct sulfated steroids to the vomeronasal organ while recording optical signals from the AOB glomerular layer using an objective-coupled planar illumination (OCPI) microscope. Fast volumetric imaging demonstrated reliable increases in fluorescence intensity in the AOB glomerular layer over multiple interleaved applications of specific sulfated steroids at 10 μM concentration. The sizes and shapes of the active regions matched those reported for individual glomeruli. Many of the active regions displayed steroid response patterns consistent with recently-identified VSN "processing streams", suggesting many of these functionally-defined populations are maintained in the glomerular layer.
We found that sulfated steroids stimulate glomeruli at many locations across the anterior portion of the AOB, consistent with activation of members of the V1R family of vomeronasal receptors. We analyzed the spatial activation patterns of multiple sulfated steroid processing streams, finding stereotypy in some patterns across multiple animals. Our results indicate that responses to sulfated steroids are transmitted broadly across the anterior AOB, and provide a new framework for studying functional connectivity in this mammalian social olfactory pathway.

Disclosures: J.P. Meeks, None; **G.F. Hammen**, None; **D. Turaga**, None; **T.E. Holy**, None.

Nanosymposium

124. Neural Correlates of Olfactory Behavior

Location: Room 1B

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 124.4

Topic: D.01. Chemical Senses

Support: NIH Grant DC006885

NIH Grant DC009413

Title: Molecular mechanisms of pheromone mediated behavior

Authors: *L. T. STOWERS;
Scripps Res. Inst., LA JOLLA, CA

Abstract: The sense of olfaction is composed of a subset of neurons that are activated by pheromones and lead to the regulation of stereotyped innate social behaviors such as aggression and maternal behaviors. Pheromone stimuli are known to elicit a variety of specific innate behaviors in rodents, providing a unique opportunity to study the detection-perception-behavior pathway at the molecular and cellular levels. We expect that elucidating the stimulating ligands and responsive neurons will enable us to activate, study, and identify the mechanisms underlying neural information coding of defined behaviors. We have been isolating the chemical ligands, pheromones, which specifically govern social behaviors such as aggression, fear, and maternal-infant behavior. Isolation of these pheromones enables us to specifically activate, and thereby identify, the population of sensory neurons dedicated to promoting each behavior. Identifying these molecules will allow us to investigate the kinetics of their response as well as genetically manipulate their properties to validate their role in promoting behavior. We expect that our studies will provide the tools to expand our understanding of the logic of neuronal coding of innate behaviors.

Disclosures:

Nanosymposium

124. Neural Correlates of Olfactory Behavior

Location: Room 1B

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 124.5

Topic: D.01. Chemical Senses

Support: Howard Hughes Medical Institute

Title: Genetic analysis of olfactory processing and function in mice

Authors: *A. FLEISCHMANN, D. L. SOSULSKI, R. AXEL;
Dept. of Neurosci., Columbia Univ., New York, NY

Abstract: We are using a set of defined genetic perturbations in the expression of the odorant receptor gene repertoire to characterize the functional properties of neural circuits underlying olfactory sensory processing. We have generated transgenic mice with a "monoclonal nose" in which greater than 95% of the sensory neurons express the acetophenone-responsive odorant receptor, M71. In assays for associative olfactory discrimination, we found that M71 transgenic mice fail to detect the M71 receptor ligand acetophenone despite a 1000-fold increase in sensory neurons responsive to this odor. However, these mice readily detect other odors in the presence of acetophenone (Fleischmann et al., 2008). These results suggest a model of olfactory processing in which the recognition of patterns of neural activity, or contrast, is critical for odor detection.
To test this model, we have generated additional transgenic mouse lines. In these mice, the frequency of acetophenone-responsive sensory neurons is increased 15-, 100- and 500-fold compared to wild-type mice. Histological analysis has shown that in these transgenic mice, M71 expressing fibers converge onto distinct glomeruli. The number of glomeruli innervated by M71 expressing fibers is proportional to the frequency of sensory neurons expressing the M71 OR. We have used in vivo two-photon microscopy to reveal how these genetically defined patterns of glomerular activity are transformed into piriform odor representations, and we have characterized the behavioral consequences of such perturbations on odor detection and discrimination. Preliminary results suggest that the recognition of contrast may involve inhibition at multiple stations within the olfactory pathway. This genetic approach provides an opportunity to reveal the principles of olfactory processing that link the generation of patterns of odor-evoked neural activity with olfactory-driven behaviors.

Disclosures: A. Fleischmann, None; **D.L. Sosulski**, None; **R. Axel**, None.

Nanosymposium

124. Neural Correlates of Olfactory Behavior

Location: Room 1B

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 124.6

Topic: D.01. Chemical Senses

Support: NIDCD grant DC007725

NIDCD grant DC009948

Title: Representational learning mechanisms within the olfactory bulb

Authors: *T. A. CLELAND;
Cornell Univ., ITHACA, NY

Abstract: Olfactory learning is a distributed phenomenon involving changes in synaptic weights and gene expression profiles throughout the central nervous system, thereby underlying a number of potential changes in animal behavior. I have sought to identify an aspect of this systemic odor learning that can be localized within the olfactory bulb (OB). I here present the case for olfactory generalization gradients, in both their nonassociative (cross-habituation) and associative (rewarded) forms, as plastic behavioral measures dependent on learning and attention and regulated substantially by OB neural circuitry. Olfactory generalization gradients are regulated by multiple determinants of learning including the amount of training, CS salience, US reward value, and training-testing latency, as well as by cholinergic and noradrenergic neuromodulator activity within the OB. Whereas all forms of learning evoke progressively sharper generalization gradients, different task determinants and neuromodulators regulate these gradients via qualitatively different transformations that can be investigated more deeply at the neural circuit level with the aid of computational systems modeling.
As OB learning influences the shape and structure of odor representations, it offers advantages for the study of integrated learning systems in the brain that preparations limited to a single learning variable cannot provide. In particular, it enables the study of perceptual learning through a Bayesian perspective as outlined by Barlow (2001), that neural representations should be regarded as "approximate estimates of the probable truths of hypotheses about the current environment" rather than as simple reflections of stimulus properties. Moreover, OB learning offers a powerful model system for integrating across cellular, circuit, and behavioral levels of analysis, an important advantage for the study of early-onset aspects of cortical dementia, including systems-level compensatory responses that can obscure the expression of accumulating cognitive deficits.

Disclosures:

Nanosymposium

124. Neural Correlates of Olfactory Behavior

Location: Room 1B

Time: Sunday, November 14, 2010, 8:00 am - 9:45 am

Program Number: 124.7

Topic: D.01. Chemical Senses

Support: NSF Postdoctoral Fellowship

Title: The genetic and neuronal basis of food preference behavior in Caenorhabditis elegans

Authors: *E. E. GLATER[1], C. I. BARGMANN[2];
[1]The Rockefeller Univ., New York, NY; [2]Howard Hughes Med. Institute, The Rockefeller Univ., New York, NY

Abstract: Understanding the genetic basis of behavior and nervous system function is relevant for understanding inherited human neurological and psychiatric disorders. Although a few of these disorders have been traced to single genes, most are likely to have a complex genetic basis as well as gene-environment interactions. Using model organisms that have genes we can easily manipulate is critical to gaining a better understanding of how genetic polymorphisms contribute to complex phenotypes. Our research focuses on the genetic basis of differences in the food preference behavior between two strains of the nematode *Caenorhabditis elegans. C. elegans* can use olfaction to discriminate among pure volatile chemicals and among different kinds of bacteria, which are its major food source. We have found that strains of *C. elegans* that have been isolated from different locations around the world have distinct bacterial preferences. Specifically, in a bacterial choice assay between *Serratia marcescens*, a pathogenic soil bacteria, and *Escherichia coli* HB101, a common laboratory food source for *C. elegans,* the N2 Bristol strain had a stronger preference for *Serratia* than the CB4856 Hawaii strain did. We have found that this variation in food preference behavior has a complex genetic basis, involving at least five quantitative trait loci (QTL). We have begun to characterize the neural circuit that underlies this food choice and found that one chemosensory neuron, AWC^{on}, is important for discrimination between these two bacterial species. Understanding the genetic basis of this natural variation will provide insights into the mechanisms by which *C. elegans* tunes its responses to different complex stimuli, and more generally, into the evolution of the genome and behavior.

Disclosures: E.E. Glater, None; **C.I. Bargmann**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.1

Topic: D.03. Multisensory

Support: UCLA Faculty Grants Program

UCLA Career Development Award

UCLA Thesis Year Fellowship

Title: One-shot recalibration of auditory space by vision

Authors: *L. SHAMS[1], D. WOZNY[2,3];
[1]UCLA, LOS ANGELES, CA; [2]Biomed. Engin., UCLA, Los angeles, CA; [3]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Background: It is known that human sensory systems continue to adapt to the environment, even in the mature stage of life. For example, after repeated exposure to auditory and visual stimuli with a certain spatial offset, the perceived auditory space gets shifted in the direction of the previously experienced visual stimuli. However, this class of recalibration has been reported only as a result of an extensive amount of exposure (hundreds/thousands of trials) to discrepant stimuli. Purpose: Here we investigated whether recalibration requires a substantial amount of evidence for error to be triggered or whether recalibration can occur even after a single exposure to discrepancy. Methods: Unisensory stimuli (presented at varying locations) and auditory-visual stimuli (with varying degrees of spatial discrepancy) were randomly intermixed across trials. Thus, on bisensory trials, discrepancy randomly changed from trial to trial in both magnitude and direction. Auditory and visual stimuli were noise bursts and white disks on a dark background presented simultaneously for 35ms at one of five spatial locations along azimuth. The locations of auditory and visual stimuli were independent; i.e., could have a discrepancy varying from 0 to 26 degrees. Fifteen trials of each of the 35 stimulus conditions (10 unisensory conditions and 25 bisensory conditions) were presented in a pseudorandom order. The observers (N=146) reported the location of the stimulus by moving a cursor on the screen. The initial location of the cursor was randomized. No feedback was provided. For each unisensory auditory trial, “the auditory aftereffect” was measured as the difference between the response on that trial and the observer’s average response for the given auditory stimulus location. We plotted the “auditory aftereffect” as a function of the AV spatial discrepancy on the previous bisensory trial. Results: The analysis revealed a significant aftereffect that increased in magnitude as a function of previous trial discrepancy. For all non-zero discrepancy conditions, a significant aftereffect was found in the direction of the preceding visual offset. Conclusions: These results suggest that crossmodal sensory recalibration can occur after a single presentation of a brief stimulus lasting just a few milliseconds, and can occur in the absence of feedback or reinforcement. These findings suggest an impressive degree of plasticity in a basic perceptual map induced by a crossmodal error signal. Therefore, it appears that modification of sensory maps does not necessarily require accumulation of substantial amount of evidence of error to be triggered, and is continuously operational.

Disclosures: L. Shams: None. **D. Wozny:** None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.2

Topic: D.02. Auditory

Support: NEI Grant 303-8779

Title: Better late than now: Visual stimuli after sounds shift auditory space in humans and monkeys

Authors: *D. S. PAGES, J. M. GROH;
Duke Univ., DURHAM, NC

Abstract: Visual stimuli affect the perceived location of sounds. It has been assumed that the neural mechanism supporting visual recalibration of perceived sound location involves a simple Hebbian mechanism, where simultaneously presented auditory and visual stimuli excite a common population of neurons and 'wire' the auditory stimulus to a new location. However, an alternative possibility is that visual error after auditory localization could be used to 'update' auditory space via a feedback mechanism. Under this view, what you see after you make an eye movement to a sound would play a critical role in whether/how you adjust your sense of sound location.
Previous studies of the effects of vision on sound localization have allowed for both possibilities, because visual and auditory stimuli have generally been presented simultaneously, potentially permitting Hebbian associations to form, and have also been left on long enough for visual feedback to be provided following any orienting movements to the sounds, permitting plasticity to be guided by visual reinforcement. Prism adaptation experiments such as those conducted in barn owls could involve either or both mechanisms.
In the present study we seek to distinguish between these possibilities - using tasks permitting only one of these mechanisms to operate. In both tasks, we attempted to induce a persistent shift in the reported location of sounds by presenting spatially mismatched visual stimuli. The Hebbian task involved simultaneous but short-duration visual and auditory stimuli. The visual and auditory stimuli were both turned off prior to the completion of a saccadic eye movement to the sound. In contrast, in the feedback task, the visual and auditory stimuli were never on simultaneously. Rather, the sound played first and a visual stimulus was turned on during the saccade to the sound. We tested the impact of the exposure to these two types of mismatched visual-auditory trials on the accuracy of sound localization on interleaved auditory-only trials in human and nonhuman primates.
We found a robust shift in auditory localization in the feedback paradigm, typically about 20% of the 6 degree shift, whereas shifts in the Hebbian/simultaneous task were smaller. Our results indicate that a feedback signal is used for visually-guided auditory plasticity in the human and

nonhuman primate, and that coincident stimuli are not necessary. More broadly, our results show that important and behaviorally relevant interactions between sensory modalities do not require the presence of stimuli that are coincident in time.

Disclosures: D.S. Pages, None; **J.M. Groh**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.3

Topic: D.02. Auditory

Support: Max-Planck Society (NKL, CK, CP)

German Research Foundation (DFG: CK)

Newcastle University Faculty of Medicine (CIP) and MRC (CIP)

Title: A brain region consisting of neurons with moderate sensitivity for voices

Authors: C. PERRODIN[1], C. KAYSER[1], N. K. LOGOTHETIS[1,2], *C. I. PETKOV[3,1];
[1]Dept. Physiol. of Cognitive Processes, Max Planck Inst. for Biol. Cybernetics, Tuebingen, Germany; [2]Imaging Sci. and Biomed. Engin., Univ. of Manchester, Manchester, United Kingdom; [3]Newcastle Univ., Newcastle, United Kingdom

Abstract: A region of 'voice' clusters has recently been identified in the macaque auditory cortex with functional magnetic-resonance imaging (fMRI). These clusters show a strong fMRI activity preference for the voice of conspecifics and appear to functionally correspond to those from the known human voice region. In the *visual* system fMRI has been used to guide electrophysiological recordings from neurons in the monkey brain that were shown to be highly selective for faces [1]. We investigated whether fMRI-guided electrophysiology would reveal comparable levels of selectivity in one of the recently identified monkey voice clusters [2]. During fMRI acquisition and electrophysiological recordings, three categories of 12 sounds were used for stimulation: macaque vocalizations (MVocs), other animal vocalizations (AVocs), and natural sounds (NSnds). The sound categories were comparable in their low-level acoustical features, having been selected for this from a large set of sounds. We first used the stimuli during fMRI, as we have previously done, to identify the clusters with a strong activity preference for MVocs. Then electrophysiological responses to the auditory stimuli were recorded from the

anterior voice cluster in two awake macaques (total of 193 responsive single- and multi-units, from 125 sites). Both monkeys showed moderate neuronal response preferences for MVocs over the other sound categories (respectively, 41% and 29% preference for MVocs in the unit activity of each animal), even if the analysis focused on the focal cluster in each animal with maximal selectivity for MVocs (respectively, 72% and 73% preference for MVocs). Our results suggest that a strong fMRI activity preference need not result from a large proportion of highly selective neurons. This is the case even if methodological differences may have somewhat affected the neuronal selectivity differences observed between our study and the previous macaque work on face processing, which resulted in 96% and 84% selectivity for faces in two animals [1]. In all cases, our results may reflect evolutionary differences that have affected voice and face selectivity. Namely, the visual system appears to have specialized during vertebrate evolution to represent canonical facial features (e.g., two eyes, a nose and a mouth). By contrast, the auditory system could have had less opportunity to specialize, given that many animals modify the acoustics of their vocalizations to be distinct from those of other animals and to circumvent environmental noise.
[1] Tsao, D.Y., et al. (2006) Science 311,670-74.
[2] Petkov, C.I., et al. (2008) Nat Neurosci 11:367-74.

Disclosures: C. Perrodin, None; **C. Kayser**, None; **N.K. Logothetis**, None; **C.I. Petkov**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.4

Topic: D.02. Auditory

Support: Max-Planck Society (CP, CK, NKL)

German Research Foundation (DFG: CK)

Newcastle University Faculty of Medicine (CIP)

MRC (CIP)

Title: Visual influences on voice-sensitive neurons

Authors: *C. PERRODIN[1], C. KAYSER[1], N. K. LOGOTHETIS[1,2], C. I. PETKOV[1,3];
[1]Physiol. of Cognitive Processes, Max Planck Inst. For Biol. Cybernetics, Tuebingen, Germany;

[2]Div. of Imaging Sci. and Biomed. Engineering, Univ. of Manchester, Manchester, United Kingdom; [3]Inst. of Neuroscience, Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Many animals depend upon vocal and facial communication signals for survival and social interactions, but it remains unclear how voices and faces are integrated by the brain. Most studies have evaluated the unisensory processing of either vocal or facial information in brain regions thought to be 'voice' or 'face' sensitive. Other studies have described multisensory interactions in the brain for voices and faces, but only for a few brain regions, such as those close to the primary auditory cortex or in the prefrontal cortex. This work aims to address whether the responses of neurons in a voice-sensitive brain region, which was recently identified in monkeys with functional MRI, are influenced by faces.
Extracellular recordings were conducted in two awake rhesus macaques. We targeted the anterior voice-sensitive cluster on the superior temporal plane, which was first localized for each animal with fMRI [please see the linked presentation] and resides ~5 mm anterior to the tonotopically organized field RT. For stimulation we used movies of vocalizing monkeys and humans that were matched in their low-level auditory and visual features. These dynamic face and voice stimuli were presented in auditory only, visual only or audio-visual stimulation conditions. Neuronal responses to the stimuli yielded a total of 318 local-field potential (LFP) sites and 208 single- and multi-units. Significant multisensory interactions were observed in 70% of the LFP sites and in 33% of the single- and multi-unit responses. We observed both suppression and enhancement of the neuronal responses to the audio-visual condition compared to the auditory condition, as previously noted for neurons in other brain regions. Notably, human voices were as efficient in driving the neuronal responses as were the monkey voices and elicited similar audiovisual interactions, questioning the species-specificity of the voice-sensitive regions.
Our results provide evidence for visual influences in what has been characterized as an auditory 'voice' region. This suggests that, rather than conducting strictly unisensory processing, neurons in the voice region (and potentially also the face region) form an integral part of a network engaged in the processing of communication signals from the different sensory modalities.

Disclosures: C. Perrodin, None; **C. Kayser**, None; **N.K. Logothetis**, None; **C.I. Petkov**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.5

Topic: D.03. Multisensory

Support: CRCNS grants R01 NS50942

Korea Research Foundation Grant KRF-2008-356-H00003

Title: Different coding formats in the same primate SC neurons: A visual place code but auditory rate code

Authors: *J. LEE, J. M. GROH;
Ctr. for Cognitive Neurosci., Dept. Psychol. and Neurosci., Dept. Neurobio., Duke Univ., DURHAM, NC

Abstract: In everyday life, perception and behavior are often guided by both visual and auditory stimuli. The superior colliculus is thought to be involved in integrating such signals. Specifically, this structure has been thought to contain neurons with visual and auditory receptive fields in register with one another, a property thought to be important for detecting that a sight and sound are coincident in space.
However, quantitative information on how the SC's auditory receptive fields compare to visual receptive fields is lacking. Furthermore, before signals reach the SC, the auditory system encodes locations in a different format from that used by the visual system, where, neurons have circumscribed receptive fields (a "place code" for stimulus location). In contrast, neurons in the early auditory pathway seem to use a "rate code" for sound location: auditory responses increase with increasing eccentricity of sound locations in the contralateral field in both the IC and auditory cortex (Groh JM et al., 2003; Werner-Reiss U and Groh JM, 2008). If SC neurons use a rate code for sound, then their visual and auditory responses cannot be "in register" in the conventional sense. Accordingly, we sought to determine whether SC auditory responses resemble a rate-code, like their auditory inputs, or a place code, like their visual responses.
We assessed the sensory and saccade-related responses of 180 SC neurons in two monkeys to visual and auditory targets spanning a horizontal range of +/- 24 degrees. We fit each neuron's responses to Gaussian and sigmoid curves. A circumscribed receptive field (place code) should be substantially better fit by a Gaussian than a sigmoid, whereas a monotonic response pattern (rate code) should be equally fit by either a (half) Gaussian or sigmoid function.
We found that most neurons have rate-coded responses to auditory stimuli, even though the same neurons have circumscribed receptive fields to visual stimuli. Across the population, the sigmoid functions were as good as Gaussians at fitting the auditory responses. In contrast, the Gaussians were significantly better than sigmoids in fitting the visual responses. This was true for both sensory and saccade-related activity.
Lastly, we showed that this discrepancy can be reconciled by a read-out algorithm in which both the site and level of activity in the SC contribute to convert the visual and auditory signals into a saccade command (Groh and Sparks, 1992; Groh, 2001; Porter and Groh, 2006). This algorithm can account for systematic difference between the accuracy of visual and auditory saccades. This suggests that different codes in the brain can nevertheless produce appropriate multimodal saccade.

Disclosures: J. Lee, None; **J.M. Groh**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.6

Topic: D.03. Multisensory

Support: NIH Grant EY016178

Title: A normalization model of multisensory integration

Authors: *T. OSHIRO[1], D. E. ANGELAKI[2], G. C. DEANGELIS[1];
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Abstract: A basic question in multisensory integration is: how do neurons combine their multisensory inputs? Many physiological studies have revealed a set of empirical principles that govern how neurons combine multisensory inputs, including the principle of inverse effectiveness and the spatial principle for multisensory enhancement. We propose that a multisensory version of divisive normalization, inspired by the work of Heeger and colleagues, can account for a wide variety of findings on multisensory integration.
Each multisensory neuron in our model receives inputs from two different sensory modalities, and performs a linear weighted sum of those inputs followed by an expansive static nonlinearity. The response of each model neuron is then divided by the summed activity of all other neurons in the pool, including neurons with a wide variety of receptive field locations and tuning properties.
Our normalization model neurons exhibit many of the classic response properties described in the physiology literature, including: 1) the principle of inverse effectiveness, 2) the spatial principle for multisensory enhancement, 3) multisensory suppression in unimodal neurons, 4) super-additive interactions among weak multimodal inputs (e.g., visual and auditory), and 5) additive or sub-additive interactions among weak unimodal inputs (e.g., two visual inputs). In addition, the normalization model naturally accounts for a puzzling finding that we reported for visual-vestibular neurons in area MSTd of macaque monkeys: bimodal responses were well fit by a weighted linear summation of unimodal responses, but the linear weights appeared to change with stimulus reliability (Morgan et al., 2008).
To test for the existence of a multisensory stage of normalization, we recorded from neurons in area MSTd, where visual (optic flow) and vestibular cues to self-motion are integrated. By manipulating visual and vestibular stimulus directions, we tested a key prediction of the model which is analogous to the spatial principle: a non-optimal stimulus for one modality, which is excitatory on its own, should be able to suppress a near-optimal response to the other modality when the cues are combined. Preliminary results from area MSTd confirm this prediction. This

finding is readily explained by normalization at the network level, but cannot be explained by alternative multisensory models that incorporate a sigmoidal static nonlinearity in each neuron and subtractive lateral inhibition among neurons, instead of divisive normalization.
We suggest that normalization may provide a unifying computational framework for understanding multisensory integration in single neurons.

Disclosures: T. Oshiro, None; **D.E. Angelaki**, None; **G.C. DeAngelis**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.7

Topic: D.03. Multisensory

Support: UCLA Faculty Grants Program and Career Development Grant

David and Lucile Packard Foundation

Betty and Gordon Moore Foundation

Title: Bayesian priors are encoded independently from likelihoods in human multisensory perception

Authors: *U. R. BEIERHOLM[1], L. SHAMS[2];
[1]Gatsby Unit, London, United Kingdom; [2]Psychology, UCLA, Los Angeles, CA

Abstract: It has been shown that human combination of crossmodal information is highly consistent with an optimal Bayesian model performing causal inference. These findings have shed light on the computational principles governing crossmodal integration/segregation. In a Bayesian framework, intuitively, priors represent a priori information about the environment, i.e., information available prior to encountering the given stimuli, and are thus not dependent on the current stimuli.
While this interpretation is considered as a defining characteristic of Bayesian computation by many, the Bayes rule per se does not require that priors remain constant despite significant changes in the stimulus, and therefore, the demonstration of Bayes-optimality of a task does not imply the invariance of priors to varying likelihoods.
It has also been noted that performance which is consistent with Bayesian inference may be achieved through other processes that do not involve combining priors and likelihoods. Because

we have a method of estimating priors and likelihoods, we were able to address this question empirically.
We investigated the independence of the priors from the likelihoods by strongly manipulating the presumed likelihoods and examining whether the estimated priors change or remain the same.
Methods: We examined the auditory-visual spatial perception by having observers localize simple auditory and visual stimuli, each presented at one of 5 possible locations along azimuth. Unisensory auditory, and visual trials were intermixed with bisensory trials in which the stimuli were either at the same location or at different locations. 19 observers participated in two sessions that were spaced one week apart. In one session the visual stimuli were high-contrast, and in another session the visual stimulus were low-contrast. All else (including the task and auditory stimuli) were identical between the sessions. The priors and likelihoods of individual observers were estimated for each of the two sessions. Likelihood and prior parameters were compared between the sessions using paired t-tests.
Results: Whereas the difference between the visual likelihoods of the two sessions was substantial and highly statistically significant, no statistically significant change was found in either auditory likelihood or prior parameters. Power analyses revealed a moderate to high experimental power.
Conclusion: The results suggest that the estimated prior probabilities are indeed independent of the immediate input and hence, likelihood. These findings provide evidence that the human nervous system follows Bayesian inference in this perceptual task.

Disclosures: U.R. Beierholm, None; **L. Shams**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.8

Topic: D.03. Multisensory

Support: ERC

SSF

Human Frontier Science Programme

Title: Multisensory integration and the space near the hand: An fMRI study

Authors: G. GENTILE, C. BROZZOLI, V. I. PETKOVA, *H. EHRSSON;

Karolinska Inst. Dept. Neurosci., Stockholm, Sweden

Abstract: In the macaque brain a number of multisensory areas have been described where neurons respond to visual, tactile and bimodal visual-tactile stimulation of the upper limb. A key feature of these neurons are that many of them discharge preferentially to visual stimuli near the hand indicating that they perform multisensory integration in near-personal space. However, little is known about the possible existence of such multisensory areas in the human brain. The purpose of this study was to investigate the neurophysiological basis for multisensory perception of the hand and the space surrounding the hand in humans. In a first 3T-fMRI experiment with 24 participants, we employed tactile, visual and visuotactile stimulation of the right hand in a setup where participants were directly looking at their upper limb. We identified brain regions that were activated by both visual and tactile stimuli as well as areas showing greater activity in the visuotactile condition than in both unimodal conditions, either in an additive or in a superadditive fashion. The bilateral ventral premotor cortex, the cortex lining the left post-central sulcus, the superior parietal gyrus, the parietal operculum and the cerebellum all showed evidence of multimodal convergence and multisensory integration in an additive fashion ($p<0.05$ corrected for multiple comparisons). Superadditive effects were observed in the left posterior parietal cortex, insula and putamen ($p<0.001$ uncorrected). In a second experiment with 18 participants, we employed an event-related fMRI-adaptation paradigm to test the hypothesis that specific groups of neurons in multisensory areas are selective for visual stimulation close to one's own limb. In this second experiment the participants had their right hand placed visibly in front of them or the arm retracted, serving as control. While they maintained central fixation, four types of visual stimuli (a moving ball) were presented: 6 seconds of stimulation close (2 cm) to the hand or far (100 cm) from it, or 3 seconds close to the hand followed by 3 seconds in far space or viceversa. We found active areas in the anterior intraparietal sulcus and in the ventral premotor cortex that exhibited significant BOLD-adaptation only for visual stimulation in near-personal space ($p<0.05$ corrected). Further, a direct contrast between near and far stimuli showed significant in corresponding regions ($p<0.001$ uncorrected). These results are important because they identify areas in the human brain that perform integration of visual and somatosensory signals from one’s own hand and that contain groups of neurons that respond selectively to the space surrounding the body.

Disclosures: G. Gentile, None; **C. Brozzoli**, None; **H. Ehrsson**, None; **V.I. Petkova**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.9

Topic: D.03. Multisensory

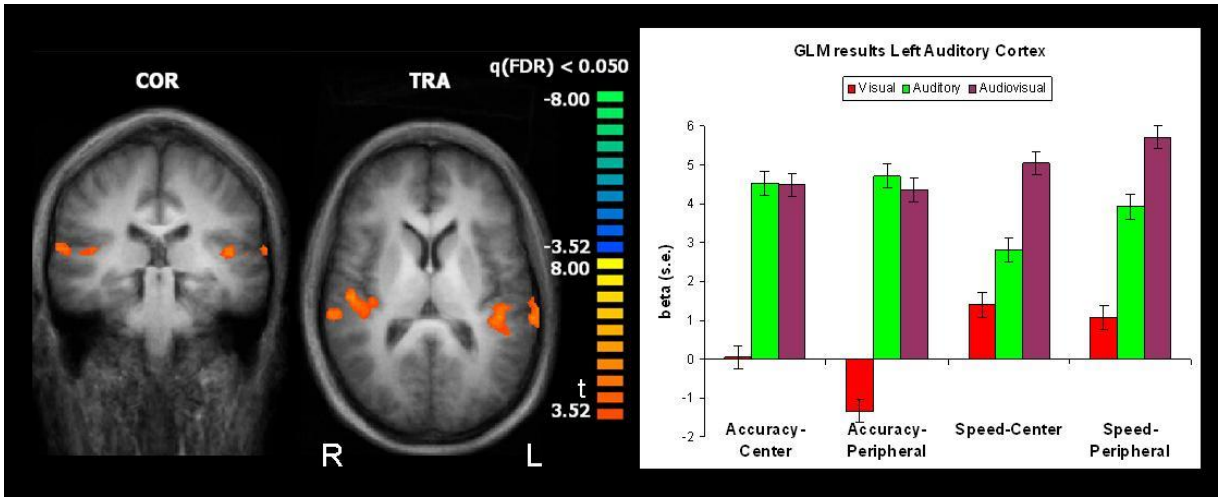
Support: EU FP7-PEOPLE-2007-4-1-IOF Grant # 221187

NWO (Netherlands Organisation for Scientific Research) Grant # 451-07-020

Title: Multisensory integration in auditory cortex depends on behavioral goal

Authors: *N. VANATTEVELDT[1,2], B. S. PETERSON[1], B. VAN DOORN[1,2], B. GUNTER[1], D. SEMANEK[1], C. E. SCHROEDER[1,3];
[1]Psychiatry, Columbia Univ. Col. of Physicians and Surgeons, New York, NY; [2]Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands; [3]Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY

Abstract: The traditional assumption that the sensory systems operate independently of each other until a late processing stage has been challenged by recent demonstrations of direct anatomical connections between early low level sensory cortices in animals. Moreover, recent functional studies show that audiovisual integration can occur in auditory cortex. Using functional magnetic resonance imaging (fMRI) in humans, the present study systematically investigated the conditions promoting early vs. later integration. We hypothesized that integration in low level sensory cortex occurs for peripheral stimuli when integration should be fast.
To address this hypothesis, we manipulated stimulus and task properties in a 3x2x2 design. Stimuli were object images and sounds, presentation of which was manipulated in two ways: unimodal (A, V) vs. bimodal (AV) and central vs. peripheral. Subjects (n = 9) performed a speeded detection task and a categorization task focused on accuracy in different blocks. Scanning was performed on a 3T MRI system (GE Signa) using a Gradient Echo EPI pulse sequence (TR/TE 1250/27ms; 22 4mm slices; in-plane voxelsize 3.5mm^2).
General Linear Model analysis revealed that in bilateral auditory cortex (AC), multisensory integration ($AV > A \cap AV > V$) was exclusively expressed during the speeded detection task and not during the more complex categorization task (fig. 1). Moreover, auditory cortex activation by unimodal visual stimuli was more pronounced during the speeded task. Interestingly, different auditory regions seemed to be specialized in integrating centrally (posterior/lateral AC) or peripherally (anterior/medial AC) presented AV stimuli.
These results support the hypothesis that direct interaction of visual and auditory information in auditory cortex occurs when integration should be fast (speeded task) but not when the goal is accuracy (categorization task). Future analyses will include effective connectivity modeling to further investigate the functional complementary dual pathway hypothesis.



Disclosures: N. Vanatteveldt, None; **B.S. Peterson**, None; **B. van Doorn**, None; **B. Gunter**, None; **D. Semanek**, None; **C.E. Schroeder**, None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.10

Topic: D.03. Multisensory

Support: BMBF Grant 01GW0562

Janggen-Pöhn Stiftung

Jubiläumsstiftung der BLKB

Title: Left fusiform gyrus integrates visual, auditory, and tactile cues of manipulable objects

Authors: *T. KASSUBA[1,2,3], C. KLINGE[2], C. HÖLIG[2], M. MENZ[2], M. PTITO[1,4], B. RÖDER[5], H. R. SIEBNER[1,2,3];
[1]Danish Res. Ctr. for Magnetic Resonance, Copenhagen Univ. Hosp. Hvidovre, Hvidovre, Denmark; [2]NeuroImageNord/Department of Systems Neurosci., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [3]Dept. of Neurol., Christian-Albrechts-University Kiel, Kiel, Germany; [4]Ecole d'Orphométrie, Univ. de Montréal, Quebec, Canada, Montreal, QC, Canada; [5]Biol. Psychology and Neuropsychology, Univ. of Hamburg, Hamburg, Germany

Abstract: Manipulable man-made objects can be identified not only by vision but also by touch or object-related sounds. While previous neuroimaging studies have shown that the dorsal part of the lateral occipital complex (LO) is relevant for visuo-tactile and the posterior superior temporal sulcus (pSTS) for audio-visual integration (Amedi et al. Exp Brain Res 2005), little is known about the functional neuroarchitecture of object-specific audio-tactile or audio-visuo-tactile integration. Here we performed two complementary fMRI experiments in the same group of healthy individuals (n = 18) which were designed to identify brain areas processing object-specific information within all three modalities. In the first experiment, participants were exposed to unimodal auditory, visual, or tactile object or non-object stimuli. They had to respond to the repetition of a stimulus in consecutive trials. In the second experiment, object stimuli were presented simultaneously in two or three modalities, and participants had to respond to incongruent object input from the different modalities. The left fusiform gyrus (FG), LO, and pSTS responded to uni- and crossmodal object input from more than one modality but only the FG displayed a consistent object-related response to stimuli from all three modalities. While the left LO was activated during the processing of visual/tactile (but not auditory) object information, the left pSTS responded to auditory/tactile (but not visual) object-related input. The present results significantly extend previous neuroimaging studies (e.g. Stevenson et al. Exp Brain Res 2009; Naumer et al. Cereb Cortex 2009) by showing that the left FG processes uni- and crossmodal object related information in the visual, auditory and tactile modality. Based on the modality-specific spatial distribution of object-related activity within FG and adjacent inferior temporal gyrus, we put forward the hypothesis that in this part of the ventral stream, object-related information is handled in three functionally interacting but spatially segregated modules. We propose that the trimodal integration within FG and adjacent inferior temporal gyrus is critical to the identification of a specific manipulable object.

Disclosures: T. Kassuba: None. **C. Klinge:** None. **C. Hölig:** None. **M. Menz:** None. **M. Ptito:** None. **B. Röder:** None. **H.R. Siebner:** None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.11

Topic: D.03. Multisensory

Support: Mind Science Foundation grant

Title: A large-scale analysis of synesthesia reveals clustering of subtypes and influence of

grapheme shape on color associations

Authors: *D. M. EAGLEMAN;
Neurosci., Baylor Col. of Med., HOUSTON, TX

Abstract: In synesthesia, normal sensory stimulation triggers an anomalous sensory experience. For example, a sound may not only be heard--but also seen, tasted, or felt as a touch. Synesthesia results from increased crosstalk between sensory areas, but the neural details remain unclear. Our laboratory has designed the Synesthesia Battery (synesthete.org), a free platform for screening and quantifying synesthesia. We have rigorously verified over 12,000 synesthetes across 22 types of synesthesia and 8 languages. In this talk we will discuss some of the most important findings from these data. For example, using factor analysis we have found that synesthesia types factor into at least five clusters that may represent different genetic bases_e.g., a synesthete with colored weekdays is highly likely to have colored months, but no more likely than chance to possess color triggered by smell. The most prevalent cluster, which we term colored sequence synesthesia, is characterized by the triggering of color experiences by ordinal sequences such as letters, numbers, weekdays and months. Multidimensional scaling of the data from over 6,000 rigorously verified colored sequence synesthetes reveals that the colors of letters are not randomly distributed, but instead are colored, at least in part, based on shape similarity to other letters. Combining these psychometric data with our parallel work in neuroimaging and genetics, we will argue that synesthesia is not a single phenomenon, but is instead an umbrella term that covers several different phenomena. All have in common an increased crosstalk between senses, but may be underpinned by different neural and/or genetic bases. We present a new anatomical hypothesis that brings together scattered findings in the literature into a parsimonious framework.

Disclosures: D.M. Eagleman: None.

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.12

Topic: D.03. Multisensory

Support: Frankfurt Institute for Advanced Studies

Barbara Wengeler Foundation

Title: Exploring the experienced unity of consciousness and its binding mechanism by neuroscientific synesthesia research

Authors: *A. MROCZKO[1,2];
[1]Max Planck Inst. For Brain Res., Frankfurt am Main, Germany; [2]Neurophilosophy, Johannes Gutenberg Univ., Mainz, Germany

Abstract: Synesthesia is a neurologically-based condition, in which stimulation of one sensory or cognitive pathway leads to automatic experiences in a second sensory or cognitive pathway. This phenomenon with various experiential levels: perceptual, bodily, emotional, and cognitive, permanently and perceptually bound within a single unified experience, provides remarkable glimpses also into normal brain and mind functioning. However, the explanatory potential of the neuroscientific synesthesia research has not been fully realized in multidisciplinary approaches to consciousness and cognition.
For synesthetes abstract concepts are concrete, e.g. letters have certain personalities, time units or musical notes are colorful, personalities can smell. Such integrated and encompassing conscious experiences transgress the boundaries of diverse mental capacities and settle themselves beyond the perception/cognition dichotomy.
A connected target phenomenon is the synchronic unity of consciousness which refers to the simultaneously appearing and interrelated multimodal conscious contents. Sometimes it has evoked skepticism. Especially in certain pathological states like: split-brain syndrome or dissociative identity disorder, this unity has been claimed to be broken down (Dennett 1992; Gazzaniga 2000). Nevertheless, such an apparent breach affects a failure of one of many different unity *forms* and may demonstrate only one extreme on the continuum of the unity of consciousness. This continuum should be understood as the domain of a qualitative universal ('experiential coherence') - a general phenomenal property, instantiated and differentiated by particular conditions from neuropsychopathology, normal and extraordinary perception, exhibiting different degrees of coherence in unifying selected conscious states.
In such a pluralistic framework for the unity of consciousness, the phenomenon of synesthesia mirrors the other side of the continuum, where conscious experiences seem to be hypercoherent, i.e. more strongly unified than in ordinary situation, especially in the case of projectors (Dixon et al. 2004; Rouw & Scholte 2010). Therefore, synesthesia seems to be one of the best model phenomena to compare the varying distribution of phenomenal coherence between different neuropsychological conditions. Additionally, the phenomenological feature of synaesthesia and the synchronic unity of consciousness seems to be supported by the similar neurophysiological mechanisms: of binding (Singer 2009), focused attention and multimodal feature integration (Treisman 2005) underlying the distinctive functional coherence of both phenomena.

Disclosures:

Nanosymposium

125. Multisensory Interactions

Location: Room 33C

Time: Sunday, November 14, 2010, 8:00 am - 11:15 am

Program Number: 125.13

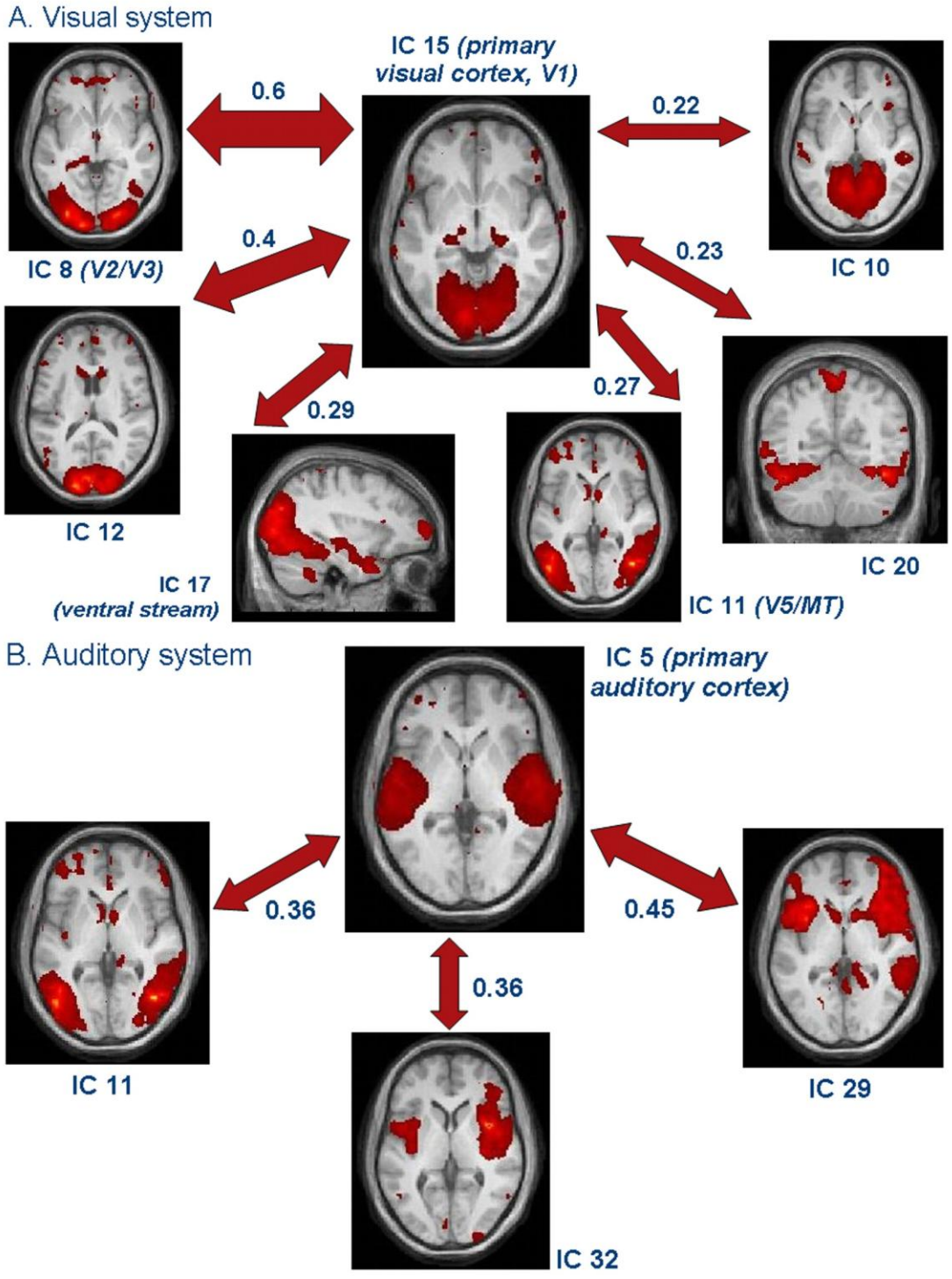
Topic: D.03. Multisensory

Title: Brain networks observed with fMRI during movie presentation

Authors: *A. KONRAD[1], G. VUCUREVIC[2];
[1]Dept. of Psychiatry and Psychotherapy, [2]Inst. of Neuroradiology, Univ. Med. Ctr., Mainz, Germany

Abstract: ***Objective:*** Previous functional magnetic resonance imaging (fMRI) studies mostly used abstracted paradigms to investigate different aspects of human brain function. Though, the application of those particular tasks underlie several restrictions, subjects have to be compliant and without relevant cognitive impairment. Therefore, we decided to investigate human brain function in more natural conditions in subjects which freely viewed a film.
Methods: We investigated 7 healthy subjects which freely viewed a 75 min film of the *Carmen* Opera during fMRI acquisition on a 3 T scanner. Preprocessing steps included realignment, optimization of signal-to-noise ratio, single independent component analysis (ICA) and removal of artifical ICs, normalization with *DARTEL*-template, segmentation of brain surface with binary mask, and smoothing. Group ICA was performed using *GIFT* software to get 40 different ICs and their activation time courses. We then used *ICASSO* software to test the ICs reliability and for the visualization of the ICs clustering. In addition, network interconnectivity was calculated.
Results: ICA provided 40 brain network patterns activated during our paradigm. The primary visual cortex IC showed high functional connectivity with several other higher order visual association networks *(Figure 1, A).* However, we were able to show that less ICs are connected to the primary acoustical cortex IC *(Figure 1, B).* The highest functional connectivity was seen between 5 ICs representing several motoric networks.
Conclusions: We were able to demonstrate a large number of brain networks, in particular the visual and auditory system and their associated information processing networks. We suggest that this method is more suitable for the investigation of cerebral functional connectivity between sensory and higher brain processing networks than resting state fMRI. Moreover, the simple experimental design may be particularly useful in the examination of subjects not compliant to follow *"abstract"* fMRI paradigms like children or neuropsychiatric patients.

Figure 1.
Primary sensory networks and connectivity with association networks



Disclosures: A. Konrad, None; **G. Vucurevic**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.1

Topic: D.04. Vision

Support: NIH Grant R01-EY11001

NIH Grant T32-GM07367

Title: Normalization in a nonlinear circuit model of V1

Authors: *D. B. RUBIN, K. D. MILLER;
Dept. of Neurosci., Columbia Univ., NEW YORK, NY

Abstract: The V1 neuronal population response to multiple stimuli is a sublinear sum (roughly, the average) of the responses to the individual stimuli alone (MacEvoy et al. Nat Neuro 12:637 2009, Busse et al. Neuron 64:931 2009). Similarly, in V4, the response to two stimuli in different portions of the receptive field is roughly the average of the individual responses. These and other nonlinear response properties have been phenomenologically described as "normalization" but may have diverse mechanistic origins. Many are present in the feedforward inputs to cortex and/or can arise through nonlinearities of cells and synapses, rather than circuit properties (Kayser et al. J Neurophys 85:2130 2001, Lauritzen et al. J Neurophys 86:1803 2001, Carandini et al. J Neuro 22:10053 2002, Priebe & Ferster Nat. Neuro. 9:552 2006, Li et al. J Neurophys 96:1755 2006). Nonetheless, cortical circuits may show averaging-like behavior.
Here we present a simple circuit model of this behavior. A ring of excitatory (E) and inhibitory (I) firing-rate neurons have preferred orientations given by their location on the ring. Each cell has an identical thresholded power-law input-output function (the only circuit nonlinearity) and receives inhibition and excitation with identical orientation tuning. Inputs to E and I cells have identical tuning, but E cells receive weaker excitation and stronger inhibition. Surprisingly, this simple circuit closely replicates the results of MacEvoy et al. and Busse et al. The response to two orthogonal identical-contrast gratings is roughly 0.6 times the sum of individual responses; responses shift to "winner take all" with increasingly unequal contrasts; and behavior mimics experiments as orientation difference is varied. Some parameter regimes show supralinear summation at very low contrasts with sublinear addition at higher contrast. Neither lateral inhibition, nonspecific inhibition, nor differences in E vs. I cellular properties are required. Through analysis and simulation we are isolating key requirements for this behavior. It is likely tied to our previous result (Ozeki et al. Neuron 62:578 2009) that surround suppression is "de-amplification" rather than inhibition: a network with strong, destabilizing excitation stabilized by

feedback inhibition produces “balanced amplification” (Murphy and Miller Neuron 61:635 2009); a surround stimulus provides input biased towards inhibitory cells, decreasing amplification. Network nonlinearities may tilt the network toward inhibition with increasing stimulus magnitude, yielding decreased amplification for two inputs vs. one and hence sublinear addition.

Disclosures: D.B. Rubin, None; **K.D. Miller**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.2

Topic: D.04. Vision

Support: NIH Grant R01 - EY11001

NIH Grant T32 - GM07367

Title: Contrast dependence of summation field size and surround properties in a nonlinear circuit model of V1

Authors: *K. D. MILLER, D. B. RUBIN;
Cntr Theoretical Neurosci, Columbia Univ., NEW YORK, NY

Abstract: In many V1 neurons, surround stimuli are neutral or facilitatory at low center contrast but suppressive at high center contrast; and with increasing stimulus contrast, summation fields shrink and length-tuning curves can develop multiple peaks. What mechanisms underlie these contrast-dependent changes?
Recently we argued (Ozeki et al. Neuron 62:578 2009) that surround suppression in V1 is “de-amplification” rather than inhibition - a lowering of the degree of amplification in a network with strong, destabilizing recurrent excitation stabilized by strong feedback inhibition (an “inhibition-stabilized network” or ISN). Additionally, we demonstrated (SFN, 2009) that such a network, along with spatial connectivity typical of cortex, creates excitatory and inhibitory activity that is periodic over retinotopic space, and that this periodicity is necessary to achieve surround suppression of both excitatory and inhibitory neurons. Such periodicity explains the simple-cell-like spatial structure of the combined classical and extra-classical receptive fields that has been observed in response to contrast-modulated sinusoidal gratings (Tanaka and Ohzawa J Neurophys 101:1144 2009), and the periodic length tuning of inhibitory conductances to high-

contrast stimuli (Anderson et al. J Neurosci 21:2104 2001). These results arise because the ISN model produces a network that "resonates" over a narrow range of spatial frequencies (SFs). These studies used a linear model, in which responses scale with contrast. We now introduce a power law input/output nonlinearity in the neurons of this network, identical for excitatory and inhibitory neurons. Dynamic regime (ISN or non-ISN) becomes a contrast-dependent network property: at low contrast, neuronal gain is shallow, so effective connectivity is weak and the network operates in the non-ISN regime. With increasing contrast, the gain of cells strengthens, driving the network to the ISN regime. Along with this transition, there is a transition from zero-SF (spatially flat) activity patterns to increasingly higher-SF patterns. This yields the contrast-dependent shrinking of summation fields, the transition from facilitatory to suppressive surround, and a transition from facilitating or single-peaked length tuning curves to multiple-peaked curves (as also observed with increasing contrast by Anderson et al., 2001, and others). This transition between dynamic regimes may represent a novel mechanism underlying changes in response properties with increasing stimulus magnitude in many cortical circuits.

Disclosures: K.D. Miller, None; **D.B. Rubin**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.3

Topic: D.04. Vision

Support: NIH Grant EY12816

NIH Grant EY18322

Title: Moiré interference of retinal ganglion cell mosaics generates periodic orientation maps in visual cortex

Authors: *S.-B. PAIK[1], D. L. RINGACH[2];
[1]Neurobio., [2]Neurobio. & Psychology, Univ. of California at Los Angeles, Los Angeles, CA

Abstract: The primary visual cortex of higher mammals is organized into columns of cells with similar preference for the orientation of visual stimuli. The preferred orientation changes systematically across the cortical surface, forming an orientation map. Despite extensive study of this cortical structure, it is still unknown how orientation maps develop in the absence of visual experience, what function they play in normal visual processing, and why some species lack

them.
Here we continue with the investigation of the statistical connectivity hypothesis (Ringach 2004, 2007). The basic idea of the model is that orientation tuning in the cortex is constrained by the spatial arrangement of ON- and OFF-center receptive fields originating in the retina and mirrored in the LGN, a notion that goes back to the work of Wässle, Soodak, and collaborators (Wässle et al. 1981; Soodak 1987).
We address a question that was not answered in previous studies: what causes the model to generate orientation maps that are periodic in nature? We show that the periodicity of the map arises from the moiré interference of retinal ganglion cell (RGC) mosaics. This insight opens the door to the underlying explanations of experimental results and derivation of new predictions based on the model. First, the period of the cortical orientation map is shown to depend on the ratio of densities between ON- and OFF-center RGCs and their spatial alignment. Second, at some locations in the visual field, the visual cortex is unable to represent stimuli of a specific orientation, which we refer to as orientation scotomas. Third, there is a parameter regime where orientation tuning in individual cells arises without the emergence of an orientation map; this extends the theory to include species such as rats and mice. Finally, the model provides a lucid and cohesive explanation for several findings in developmental studies, such as the need for both ON and OFF channels in the development of orientation tuning, the restoration of map structure after reverse occlusion, and the emergence of simple cell structure without an intermediate phase of subregion overlap.
References:
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Soodak, R.E., Proc. Natl. Acad. Sci. USA, 84, 3936-3940 (1987).
Wässle, H., Boycott, B.B. & Illing, R.B., Proc. R. Soc. Lond. B. Biol. Sci., 212, 177-195 (1981).

Disclosures: S. Paik, None; **D.L. Ringach**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.4

Topic: D.04. Vision

Support: NIH Grant EY010742

NIH Grant MH063912

NIH Grant NS069464

Kavli Institute for Brain and Mind, Innovative Research Grant

Predoctoral Fellowship, Institute for Neural Computation at the University of California San Diego

Bridge to the Doctorate Fellowship, NSF

Title: Mapping the monosynaptic inputs to a single visual cortical neuron in vivo

Authors: *J. H. MARSHEL[1,2], T. MORI[1], K. J. NIELSEN[1], E. M. CALLAWAY[1,2];
[1]Systems Neurobio. Labs., The Salk Inst. for Biol. Studies, La Jolla, CA; [2]Neurosciences Grad. Program, UCSD, La Jolla, CA

Abstract: Neocortex contains a convoluted web of connections between millions of neurons, confounding attempts to determine the pattern of connectivity between single neurons in the intact brain. Understanding connectivity between neurons at this level of detail is essential to understanding how information is processed by single neurons organized into precisely connected neuronal networks to give rise to both the stereotyped and diverse response properties of neurons throughout the brain. To better understand the mechanisms underlying these computations, it is also necessary to probe each element of the circuit to determine the cause-effect role each element has on the computations the network performs. To open the possibility for studying single neural networks with this level of precision, we developed and validated a novel strategy to genetically target and trace the monosynaptic inputs to a single mammalian neuron both *in vitro* and *in vivo*. The strategy independently targets a postsynaptic neuron and its presynaptic network for specific gene expression and fine-scale cell labeling by using single cell electroporation of plasmid DNA to target infection and monosynaptic retrograde spread of a genetically modifiable rabies virus. The technique is highly reliable, with transsynaptic labeling occurring in every electroporated neuron infected by the virus. This led to an overall *in vivo* single network labeling rate of over one third of single cell electroporation attempts (n = 6/17). Applying this approach to target single primary visual cortical neuronal networks *in vivo*, we found clusters of both spiny and aspiny neurons surrounding the electroporated layer 2/3 neuron in each case, in addition to intricately labeled distal inputs from cortical and subcortical structures. We are presently extending this work by using *in vivo* two-photon calcium imaging to identify the visual receptive fields of neurons within these connected networks. The broad applicability of this technique to probe and manipulate single neuronal networks with single cell resolution *in vivo* may shed new light on fundamental mechanisms underlying circuit development and information processing by neuronal networks throughout the mammalian brain.

Disclosures: J.H. Marshel: None. **T. Mori:** None. **K.J. Nielsen:** None. **E.M. Callaway:** None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.5

Topic: D.04. Vision

Support: NIH grant EY01778

NIH grant HD15052

NIH grant EY014860

Title: Functional role of pulvinar input to primary visual cortex in the primate

Authors: *G. PURUSHOTHAMAN[1], R. MARION[2], S. WALSTON[2], K. LI[2], D. YAMPOLSKY[3], Y. JIANG[2], V. CASAGRANDE[2];
[1]Cell and Developmental Biol., Vanderbilt Univ., NASHVILLE, TN; [2]Vanderbilt Univ., Nashville, TN; [3]Vanderbilt Univ., Nashville, YT

Abstract: Pulvinar is the largest dorsal thalamic nucleus in primates. It has well differentiated sub-nuclei and reciprocal connections with most visual cortical areas. Architectonic subdivisions, cell types, and cortical connections of pulvinar are known in detail but relatively little is known about its function. Pulvinar receives its main drive from layer 5 of the primary visual cortex (V1). It sends an intricate pattern of outputs to supragranular layers of V1 which, in turn, send outputs to extrastriate ventral visual stream. Pulvinar is therefore assumed to modulate the information transmitted from V1 to extrastriate areas but the function of pulvino-V1 signals remains unknown. We studied neural activities in supragranular layers of V1 before and after the inactivation of pulvinar in anesthetized, paralyzed primates (*Otolemur garnettii*, N=2). V1 responses to drifting sinusoidal gratings were recorded using a 100-electrode array implanted in supragranular layers. Muscimol and Dextran were injected into a retinotopically matched region of pulvinar. Tangential sections of CO-stained cortex confirmed the array location to be V1 and electrode tips to be in supragranular layers. Fluorescence microscopy showed some transported Dextran in the region of the array in V1. Before pulvinar inactivation, V1 neurons exhibited a strong phasic response to stimulus onset, as expected. After pulvinar inactivation, this response was significantly attenuated in 98% of all V1 cells studied (N=64). Spike density analysis showed that the average instantaneous spike rate of the phasic response was 2.17 times that of the maintained response before pulvinar inactivation. This ratio decreased to 1.27 after inactivation. Distributions of this ratio before and after pulvinar inactivation were significantly different (Rank-Sum, $P<0.000009$). Because of the low spike rate of individual V1 neurons after pulvinar inactivation, we examined for any dynamic effects of this inactivation using multiunit activities. Multiunit activities (N=73) after pulvinar inactivation showed a biphasic response with

an initial suppressive phase that dipped 389±30 msec after stimulus onset. This suppression was significant in 71% of units (Rank-Sum, P<0.01). This was followed by slow recovery that peaked at 1035±34 msec, significant in 95% of units (Rank-Sum, P<0.01). These results show that pulvinar inactivation profoundly affects activities in the "output" layers of V1 and implicate pulvinar much more strongly than previously thought in visual information processing through a potentially crucial gating role in transmitting information from V1 to the ventral stream.

Disclosures: G. Purushothaman, None; **R. Marion**, None; **S. Walston**, None; **K. Li**, None; **D. Yampolsky**, None; **Y. Jiang**, None; **V. Casagrande**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.6

Topic: D.04. Vision

Support: EU FET 'SECO' grant to KACM

Title: Recurrent excitatory and inhibitory connections in layer 4 of cat visual cortex

Authors: *O. OHANA[1], H. PORTNER[2], D. KUHL[1], K. A. C. MARTIN[3];
[1]Inst. for Mol. and Cell. Cognition, ZMNH, Hamburg, Germany; [2]Inst. of Terrestrial Ecosystems, ETH, Zürich, Switzerland; [3]Inst. of Neuroinformatics, UZH/ETH, Zürich, Switzerland

Abstract: This study characterizes and quantifies synaptic connections within L4 of cat visual cortex in vitro and emphasizes the mechanisms and functional role of their kinetics. Sensory information arriving in layer 4 (L4) of primary visual cortex is immediately transformed at the first synapses, such that L4 neurons possess new properties, such as orientation and direction selectivity and response normalization, that are not present in their thalamic afferents. The numerous synaptic connections between neighboring excitatory and inhibitory L4 neurons are thought to play important roles in shaping neural responses in L4 and enabling the emergence of these sensory transforms. To understand better the properties of these recurrent connections we made visually-guided patch clamp recordings from pairs of L4 neurons in acute slices of cat V1. During recordings the neurons were filled with biocytin and subsequently reconstructed in 3D. We measured from 3 groups of connections; Excitatory-to-excitatory (E-E, n=11), excitatory-to-inhibitory (E-I, n=8) and inhibitory-to-excitatory (I-E, n=9). Analysis of the postsynaptic potentials (PSPs) in response to slow-rate repetitive presynaptic action potentials revealed that all

three connection types were moderately variable and had similar CV (range 0.3-0.45, for all connections) and failure rates. In amplitude the E-I PSPs were significantly larger (amp = 1.54±1.55 mV) than E-E EPSPs (0.38±0.35 mV) or I-E IPSPs (0.6±0.4 mV). We measured the rise and decay kinetics of the PSPs and their latencies and found that E-I connections were evoked with the shortest latencies and fastest kinetics. We used compartmental modeling of PSP propagation in realistic trees of the reconstructed neurons together with light microscopy to provide a plausible explanation to the differences in PSP kinetics, based on the dendritic location of the various PSPs. We simulated L4 by a network of integrate-and-fire neurons and showed that the experimentally observed differences in PSP kinetics and latencies within the recurrent network might profoundly affect the firing rates and patterns of activity within the network.

Disclosures: O. Ohana, None; **H. Portner**, None; **D. Kuhl**, None; **K.A.C. Martin**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.7

Topic: D.04. Vision

Support: NIH Vision Training Grant- # T32 EY0007024

Pew Scholars Program

James S. McDonnell Foundation

Title: Layer-specific correlated variability and local synchronization

Authors: *B. J. HANSEN, V. DRAGOI;
Neurobio. & Anat., Univ. Texas Med. Sch., Houston, TX

Abstract: Despite the fact that strong trial-by-trial correlated fluctuations in response strength have been reported in many cortical areas, recent evidence suggests that neuronal correlations are much lower than previously thought. We revisited this issue by performing laminar recordings in primary visual cortex (V1) to record from all cortical layers simultaneously. Thus, it has long been known that each cortical layer contains a unique pattern of connections with other cortical and subcortical regions. The granular layer (layer 4) receives feedforward thalamic projections. Subsequent, neuronal impulses from granular layer are transmitted to neurons in supragranular layers (layers 2-3) and then to infragranular layers (layers 5-6), which constitute the outputs of

V1. We used laminar probes (16 contacts with a diameter of 25µm and an inter-contact distance of 100µm) to record single-units and local field potentials (LFPs) in V1 from two monkeys during a fixation task. Cortical layers were identified by measuring the evoked-response potentials (ERPs) and computing the current-source density to locate a sink-driven inversion in the amplitude of the ERP. We examined spike count correlations (rsc) and spike-field coherence (SFC) as measures of network coding in V1 in different cortical layers across 19 sessions. Contrary to expectation, we found that correlations between neurons depend strongly on local network context - whereas neurons in the granular layer of V1 showed virtually no correlated variability (rsc-G = 0.04), neurons in the output layers (supra- and infragranular) exhibited strong response correlations (rsc-SG = 0.34, rsc-IG = 0.27). However, despite the lack of response correlations between nearby neurons, cells in the input layer exhibited strong gamma-band synchronization (30-80 Hz) with the local network activity (SFC-G = 0.52). In contrast, neurons in the output layers were only weakly synchronized with their local population (SFC-SG = 0.30, SFC-IG = 0.25). Analysis of lower frequencies, such as alpha (8-14 Hz) and beta (14-27 Hz), failed to yield a significant layer dependency. Our findings indicate that specific cortical layers employ particular coding strategies - incoming stimuli are optimally encoded by the input network and then transferred to output neurons that process information in a context-dependent manner within local recurrent circuits.

Disclosures: B.J. Hansen, None; **V. Dragoi**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.8

Topic: D.04. Vision

Title: The impact of recurrent connections on the spatial organization of neuronal selectivities

Authors: *M. KASCHUBE;
Princeton Univ., PRINCETON, NJ

Abstract: With the advent of two-photon imaging and the development of powerful genetic tools, the mouse visual cortex has become in recent years an important model system for studying cortical plasticity. However, a theoretical framework in which the effects of synaptic plasticity on cortical circuits can be studied in mouse visual cortex has been lacking. Unlike in the visual cortices of primates and carnivores, in which most neuronal selectivities are organized into largely continuous maps, in rodents, most receptive field properties exhibit considerably less

spatial order, often indistinguishable from random spatial distribution. Here, I present a self-organization framework for the collective development and stabilization of neuronal selectivities in large networks of neurons. It is constructed on a phenomenological level using an approach combining bifurcation theory with the theory of random matrices. This framework can account for both types of architectures, continuous maps and the near disordered maps of rodent-type, depending on the structure of recurrent connections. It predicts that on the one hand, any given layout of orientation preferences is consistent with a very large set of different connectivities. On the other hand, two almost identical cortical circuits can generate very different architectures. In the extreme case, a connectivity that generates a near continuous map can, after subtle modifications, give rise to a disordered map of rodent-type. Consistently, solutions of the model show a relatively sharp transition from continuous to disordered maps. This transition occurs when i) decreasing the degree of coherence of recurrent connections or ii) when increasing the degree of sparseness of recurrent connections. I conclude that rodent visual cortex is an excellent model system for studying cortical plasticity on the network level and that due to recurrent connections the relation between connectivity and neuronal selectivities is highly non-linear.

Disclosures:

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.9

Topic: D.04. Vision

Support: NIH Grant R01EY002686 to JHK

Title: Representation of ocular dominance columns in owl monkeys and galagos

Authors: *T. TAKAHATA, J. H. KAAS;
Dept Psychology, Vanderbilt Univ., NASHVILLE, TN

Abstract: Since the first identification by Hubel and Wiesel in 1972, ocular dominance columns (ODCs) have been well characterized in Old World macaque monkeys, and also described in humans. However, it has been thought that this distinct structure is not ubiquitous in species of New World or prosimian primates. Reportedly, ODCs of marmosets disappear along the maturation of the brain. Existence and representation of ODCs vary among individuals in squirrel monkeys. Transport studies failed to reveal segregated eye-dominant inputs in visual cortex in owl monkeys. Most of these classical studies have been performed by using

cytochrome oxidase (CO) histochemistry or anterograde tracer transport methods. In spite of these observations, New World monkeys can discriminate stereoscopic vision, therefore, ODC has been thought to be the outcome of development and have no functional significance in mature animals. Recently, we revealed distinct structures within ODCs in macaques by performing *in situ* hybridization (ISH) histochemistry in the striate cortex (V1) for immediate early-genes, such as *c-fos* and *zif268*, after brief monocular inactivation: supragranular CO patches continued into infragranular layers, and there were "border strips" in the vicinity of boundaries of ODCs. These functional structures were not clear with the classical CO histochemistry or transport studies in the past. Therefore, this method appears to be more sensitive to functional ocular dominance structures in the visual cortex than other classical methods. We attempted ISH after monocular inactivation in owl monkeys (*Aotus trivirgatus*) and galagos (*Otolemur garnetti*), in which the overall representations of ODCs have not been revealed previously. As a result, obvious ODC patterns showed up in all the cortical layers in both species. Presumptive border strip structures were also observed in layer 4 as in macaques. However, there were some differences in the representations of ODCs. Owl monkey ODCs were wider and courser than those of macaques. Besides, the shape was different across layers. Surprisingly, the ODC pattern crossed the V1/V2 boundary and extended into the extrastriate cortices in owl monkeys. The representation of ODCs in galagos was patchy, while those of macaques and owl monkeys were stripes. These results suggested that the existence of ODCs is common in all the primate species and provide clues to study the evolution and functional significance of ODCs.

Disclosures: T. Takahata, None; **J.H. Kaas**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.10

Topic: D.04. Vision

Support: NIH grant EY018119

Title: Plasticity of inhibitory axonal arbors in visual cortex following retinal lesions

Authors: *S. A. MARIK, H. YAMAHACHI, C. D. GILBERT;
The Rockefeller Univ., NEW YORK, NY

Abstract: Cortical circuits in the adult brain are highly dynamic, undergoing continuing synaptic

turnover throughout life. While synaptogenesis and synapse elimination occurs continuously, the functional topography of sensory maps is stable in the absence of alterations of sensory experience. However, perceptual learning and lesions of the central nervous system induce functional changes in cortical neurons. In the visual system, binocular retinal lesions induce functional reorganization in the lesion projection zone (LPZ) and the surrounding cortical areas (the peri-LPZ). We have previously shown that alterations in visual input by retinal lesions, cause axons of excitatory neurons located within the peri-LPZ to undergo a dramatic and dynamic restructuring of their projections into the LPZ. The initial exuberance of axonal growth and the concomitant pruning into the LPZ follows the time course and extent of reorganization of cortical topography, and is therefore a likely substrate of the cortical remapping. While most of the anatomical studies have focused on excitatory neurons, the contribution of inhibitory interneurons is less well characterized. To study the dynamics of inhibitory neurons in the LPZ and peri-LPZ following retinal lesions we combined cell-specific labeling with viral vectors and longitudinal two-photon microscopy. Inhibitory interneurons were labeled with a genetically modified AAV where GFP expression was driven by the GAD65 promoter. Inhibitory interneurons were imaged over a period of several weeks before and after the placement of the retinal lesion. In the absence of a lesion, the axonal structure of inhibitory neurons was stable. Following retinal lesions, however, inhibitory interneurons located within the LPZ underwent rapid and massive axonal growth and restructuring. The newly formed axons extended towards the peri-LPZ, increasing beyond their normal territory. These results indicate that, as with excitatory axons, the axons of inhibitory interneurons undergo dynamic changes following sensory loss and they play an important role in the cortical functional reorganization.

Disclosures: S.A. Marik, None; **H. Yamahachi**, None; **C.D. Gilbert**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.11

Topic: D.04. Vision

Support: A For Women In Science Fellowship from L'Oreal USA and the American Association for the Advancement of Science

NIH F32 EY017766

NIH R01 EY019022

Title: Network mechanisms for sleep-dependent consolidation of plasticity in the visual cortex

Authors: *S. J. ATON, J. SEIBT, M. DUMOULIN, T. COLEMAN, M. G. FRANK;
Univ. Pennsylvania, Philadelphia, PA

Abstract: Ocular dominance plasticity (ODP) in the primary visual cortex (V1) is a canonical form of *in vivo* synaptic remodeling initiated when one eye is deprived of sight (monocular deprivation; MD). Following brief MD, ODP is consolidated, through sleep-dependent mechanisms that mimic long-term potentiation (LTP) of glutamatergic synapses *in vitro*. These sleep-dependent cellular events are associated with enhanced multiunit activity across the visual cortex. It is possible that enhanced multiunit activity reflects increased firing rate and/or synchrony among individual V1 neurons, or changes in spike timing between neurons, all of which could facilitate activation of LTP-like intracellular pathways and functional potentiation of non-deprived eye responses. To clarify the network-level mechanisms involved in sleep-dependent consolidation of ODP, we recorded populations of individual V1 neurons in freely-moving, freely-sleeping animals over a baseline period of *ad lib* sleep and waking, 6 h of waking MD (or waking visual experience without MD), and 6 h subsequent sleep, using custom-made stereotrode arrays. At intervals (following baseline, following MD (or control experience), and following subsequent sleep), visual responses and were assessed in these neurons during presentation of stimuli to the left (non-deprived) and right (deprived) eyes, in order to track the consolidation of ODP.
Our preliminary findings suggest that ODP consolidation during post-MD sleep occurs in regular-spiking (putative glutamatergic) V1 neurons, but does not occur in fast-spiking (putative GABAergic) neurons. Regular-spiking neurons also show enhanced firing rates during the first 2 h of post-MD sleep (relative to baseline), while firing rates are unchanged in fast-spiking neurons. Moreover, synchrony of firing between pairs of regular-spiking and fast-spiking neurons is reduced during post-MD sleep. These data suggest a change in the balance of excitation and inhibition across the cortex during consolidation of ODP. In addition to these changes, our preliminary recordings suggest that patterns of neuronal activation across V1 during waking MD are reiterated during subsequent sleep. Altogether, our data suggest that consolidation of ODP during post-MD sleep is associated with: 1) changes in the balance of activity between putative glutamatergic and GABAergic neuronal populations, and 2) "replay" of network activity patterns associated with prior visual experience.

Disclosures: S.J. Aton, None; **J. Seibt**, None; **M. Dumoulin**, None; **T. Coleman**, None; **M.G. Frank**, None.

Nanosymposium

126. Striate Cortex: Functional Organization and Plasticity

Location: Room 5B

Time: Sunday, November 14, 2010, 8:00 am - 11:00 am

Program Number: 126.12

Topic: D.04. Vision

Support: NIH NEI NIBIB Grant R01EB000843

Title: Functional reorganization of visual cortex with central pathology

Authors: *D. C. REITSMA[1], M. J. MACIEJEWSKI[2], V. SZEDER[2], J. L. ULMER[2], W. M. MUELLER[2], B. F. REMLER[2], E. A. DEYOE[1];
[1]Radiology, [2]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The degree to which cortex reorganizes in response to central pathology remains controversial. Consequently, we used BOLD fMRI and conventional visual field mapping techniques with checkered rings and wedges to study cortical organization in a group of 36 subjects, (60 clinical hemispheres of subjects with widespread pathology and 12 healthy controls), in order to identify potential cases of reorganization. To dissociate reorganization from pathology-related deletions, we focused our analysis on increases in representation, specifically, of the ipsilateral visual field. Patients were divided into two distinct groups: *Group-A) Hemispheres without evidence for reorganization (n = 54).* Retinotopic maps within these hemispheres may have had deletions, yet the pattern of remaining topography appeared normal. *Group-B) Hemispheres with evidence for reorganization (n = 6).* These hemispheres all had increased representation of the ipsilateral field beyond the range of normal subjects, despite pathology-related loss in other portions of the field. A quantitative analysis revealed that for *Group-B* the number of fMRI voxels in each hemisphere representing the ipsilateral visual field averaged 2X the number in healthy controls ($p < 0.001$) and 4X the number in *Group-A* patients ($p < 0.0001$). *Group-B* hemispheres were from 5 different patients with diverse pathologies, including: recurrent stroke, temporal lobectomy, anaplastic astrocytoma, congenital hydrocephaly, and congenital cerebral malformation. Thus, we found no obvious correlation between type of pathology and occurrence of reorganization. Since we chose to focus our analysis on increased representation of the visual field, our results do not exclude other possible reorganizational effects. Cortically, the ipsilateral representation mainly extends away from the vertical meridian representation which itself is associated with transcallosal connections. So, a disruption of inter-hemispheric competition might lead to expansion of the existing ipsilateral representation further away from the vertical meridian. This is consistent with the patterns observed for *Group-B.* However, one subject was congenitally acallosal, perhaps suggesting different mechanisms of reorganization for congenital versus late onset pathologies.

Disclosures: D.C. Reitsma, None; **M.J. Maciejewski**, None; **V. Szeder**, None; **J.L. Ulmer**, None; **W.M. Mueller**, None; **B.F. Remler**, None; **E.A. DeYoe**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.1

Topic: F.01. Human Cognition and Behavior

Title: Reward-driven prioritization of attentional allocation

Authors: *J. LEE, S. SHOMSTEIN;
George Washington Univ., Washington, DC

Abstract: Most of the recent evidence suggests that stimuli that are rewarded strongly attract visual attention and consequently modulate neural activity in the visual cortex. This raises a possibility that reward and attentional systems in the brain are greatly interconnected. However, to date, control mechanisms of attentional and reward systems have mostly been investigated independently, and the nature of this relationship remains poorly understood.
To investigate the neural mechanisms of reward and attention, using event-related fMRI, we employed a variant of the Egly, Driver and Rafal (1994) paradigm complemented with three different monetary reward schedules: (i) reward delivered randomly to either the same- or different-object target; (ii) higher reward delivered to the same-object target; and (iii) higher reward delivered to the different-object target. Since the exact same visual stimuli are presented in all three experiments, any differences in neural activity can only be attributed to the reward manipulation.
It was observed that reward schedule exclusively modulated activation in the early visual areas, as evidenced by differences in the temporal profile of the BOLD response for same- versus different-object target locations. BOLD response was enhanced for the same-object location as compared to the different-object location when reward schedule was biased toward the same-object location (same as the traditional object-based effect). On the contrary, reward schedule biased toward the different-object location reversed the traditional object-based effect, exhibiting enhanced BOLD activation for the different-object location as compared to the same-object location. Moreover, this reward modulation was cue related as well as target related. Behavioral results also supported the reward-based modulation effect, as evidenced by faster RTs for object locations with higher reward, independently of whether such location was in the same- or different-object. Importantly, the magnitude of the object-based effect was not modulated by reward schedule differentially (neither behaviorally nor in BOLD response). These results indicate that reward priority exclusively guides attention, and suggest the possibility that the control mechanisms of reward and attentional systems in the brain could be the same.

Disclosures: J. Lee, None; **S. Shomstein**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.2

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust Grant WT080568MA

Title: The role of visual cortex in resolving response competition

Authors: *T. A. KELLEY, G. REES, N. LAVIE;
Inst. for Cognitive Neurosci., Univ. Col. London, London, United Kingdom

Abstract: We report data establishing the effects of response-competing distractors on the representations of task-relevant stimuli ("targets") in early retinotopic visual cortex. Subjects participated in a functional magnetic resonance imaging study where they were asked to make a speeded choice response, classifying a target object image into one of two categories (e.g. fruits, animals). An irrelevant distractor image that was either congruent (same image as target), incongruent (image from opposite category as target), or neutral (image from task-irrelevant category, e.g. household items) was present on each trial. Target and distractor images were always presented in different quadrants of the visual field, allowing for the separate assessment of the retinotopic visual cortex response to target versus distractor images. The results demonstrated a distractor congruency effect on the visual response to targets. Retinotopic areas V1 and V4 showed an increased response to target images in the presence of incongruent compared to neutral distractors. Moreover, the increased target response in the presence of incongruent (vs. neutral) distractors predicted the magnitude of behavioural interference effects. A larger increase in the retinotopic response to targets was strongly correlated with smaller interference effects of incongreunt (vs. neutral) distractors on the behavioural reaction times to targets. The results suggest a component of distractor conflict resolution in the retinotopic visual cortex response to targets; we discuss the implications to the current models of cognitive control in the face of competing distractors.

Disclosures: T.A. Kelley, None; **G. Rees**, None; **N. Lavie**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.3

Topic: F.01. Human Cognition and Behavior

Support: NIH R01MH087214

Title: The functional significance of spatially global feature-based attention

Authors: *E. F. ESTER[1], J. T. SERENCES[2], E. AWH[1];
[1]Psychology, Univ. of Oregon, Eugene, OR; [2]Dept. of Psychology, UCSD, San Diego, CA

Abstract: Visual selection enables the prioritization of behaviorally relevant information. This selection can occur in myriad ways. For example, one may choose to select stimuli on the basis of spatial location. Alternately, one may choose to select stimuli that contain specific features, such as a particular color or orientation. At the neural level, feature-based attention amplifies the response of a neuron when attention is directed to the neuron's preferred feature, and suppresses the response when attention is directed to the neuron's non-preferred feature. In recent years, several neurophysiological studies in non-human primates and neuroimaging studies in humans have demonstrated that feature-based selection spreads to stimuli outside the focus of spatial attention and to unstimulated regions of the visual field. However, the functional significance of these spatially global effects remain unknown. One possibility is that they reflect the operation of top-down (i.e., volitional) factors that serve to maximize the precision of a perceptual representation by recruiting additional neurons that are tuned to an attended feature. Alternatively, these effects may simply reflect the passive spread of activity across similarly tuned neurons in adjacent visual areas. Here, we use fMRI and multivariate analyses to distinguish between these possibilities. On each trial of our task, subjects were instructed to monitor the orientation (0-160° in 20° increments) or color (green or yellow) of a grating presented in the left or right visual field. Within each condition, we generated orientation voxel-based tuning functions (VTFs; Serences et al., 2009) using responses observed in cortical ROIs contralateral and ipsilateral to the spatial location of the monitored stimulus. When orientation was monitored, robust orientation VTFs could be generated in ROIs both contralateral and ipsilateral to the stimulus. However, when color was monitored, robust orientation VTFs could only be generated in regions contralateral to the stimulus. These findings suggest that the spatially global spread of feature-based attention is due to the operation of top-down factors, rather than the passive spread of feature-specific activity across cortical regions.

Disclosures: E.F. Ester, None; **J.T. Serences**, None; **E. Awh**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.4

Topic: F.01. Human Cognition and Behavior

Support: Hamano Life Science Research Foundation

Title: Frontal-to-parietal causal streams along the dorsal frontoparietal network exclusively mediate voluntary covert orienting of attention

Authors: *T. J. OZAKI[1], S. OGAWA[2];
[1]Lab. for Dynamics of Emergent Intelligence, RIKEN Brain Sci. Inst., Wako, Japan; [2]Kansei Fukushi Res. Ctr., Tohoku Fukushi Univ., Sendai, Japan

Abstract: Previous functional magnetic resonance imaging (fMRI) studies with effective connectivity analysis have reported that top-down causal streams along the dorsal frontoparietal network (DFPN) mediate voluntary attentional control in the human brain. However, resting-state fMRI studies with correlation analysis have proposed that the DFPN is also intrinsically configured by functional connectivity during the resting-state even when observers are required to perform no explicit task. This evidence may conflict with the findings from effective connectivity studies as stated above. In order to resolve this controversy, we performed an effective connectivity analysis based on partial Granger causality (pGC), which can factor out external and implicit influences from unmeasured variables, on an event-related fMRI data during a Posner's cueing paradigm with optimized experimental and fMRI scanning parameters for pGC analysis. Typical regions along the DFPN with greater activation during voluntary orienting than holding of attention were selected as regions of interest (ROIs). pGC analysis on the fMRI data from the ROIs and graph analysis revealed that top-down causal streams along the DFPN from frontal to parietal or visual regions appeared during voluntary orienting, whereas no causal streams from frontal to parietal regions along the DFPN were identified during other experimental epochs. Additionally, causal streams converging on the right anterior insular cortex appeared during holding and less systematic causal streams during less-attentive states. Our results demonstrated that top-down causal streams from frontal to parietal regions along the DFPN exclusively mediate voluntary covert orienting. The present findings suggest that neural representation of attention in frontal regions would be the top of the hierarchy of the DFPN for embodying voluntary attentional control.

Disclosures: T.J. Ozaki, None; **S. Ogawa**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.5

Topic: F.01. Human Cognition and Behavior

Support: Supported by the NIMH Intramural Research Program

Title: Developmental differences in the functional organization of the visual attention network

Authors: *S. R. FRIEDMAN-HILL[1], M. R. WAGMAN[2], S. E. GEX[2], A. M. SPEER[2], E. LEIBENLUFT[3], D. S. PINE[3], L. G. UNGERLEIDER[2];
[1]Div. of Developmental Translational Res., Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Lab. of Brain & Cognition, [3]Mood & Anxiety Program, Natl. Inst. of Mental Health, NIH, Bethesda, MD

Abstract: A multitude of brain imaging studies has identified a series of frontal and parietal regions commonly activated by visual selective attention tasks in healthy adults. By contrast, only a few studies have investigated the functional organization of "the Attention Network" in typically developing children. To address this question, we studied healthy children, age 8-13 (n=18) and healthy adults, age 22-40 (n=18) while they performed a face discrimination task in the presence of visual distractors of varying salience in a 3T magnet. The task was designed to look for fMRI activation associated with the filtering of irrelevant visual information; the task did not require spatial shifts of attention or response inhibition.
We found that both groups performed faster on easy discrimination trials than on difficult ones. Adults were faster than children on easy discrimination trials, but both groups had similar response times (RT) for difficult trials. RT significantly interacted with distractor salience. For easy discriminations, distractor salience had equivalent effects on RT for children and adults. For difficult discriminations, distractor salience had a larger impact on RT for children than for adults.
For easy discriminations, adults had greater activation than children in the middle occipital gyrus, whereas children had greater activation than adults in a large number of regions, including left dACC, bilateral caudate, right thalamus, right medial frontal gyrus, bilateral cingulate, and left posterior cingulate. Notably, many of the regions activated by the task in children are areas associated with the "default network" in adults, and many were negatively correlated with distractor salience. For difficult discriminations, adults had increased activation in bilateral middle occipital gyrus, bilateral inferior frontal gyrus, right precuneus, right superior parietal lobule, and left inferior parietal lobule. These regions positively correlated with distractor salience and include regions typically recruited by selective attention tasks. In contrast, for difficult discriminations, children activated right pulvinar, right caudate, left dorsolateral ACC, right medial frontal gyrus, right anterior superior frontal gyrus, and midline parietal regions.

Activation of these regions in children was not significantly affected by distractor salience. Understanding the course of typical development of brain regions involved in visual attention is an important baseline against which to measure pathological trajectories in neurodevelopmental disorders, such as ADHD or schizophrenia.

Disclosures: S.R. Friedman-Hill, None; **M.R. Wagman**, None; **S.E. Gex**, None; **A.M. Speer**, None; **E. Leibenluft**, None; **D.S. Pine**, None; **L.G. Ungerleider**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.6

Topic: F.01. Human Cognition and Behavior

Support: NIH/NIDA T90 DA022761

Title: The microgenesis of object-based vis-à-vis space-based visual attention

Authors: *L. H. MOYA[1], S. SHOMSTEIN[2], A. BAGIC[3], M. BEHRMANN[1];
[1]Psychology, Ctr. for the Neural Basis of Cognition (CNBC), Carnegie Mellon Univ., PITTSBURGH, PA; [2]Psychology, George Washington Univ., Washington, DC; [3]Neurol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Visual attention can be understood as the process by which some subset of the visual input is selected for further cognitive processing. One way of processing visual input is to attend selectively to a particular spatial location unbiased by the different features or elements that comprise the input at that location. Another way is to attend to an entire perceptual object in the scene and to process its features and elements preferentially. The former is known as space-based attention (SBA) and the latter as object-based attention (OBA). Recent cognitive neuroscience theories suggest that attention is a basic computational mechanism underlying cognitive processing, in which dynamic networks of oscillating neurons are formed to subserve a cognitive process via critical and differential contribution of oscillation frequency. A key open question is how the dynamic networks of these two forms of attention differ.
The present research utilizes a classic behavioral paradigm for uncovering OBA and SBA cognitive effects as evidenced by reaction time performance differences. At the same time, MEG, EEG and MRI neuroimaging technologies are exploited to map out with millisecond and high spatial resolution how OBA vis-à-vis SBA evolves in time, across the brain, elucidating the frequency characteristics along the way. Analysis of posterior EEG electrode brain signals

suggest that object-based and spatial attention are processed differentially first in the parietal lobes starting at 50 ms post target onset, then more laterally in the parietal-temporal areas and in the occipital lobe starting 75 ms or later suggesting medial to lateral and feedback processing, respectively. Analysis of posterior MEG magnetometer signals further suggests that object-based and spatial attention differences are particularly evident in the lateral parietal-temporal-occipital areas. While frequency has been established in extant literature to have a differential role in attended versus non-attended stimuli, the present results suggest it does not have a differential role between the two forms of attention: OBA and SBA (frequency does not interact with attention type), although its strong role in both forms of attention is strongly evident (main effects of frequency in all cases $p < 0.0001$). The present findings provide temporal data that are compatible with the account that parietal areas modulate more posterior cortical areas during OBA, and, therefore, lay the groundwork for further research into a comprehensive temporal and spatial understanding of the role of the frontal-parietal areas as the source of OBA vis-à-vis SBA and their modulation of more posterior brain regions.

Disclosures: L.H. Moya, None; **S. Shomstein**, None; **A. Bagic**, None; **M. Behrmann**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.7

Topic: F.01. Human Cognition and Behavior

Support: AA018022

AA017168

AA012388

Title: Lower frontoparietal activation predicts compromised conflict processing in alcoholism: An fMRI study

Authors: *T. SCHULTE[1], E. MULLER-OEHRING[2], E. V. SULLIVAN[2], A. PFEFFERBAUM[1];
[1]Neurosci Program Ctr. Hlth. Sci., SRI Intl., MENLO PARK, CA; [2]Dept. of Psychiatry & Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: Impaired inhibitory control functions are considered one of the key features of alcohol

abuse and dependence that are likely related to selective structural and functional brain degradation. We compared neural systems subserving conflict processing in alcoholics and controls using combined functional MRI and a Stroop Match-to-Sample task. The task included cues that either matched or did not match the relevant color feature of an upcoming Stroop stimulus. We presented trials in blocks that either required the same response or different responses to examine the contribution of response repetition to cognitive conflict processing. Groups comprised 18 alcoholics (ALC) and 17 controls (CTL), all right-handed and matched on age. Longer reaction times to incongruent (i.e., the word RED printed in green) than congruent (i.e., the word RED printed in red) Stroop stimuli in both groups indicated conflict from the task-irrelevant word content. Stroop conflict differed as a function of color cueing (match/nonmatch) and response repetition (same/mixed responses) between groups. Behaviorally, alcoholics showed longer reaction times (RTs) than controls to incongruent color nonmatch trials in mixed response blocks indicating difficulty in resolving Stroop conflict. fMRI analyses of incongruent relative to congruent trials showed that controls engaged a frontoparietal network, including anterior cingulate, dorsolateral prefrontal, and parietal cortices, more than ALC. Longer RTs to incongruent than congruent Stroop-nonmatch stimuli, i.e., greater conflict, correlated with lower activation in the left dorsolateral prefrontal cortex and left superior parietal lobe in ALC but not in CTL. By contrast, extrastriate areas and left insula were more activated in ALC than CTL when processing both incongruent and congruent trials. Here, less extrastriate activation in CTL was related to smaller behavioral Stroop conflict. Our results demonstrate that less recruitment of frontoparietal attention systems predicts impaired inhibitory control in alcoholism. This together with the enhanced activation in visual processing areas suggests a disruption of neural systems involved in the processing of bottom-up visual information and top-down attentional control and conflict resolution in alcoholism.

Disclosures: T. Schulte, None; **E. Muller-Oehring**, None; **E.V. Sullivan**, None; **A. Pfefferbaum**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.8

Topic: F.01. Human Cognition and Behavior

Title: Uncertainty and object-based attentional capture

Authors: *S. S. SHOMSTEIN[1], E. WING[3], S. LARSEN[2];
[1]George Washington Univ., WASHINGTON, DC; [2]George Washington Univ., Washington, DC;

[3]Duke Univ., Durham, NC

Abstract: Our recent behavioral investigations suggest that the degree of attentional uncertainty determines whether object representations guide attentional selection. Most of the investigations of this phenomenon, however, have been restricted to top-down attentional guidance. Here, we investigate the role of uncertainty and its relationship to object-based effects in bottom-up attentional guidance (attentional capture). In a set of two fMRI experiments, we investigated the neural mechanism of object-based attentional capture, namely the involvement of IPL and TPJ in a task with high degree of uncertainty (singleton search) and a task with a lower degree of uncertainty (feature search). Participants viewed a central rapid serial visual presentation (RSVP) stream in which a target letter was either defined by a specific color (Experiment 1, contingent capture) or could be one of four random colors (Experiment 2, singleton capture). The RSVP stream was superimposed onto a set of three objects (a cross like configuration). On critical trials, a task-irrelevant color singleton and three neutral distractors appeared in the periphery. On half of the trials the colored singleton appeared on the same object as the central target, and on the different object on the other half. Object-based effects were characterized by the degree to which a task-irrelevant distractor appearing on a same or a different object modulated attentional capture. Capture related activations were observed in IPL and TPJ, while object-based modulations of attentional capture were only observed in TPJ for the task with high degree of uncertainty (singleton capture). Furthermore, with the use of retinotopic mapping, the effects of such object-related attentional capture were examined in the early visual cortex. These results suggest that object-based representations guide bottom-up attentional orienting when the task has high degree of uncertainty. Moreover, these results provide strong evidence that uncertainty is the critical factor that determines whether objects will guide attentional selection, no matter if attention is allocated in a top-down or a bottom-up manner.

Disclosures: S.S. Shomstein, None; **E. Wing**, None; **S. Larsen**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.9

Topic: F.01. Human Cognition and Behavior

Support: NIMH-IRP

Title: Blocking cholinergic muscarinic receptors reduces response selectivity during selective attention in visual processing regions but not in parietal cortex

Authors: *M. L. FUREY[1], E. M. HOFFMAN[1], W. C. DREVETS[2];
[1]NIMH, NIH, BETHESDA, MD; [2]Dept. of Psychiatry, Oklahoma Univ. Sch. of Community Med., Tulsa, OK

Abstract: The cholinergic system modulates stimulus processing and is involved in top-down and bottom-up attention (attn) mechanisms. Here we evaluate the effects of blocking cholinergic muscarinic function on the selectivity of neural responses in ventrotemporal visual processing areas and parietal attn regions during a selective attn task that uses two competing stimuli that differ in salience. Subjects (n=24) participated in two sessions, one after an infusion of placebo (Pl), the other after 4.0 ug/kg of scopolamine (Sc) . BOLD signal was measured (n=12) using a 3T scanner as subjects performed the task. Two stimuli each comprised of superimposed images of faces and houses were shown side-by-side. Subjects performed a matching task while attending to faces (AF) or houses (AH) and were cued to shift between components every 4-7 trials. A control task included face (CF) and house (CH) pictures superimposed on phase-scrambled images. Multiple regression was used to identify responses to task, and ANOVA was used to identify processing biases towards face or house stimuli ($t > 4.5$) across infusion conditions in fusiform cortex (FC), parahippocampus (PH), inferior parietal (IP) and precuneus (PC) areas. Region-mean beta values were extracted for all conditions and analyzed with repeated measures ANOVA and t-tests. In bilateral FC drug X attn effects ($p < 0.05$) were detected, with higher responses during AF under Pl than Sc, and no change during AH. CF responses were higher than CH responses during Pl and Sc ($p < 0.05$). In PH no drug X attn effect was seen (left PH showed a trend ($p < 0.10$) towards decreased BOLD during AH under Sc). BOLD during AH was larger bilaterally than AF during Pl ($p < 0.005$) and Sc ($p < 0.02$). The magnitude of change in BOLD during Sc in left FC during AF was larger than the change in left PH during AH ($p = 0.02$). IP and PC showed larger responses during AH than AF under Pl and Sc with no change across drug conditions ($p > 0.05$) during any task. Reaction time increased under Sc relative to Pl in the AF condition ($p < 0.005$) with no change during AH ($p > 0.20$). Blocking cholinergic muscarinic receptors reduced stimulus-specific responses selectively during the AF condition in ventral visual processing areas during selective attn and not during the control task. No effect of drug on stimulus processing biases was observed in parietal areas. As faces are salient stimuli that automatically gain attn, the selective effects of cholinergic inhibition during the AF condition on task performance and on BOLD response within visual processing areas, with no effect in parietal attn regions, suggests that the cholinergic influence on selective attention is primarily via bottom-up attn mechanisms.

Disclosures: **M.L. Furey**, pending use-patent for scopolamine as an antidepressant, Ownership Interest; **E.M. Hoffman**, None; **W.C. Drevets**, pending use-patent for scopolamine as an antidepressant, Ownership Interest.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.10

Topic: F.01. Human Cognition and Behavior

Support: CIHR (EDR)

Vanier NSERC (TWS)

Title: Manipulations of perceptual load reveal age-related differences in extrastriate push-pull discriminatory signal

Authors: *T. W. SCHMITZ[1], M. L. DIXON[2], A. K. ANDERSON[1], E. DE ROSA[1];
[1]Univ. Toronto, Toronto, ON, Canada; [2]Dept. of Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Under perceptually demanding conditions, young adults are capable of filtering irrelevant information at early stages of attentional selection prior to extrastriate encoding. Healthy older adults, by contrast, exhibit impairments in early selection. In this study, we tested the hypothesis that these age differences in perceptual attention originate at the level of the extrastriate receptive fields. Prior research has shown that increases in the perceptual load of a target task yields concomitant increases in both target enhancement and distractor suppression in the extrastriate receptive fields, a pattern characterized as an interdependent push-pull mechanism. Based on this work, we deduced that an impoverished push-pull discriminatory signal originating from the extrastriate receptive fields might underlie age-related declines in perceptual attention.

To interrogate these hypotheses, we acquired fMRI while a group of healthy cognitively-intact young (avg. 21 years) and older (avg. 75 years) adults viewed superimposed face/house stimuli. Participants made sex judgments for each face, rendering houses perceptible but task-irrelevant. We manipulated perceptual load by thresholding houses at either 25% opacity (low salience) or 65% (high salience) relative to the faces. Stimuli in each condition were presented 2 times in a randomized event-related design. Our design therefore enabled us to examine, under varying conditions of perceptual load, age-related differences in attentional modulation of face repetition in the fusiform face area (FFA) as well as the neural fate of unattended house repetition in the parahippocampal place area (PPA).

In young adults, high perceptual load (65%) yielded significant FFA adaptation to repeated faces, while PPA adaptation to unattended repeated places was non-significant (despite place opacity exceeding faces). At low perceptual load (25%), the respective FFA and PPA indices of face enhancement and house suppression were less pronounced, consistent with a load-sensitive push-pull mechanism. In older adults, by contrast, the 65% condition failed to yield significant FFA adaptation to repeated faces, whereas significant PPA adaptation to unattended places was observed in the PPA. Reduced target enhancement in older adults was therefore accompanied by reduced distractor suppression. Direct age-comparisons of FFA effects revealed significantly

larger magnitudes of adaptation in young adults at high load. Collectively, our data suggest that reduced discriminatory signal originating from receptive field push-pull mechanisms underlie age-related declines in perceptual attention.

Disclosures: T.W. Schmitz, None; **M.L. Dixon**, None; **A.K. Anderson**, None; **E. De Rosa**, None.

Nanosymposium

127. Visual Attention

Location: Room 7B

Time: Sunday, November 14, 2010, 8:00 am - 10:45 am

Program Number: 127.11

Topic: F.01. Human Cognition and Behavior

Support: Brainsync FP7, 200728

Wellcome Trust

Title: Thetaburst TMS stimulation over right FEF leads to reduced BOLD signal locally but enhanced activation in ventral attention plus default networks, affecting both maintenance and shifts of spatial attention

Authors: *K. HEINEN[1], E. FEREDOES[1], C. RUFF[2], J. BROOKS[1], J. DRIVER[1];
[1]Inst. of Cognitive Neurosci., London, United Kingdom; [2]Lab. for Social and Neural Systems Res., Zurich, Switzerland

Abstract: Selection of relevant information can require not only voluntary maintenance of attention but also the ability to shift attention to unexpected stimuli. Distinct dorsal and ventral fronto-parietal networks have been associated with these different aspects of attention (Corbetta et al., 2002), leading to discussions of how they might be coordinated (Fox et al.2005), and how they might relate to other extended networks such as the 'default' system (Fox&Raichle, 2007). The current study investigated such networks in a paradigm requiring both maintenance and shifts of visual attention. We specifically tested the impact of targeting one of the main nodes in the dorsal attention network, right frontal eyefields (rFEF), with continuous thetaburst trancranial magnetic stimulation (cTBS; Huang et al, 2005) that is thought to reduce excitability of the targeted site. Combining cTBS with fMRI provides a new approach for studying interactions between extended brain networks.
During scanning, 12 participants underwent a cued covert visuospatial attention task, judging orientation for gratings on one side in a bilateral stream. After 4-8 stimuli in succession, a new

cue instructed participants whether to maintain covert attention on the same side or shift to the other. After several blocks of this task, cTBS or sham TBS was applied over the rFEF, with 600 pulses in total. This was followed by further task-blocks in the scanner.
We found reduced visual performance after real rFEF cTBS versus sham, particularly for trials when a rightward attention shift was required. Comparing pre- and post-cTBS, fMRI data revealed the expected reduction in BOLD signal for the rFEF. This coincided with an apparently reciprocal increase in BOLD signal for areas in the ventral attention network (inferior frontal gyrus and temporo-parietal junction), and in the proposed default network (superior medial gyrus and cunei).
These data not only provide new causal evidence supporting a key-role for the rFEF in direction of visuospatial attention, but also indicate some reciprocal interactions between the different networks we studied.

Disclosures: K. Heinen, None; **E. Feredoes**, None; **C. Ruff**, None; **J. Brooks**, None; **J. Driver**, None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.1

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01 NS033576

NIH Grant F32 HD056767

Title: Cortical systems for early semantic processing in word recognition

Authors: *W. W. GRAVES, S. BAILLET, R. DESAI, J. R. BINDER;
Neurol., Med. Coll Wisconsin, MILWAUKEE, WI

Abstract: Much has been discovered about the spatial distribution of neural systems for word recognition on the one hand, and the time course of word recognition on the other. Considerably less is known about how word recognition unfolds in the brain over time and space. We used magnetoencephalography (MEG) to examine the spatial distribution of neural systems for word recognition as a function of time. Word stimuli were of either high or low frequency, and rated as having either high or low imageability. We hypothesized that areas engaged in semantic (word meaning) processing would show activation for high compared to low levels of word frequency

and imageability. Participants (N = 10, mean age = 26, all right-handed, native English speakers) were asked to indicate that a letter string was an English word by tapping with the index finger of one hand, or that it was not by tapping with the index finger of the other hand. To ensure similarity in terms of orthographic and phonological characteristics, the 312 pronounceable nonwords (pseudowords) were matched to the 312 words in terms of number of letters and did not differ reliably in terms of bigram and trigram frequency or orthographic neighborhood size. Similarly, across levels of word frequency and imageability, words did not reliably differ in terms of number of letters, bigram and trigram frequency, orthographic neighborhood size, or spelling-sound consistency. Stimuli were displayed for 400 ms and replaced with fixation. The mean response time (RT) for words (827 ms) was significantly faster than for pseudowords (897 ms, $t = 11.9$, $p < 0.001$). Differences in terms of RTs and error rates between levels of word frequency, imageability, and their interaction also followed the typical pattern: a significant main effect of faster RTs for high compared to low levels of frequency and imageability, and a significant interaction, i.e. a greater effect of imageability for low frequency words and a greater effect of frequency for low imageability words. Neuronal responses to words occurred in three waves of posterior to anterior activity, each centered at about 170, 220, and 310 ms. Differences across word conditions occurred primarily in the left angular gyrus (AG), an area identified in a recent meta-analysis as a major component of the lexical semantic system. The greatest difference in this area occurred in an early time window of 110-150 ms, with greater activity for words of high frequency and high imageability compared to those of low frequency and low imageability. Although preliminary, these results suggest that semantic processing begins early in the course of word recognition, and that the left AG is prominently involved in this process.

Disclosures: W.W. Graves, None; **S. Baillet**, None; **R. Desai**, None; **J.R. Binder**, None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.2

Topic: F.01. Human Cognition and Behavior

Support: MRC Project U.1055.04.014.00001.01

Title: Rapid cortical plasticity underlying novel word learning in the human brain

Authors: *Y. Y. SHTYROV[1], V. NIKULIN[2], F. PULVERMULLER[1];
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Abstract: Humans are unique in developing large lexicons as their communication tool. To achieve this, they are able to learn new words rapidly, sometimes after just a few exposures to a novel word. However, neural bases of this so-called fast mapping are not yet understood. To address this, we exposed our subjects to familiar words and novel spoken stimuli in a short passive perceptual learning session and compared automatic ERP brain responses to these items throughout the learning exposure. Initially, we found enhanced activity for known words, indexing the ignition of their underlying memory traces. However, just after 14 minutes of learning exposure, the novel items exhibited a significant increase in response magnitude matching in size with that to real words. This activation increase reflects rapid mapping of new word forms onto the lexicon. Similar to familiar words, the neural activity subserving rapid learning of new word forms was generated in the left-perisylvian language cortex, especially anterior superior-temporal areas, as suggested by distributed source analysis of the ERP data. This first report of a neural correlate of rapid word learning demonstrates that our brain is capable of forming new neuronal circuits for linguistic events "on the fly" as it gets exposed to novel patterns of human speech. Understanding such fast learning is key to the neurobiological explanation of the human language faculty, as only humans are capable of acquiring large word vocabularies rapidly.

Disclosures: Y.Y. Shtyrov, None; **V. Nikulin**, None; **F. Pulvermuller**, None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.3

Topic: F.01. Human Cognition and Behavior

Support: Children's Hospital Boston

Title: What’s the story? An fMRI investigation of fluent reading networks

Authors: *C. F. BENJAMIN[1,1,2], M. LEE[1], R. STEINHORN[1], N. GAAB[1,2];
[1]Children's Hosp. Boston, Boston, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: Fluent readers process written language rapidly and accurately, and comprehend what they read (National Reading Panel, 2000; Wolf & Katzir-Cohen, 2001). They achieve competency in numerous reading subskills, including ‘phonological and orthographic. processes’ (Katzir et al., 2006, p54). FMRI has clarified the structures supporting these skills (Schlaggar &

McCandliss, 2007), but not their relative engagement as reading fluency varies. We hypothesized that manipulating the fluent reading network through altering reading speed would increase cognitive demands and decrease activation in higher-order network components. To this end we assessed 13 right-handed adults (12 female; mean age 24.05 [4.48] yrs) with a novel fMRI design. Participants completed two reading tasks at individually determined slow, comfortable and fast speeds. In the *fluent sentence reading* task, words constituting a sentence accrued sequentially on a screen and subjects were asked to select an image that best illustrated the meaning of the sentence from distracters. In the *letter reading* task, matched groups of identical letters and a single target were similarly presented, and subjects were asked to identify the target. Letter stimuli were matched to the sentences in overall number and letter grouping. Null periods were presented between trials.
Accuracy for *fluent sentence reading* and *letter reading* tasks exceeded 95%. The three *fluent sentence reading* contrasts (each speed > null) were associated with bilateral occipito-fusiform, left middle temporal and left inferior frontal gyral activation (bilateral in the slow condition). As speed increased, so did activity in the occipital cortex and the visual word-form area (fusiform gyrus; left > right) (contrast: fast > slow fluent sentence reading). In contrast, *letter reading* tasks (tasks > null) engaged lateral occipital cortex bilaterally, along with fronto-parietal, subcortical and frontal regions at all three speeds. Conversely, in *letter reading* increasing presentation speed elevated superior parietal and supramarginal activation. These data suggest that when reading speed is increased, components of the fluent reading network respond differently from both one another, and from speed-related changes in a more basic letter reading task. Notably, in healthy participants lower-order reading regions (e.g., fusiform gyrus) may increase their activity while higher order areas (e.g., the IFG) do not. These results have important implications when examining neurofunctional changes in dyslexia, which we are investigating in a follow-up study.

Disclosures: C.F. Benjamin: None. **M. Lee:** Employment; Children's Hospital Boston. **R. Steinhorn:** None. **N. Gaab:** None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.4

Topic: F.01. Human Cognition and Behavior

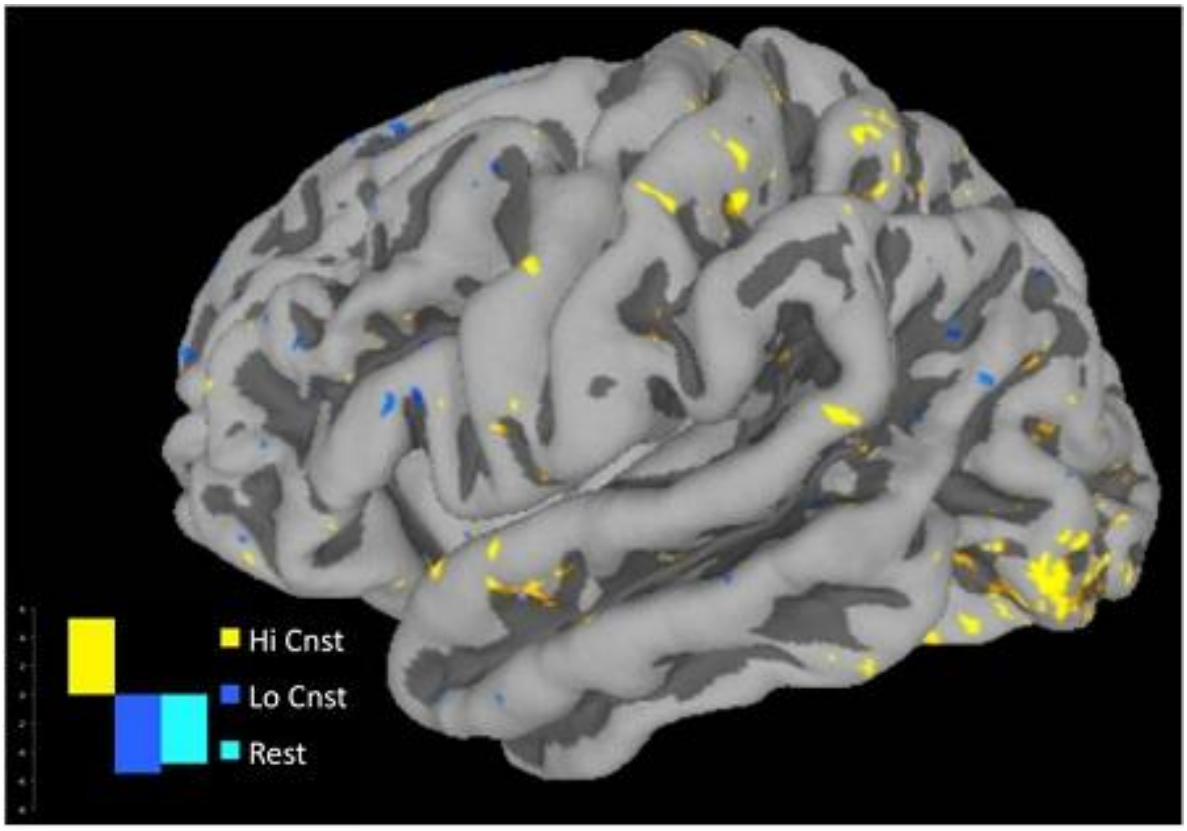
Support: NIH Grant 2 P01 HD040605

JSMF 737100401

Title: Neural correlates of reading high versus low phonologically consistent words

Authors: *A. C. RAJA BEHARELLE[1,2], E. H. MOK[2], E. ZINCHENKO[3,2], A. R. MCINTOSH[1], S. L. SMALL[2,3];
[1]Rotman Res. Inst., Toronto, ON, Canada; [2]Dept. of Neurol., [3]Dept. of Psychology, Univ. of Chicago, Chicago, IL

Abstract: The dual route cascade model of reading postulates two distinct, but interactive routes for recognizing and reading aloud words. The *indirect route* involves mapping a visual word to its auditory counterpart before accessing its meaning. Words that have consistent spelling to sound mappings (or high phonological consistency, e.g. "mint") tend to be read using the indirect route. In the *direct route,* the visual form is linked to its meaning directly. Words with inconsistent spelling to sound mappings (or low phonological consistency, e.g. "pint") are difficult to sound out and thus elicit more direct route involvement. Neuroimaging research has related parts of this theoretical framework to human neuroanatomy, postulating different neural pathways for the two routes. In particular, the visual word form area (VWFA) is argued to be active in direct route processing. However, it is unclear how these pathways interact, especially when processing words with varying phonological consistency. We investigated the dual route model by comparing words with high and low phonological consistency using a multivariate method that emphasizes the interdependencies among neural regions (partial least squares; PLS). In the fMRI scanner, 16 subjects were instructed to read covertly words presented in an event-related design. Subjects were asked to indicate whether an occasional picture or auditory word matched the previous stimulus. PLS was used to analyze which overall brain activity patterns differentiated high and low consistency words, and rest. Activity was thresholded based on a bootstrap estimated 99% confidence interval.
Several clusters were found to relate reliably to high consistency compared to low consistency words and rest including: left sup. temp. gyrus, inf. occip. gyrus and sulcus, mid. occip. gyrus, and fusiform gyrus, including the visual word form area (VWFA). Our finding suggests that involvement of the VWFA is not unique to direct route processing. When examined in the context of neural activity in the rest of the brain, VWFA shows some sensitivity to phonological processing.



Disclosures: A.C. Raja Beharelle, None; **E.H. Mok**, None; **E. Zinchenko**, None; **A.R. McIntosh**, None; **S.L. Small**, None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.5

Topic: F.01. Human Cognition and Behavior

Support: NIH K02 NS0534425

NIH R01HD057076

NIH NS61144

Title: Direct comparison of reading and matching tasks reveals task contingent stimulus effects in the reading pathway

Authors: *A. C. VOGEL[1], F. M. MIEZIN[2,3], S. E. PETERSEN[2,3,4,5], B. L. SCHLAGGAR[2,6,3,4]; [1]Dept. of Neurol., Washington Univ., SAINT LOUIS, MO; [2]Neurol., [3]Radiology, [4]Anat. and Neurobio., [5]Psychology, [6]Pediatrics, Washington Univ., Saint Louis, MO

Abstract: While there are many reasons to use functional MRI to study reading, imaging subjects when they are reading words aloud can be difficult. Therefore, many groups have opted to use surrogate tasks such as visual matching, rhyme matching, or spelling comparisons to eliminate the need for spoken output. Use of these tasks has been justified by the presumption that there is "automatic activation" of reading pathways when a word is presented, evidenced by the stroop effect and early imaging studies (i.e. Price et al., 1996, Cohen et al., 2000). We have tested the efficacy of using a non-reading task for studying "reading effects" by directly comparing BOLD activity in subjects performing a visual matching task and an item naming task on words, nonwords containing all legal letter combinations, and nonwords containing illegal letter combinations. When the matching and naming tasks are compared directly, there is significantly more activity during the naming task in "reading regions" such as the inferior frontal gyrus (IFG) and supramarginal gyrus. The only regions that showing more activity for the matching task are finger motor cortex and a single occipital region. More importantly, there are differing effects of lexicality in the naming and matching tasks. A whole-brain task (matching vs naming) by string type (word vs legal nonword vs illegal nonword) by timecourse (as 9 timepoints) analysis identifies a small set of regions, including the left IFG and left angular gyrus (AG). In the vast majority of these interaction regions (including the left IFG and left AG), there is a string type by timecourse interaction in the naming task but not the matching task. There are no regions that show an effect of string type in the matching task but not the naming task. Further, there are no differences in visual processing regions between the matching and naming tasks in this analysis. Thus, while some neural effects are consistent between reading aloud and a word-based button press task, many (including lexicality effects in regions commonly related to reading) are not consistent between the two tasks. These results argue that specific activation of the reading pathway is contingent on task (and is thus non-automatic), and this notion should be taken into consideration when designing studies intended to investigate reading.

Disclosures: A.C. Vogel, None; **S.E. Petersen**, None; **B.L. Schlaggar**, None; **F.M. Miezin**, None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.6

Topic: F.01. Human Cognition and Behavior

Support: NSERC

Canada Research Chairs (Newman)

Title: Missing-letter effect: Correlational analysis of brain activations (fMRI) reveals that reading when reading while searching for target letters is like reading

Authors: *A. J. NEWMAN[1,3], S. KENNY[3], J. SAINT-AUBIN[3], R. M. KLEIN[2];
[1]Psychology & Psychiatry, [2]Psychology, Dalhousie Univ., Halifax, NS, Canada; [3]Psychology, Univ. de Moncton, Moncton, NB, Canada

Abstract: One approach to revealing the nature of cognitive processes related to reading uses a dual task in which participants are instructed to look for a target letter while reading a text for comprehension (Smith & Pattison, 1982). This paradigm yields an index of the attention paid to words while reading, and its usefulness is highlighted by the presence of a missing-letter effect, in which the reader misses the target letter more often when it is embedded in frequent function words than in content words (Saint-Aubin, & Poirier, 1997). According to the Attention Disengagement model (Roy-Charland, Saint-Aubin, Klein, & Lawrence, 2007) the dual task constituents share the same attentional resources, making letter detection a task sensitive to the cognitive processes related to reading. The model assumes that the constituent tasks are independent, and as a result, the detection task only minimally interferes with normal reading processes. While data from eye movement recordings seem to support this assumption (Greenberg, Inhoff, & Weger, 2006; Saint-Aubin & Klein, 2001), doubts remain (Rayner & Pollatsek, 1989).
We used a novel fMRI paradigm to shed light on this question. In separate ~5 minute fMRI runs 15 subjects each performed 3 different tasks: reading sentences, searching nonwords, or reading sentences + searching for a letter (dual task). Data were analyzed by examining correlations between brain areas relevant to reading (including Wernicke's area) and the rest of the brain, and comparing these between task conditions.
Results showed strong correlations between language-related areas of both temporal lobes during both reading tasks, with no difference in these areas between reading alone and reading + searching. Conditions involving searching additionally engaged correlated activity between reading-related brain areas and anterior and posterior midline cortical and cerebellar regions implicated in attentional control. These results suggest that brain activation associated with reading is unaffected by a concurrent letter-search task, and demonstrates the ecological validity of the reading+searching dual task method as a measure of the cognitive processes involved in reading.

Disclosures: A.J. Newman, None; **S. Kenny**, None; **J. Saint-Aubin**, None; **R.M. Klein**, None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.7

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R03-HD050627

NIH Grant R01-EB006385

NIH Grant P41-RR14075

Title: Transient impairment of word processing following eye movements

Authors: *S. TEMEREANCA[1], M. S. HAMALAINEN[2], E. HALGREN[3], E. N. BROWN[4]; [1]Martinos Ctr, Harvard Med. Sch., CHARLESTOWN, MA; [2]Martinos Ctr, Harvard Med. Sch., Charlestown, MA; [3]Univ. of California at San Diego, San Diego, CA; [4]Massachusetts Inst. Technol., Cambridge, MA

Abstract: Active processing in reading requires coordination between frequent eye movements (saccades) and short fixations on the visual target. Yet, the impact of saccades on word processing remains elusive, as most studies have focused on stimulus processing during constant eye fixation. Here, we investigate the effects of saccades on word processing in 7 healthy individuals that perform a one-back word recognition task, using anatomically-constrained magnetoencephalography (MEG) and psychophysical measurements. Saccades were detected in real time based on the electrooculogram and triggered the word appearance at the new fixation with different postsaccadic delays. Word recognition was significantly slower for words presented early vs. late after saccades. Estimated neural responses with the minimum norm solution were attenuated in retinotopic visual areas, and these significant amplitude effects continued at later processing stages specific to word recognition. In addition, in several individuals, there were differences in timing of cortical activation across conditions that emerged in temporal and frontal areas.

In a second set of similarly-designed experiments, we examined whether observed postsaccadic modulation reflects in part a visual effect associated with the rapid image motion during an eye movement. The same subjects read words presented shortly after externally-generated image motion that mimics saccades or later. Analysis reveals a pronounced contribution to suppression from visual effects associated with background motion. Together, these results support an overall transient suppression of word recognition following saccades and further suggest complex postsaccadic effects at different stages of word processing that may alter perception and reading performance.

Disclosures: S. Temereanca, None; **M.S. Hamalainen**, None; **E. Halgren**, None; **E.N. Brown**, None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.8

Topic: F.01. Human Cognition and Behavior

Support: BBSRC

Wellcome Trust

Title: Chronometric TMS shows hemispheric asymmetries in the time course of ventral occipito-temporal processing consistent for both visual words and objects

Authors: *K. J. KAWABATA DUNCAN[1,2], C. J. PRICE[3], J. T. DEVLIN[1,2];
[1]Cognitive, Perceptual & Brain Sci. Res. Dept, [2]Inst. of Cognitive Neurosci., [3]Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom

Abstract: Visual object recognition engages ventral occipito-temporal cortex (vOTC) bilaterally whereas visual word recognition is believed to be primarily left lateralized. Here we used non-invasive cortical stimulation of left and right vOTC to map out the temporal flow of information during visual object and word processing. On each trial, a pair of TMS pulses separated by 40msec was delivered to either left or right vOTC or to a control site (vertex) while participants decided whether a stimulus (a word or picture) represented a living thing (e.g. "cat"). Pulses were delivered at different time points (0/40, 40/80, 80/120, 120/160, or 160/200msec post-stimulus onset).
Stimulation of either left or right vOTC slowed responses to both picture and words while stimulation of the vertex had no significant effects on response times. For both words and pictures, this slowdown first occurred in the 80/120msec time window in the left hemisphere but at 120/160msec in the right. Statistical testing confirmed that the effects of TMS occurred later in the right than left hemisphere for both words and objects. Indeed, there were no significant differences between words and objects either anatomically (left vs. right) or temporally (across the five time windows).
These findings are consistent with neurophysiological recordings in awake monkeys showing that action potentials from the ascending ventral visual stream are seen in posterior

inferotemporal cortex between 60-120msec post-stimulus onset. Here, we further demonstrate that the time course of processing in vOTC differs in the left and right hemisphere and this is replicated with remarkable consistency for both objects and words. Additional work will be necessary to investigate the basis of the temporal differences in left and right vOTC but the similarity of the timing for words and pictures suggests that vOTC is commonly involved in both visual word and object recognition.

Disclosures: K.J. Kawabata Duncan: Research Grant; BBSRC. **C.J. Price:** Wellcome Trust. **J.T. Devlin:** None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.9

Topic: F.01. Human Cognition and Behavior

Support: Dutch Science Dutch Science Foundation (NWO Rubicon 446-08-008)

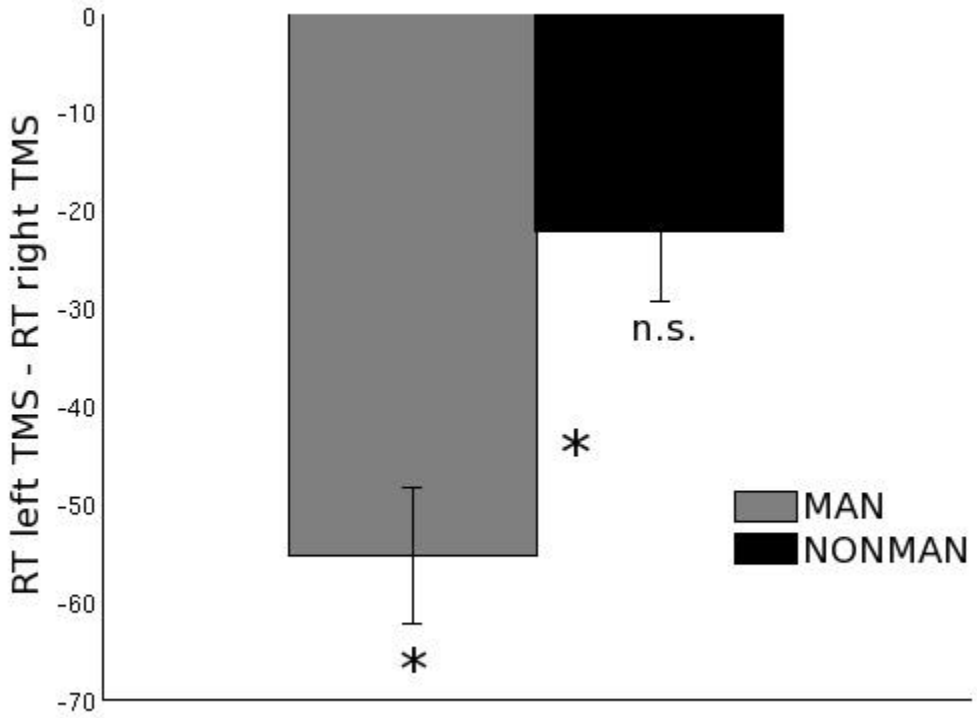
Title: Theta burst TMS over premotor cortex affects language understanding: Implications for the embodiment of concepts

Authors: *R. WILLEMS[1], L. LABRUNA[2], M. D'ESPOSITO[1], R. IVRY[2], D. CASASANTO[3]; [2]Psychology, [1]Helen Wills Neurosci. Inst., BERKELEY, CA; [3]MPI for Psycholinguistic, Nijmegen, Netherlands

Abstract: According to theories of embodied cognition, word meaning is constituted in part by activation in brain areas involved in action and perception. Previous literature has demonstrated activation of sensorimotor cortex during language understanding, but it remains unclear if this activation is functionally relevant for comprehension. Here we show that perturbation of premotor cortex activity through repetitive transcranial magnetic stimulation (rTMS) affects the online processing of action verbs, providing evidence consistent with the hypothesis that motor regions play a functional role in understanding action language.
Right-handed participants (n=12) performed a lexical decision task to manual action verbs ('to throw'), nonmanual and more abstract action verbs ('to earn'), or phonotactically legal pseudowords ('to wunger'). Manual and nonmanual verbs were matched on lexical frequency and word length.
We applied theta burst rTMS over left and right dorsal premotor cortex in two separate sessions. We predicted that rTMS applied over premotor cortex would modulate reaction times more

strongly for manual action verbs than for nonmanual verbs. Moreover, we expected that the strength of this effect would depend on whether rTMS was applied over the left or right premotor (Willems, Hagoort, & Casasanto, 2010).

Results showed a HEMISPHERE x VERB interaction (p=0.028). Responses to manual verbs were faster after stimulation of left premotor cortex than after stimulation of the right premotor cortex (p=0.03) (Fig. 1). This effect was not observed for the nonmanual verbs (|t|<1). There were no other main effects or interactions.
These results show that perturbation of the premotor cortex specifically affects lexical decision reaction times for manual action verb and challenges the skeptical view that premotor activation during linguistic comprehension is epiphenomenal. Rather, changes in premotor cortex activity causes changes in action language processing, demonstrating a functional role for premotor cortex in language understanding.



Disclosures: R. Willems, None; **L. Labruna**, None; **M. D'Esposito**, None; **R. Ivry**, None; **D. Casasanto**, None.

Nanosymposium

128. Reading Studies

Location: Room 24A

Time: Sunday, November 14, 2010, 8:00 am - 10:30 am

Program Number: 128.10

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1K99HD057522-01A1

Ellison Medical Foundation

Title: Investigating domain specificity of language-sensitive cortex using individually defined functional ROIs

Authors: *E. FEDORENKO, N. KANWISHER;
MIT, Cambridge, MA

Abstract: Several brain regions are consistently implicated in language processing. The majority view in the field is that these regions are not specific for (any aspect of) language processing, but instead that each of them is also engaged, to a similar degree, in one or more nonlinguistic functions. We argue that this conclusion is premature, because virtually all published neuroimaging studies of language have used group analyses, which are guaranteed to underestimate the functional specificity present in any individual brain. Here we circumvent that problem by functionally identifying candidate language regions in each subject individually, a method that has revealed striking functional specificity in extrastriate visual cortex. This method enables us to pool data from corresponding functional regions across subjects, rather than from corresponding locations in stereotaxic space, which may differ functionally because of the anatomical variability across subjects.
We developed a functional localizer - based on the contrast between sentences and pronounceable nonwords - that identifies key language-sensitive regions (including the classically implicated left frontal and temporo-parietal regions) in the vast majority of individual subjects. These regions (i) replicate within subjects, (ii) have clear correspondence across subjects, and (iii) possess signatures of high-level linguistic regions (similar response to language stimuli presented visually vs. auditorily). Across several studies, we scanned subjects (n=35) on both the language localizer task and one or more non-linguistic tasks that have been argued to share processing machinery with language, including arithmetic processing, musical processing, general working memory, and cognitive control tasks. Traditional group analyses reveal a picture consistent with the previous literature: many language-sensitive regions respond to a range of non-linguistic tasks. However, preliminary data analyzed with individual-subjects functional ROIs reveal a much higher degree of specificity, with some language-sensitive regions (e.g., left anterior and posterior temporal regions) responding exclusively to linguistic stimuli, and other regions responding only to some of the non-language tasks (e.g., left inferior frontal regions respond to musical processing and some cognitive control tasks, but not to arithmetic processing or general working memory tasks). Although it will be necessary to run more subjects on these and other tasks, it is clear that defining ROIs functionally can reveal greater specificity than was apparent with standard group analysis methods.

Disclosures: E. Fedorenko, None; **N. Kanwisher**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.1

Topic: F.03. Motivation and Emotion

Support: NIH Grant DC 01065

Title: Intravascular food reward

Authors: *A. J. OLIVEIRA-MAIA[1,2], C. D. ROBERTS[2], Q. D. WALKER[3], C. KUHN[3], S. A. SIMON[4], M. A. L. NICOLELIS[5,6];
[1]Champalimaud Neurosci. Program, Inst. Gulbenkian De Ciência, Oeiras, Portugal; [2]Dept. of Neurobio., [3]Dept. of Pharmacol. and Cancer Biol., [4]Departments of Neurobio. and Biomed. Engin. and Ctr. for Neuroengineering, [5]Departments of Neurobiology, Biomed. Engin. and Psychology, and Ctr. for Neuroengineering, Duke Univ. Med. Ctr., Durham, NC; [6]Edmond and Lily Safra Intl. Inst. of Neurosci. of Natal, Natal, Brazil

Abstract: Palatable stimuli, such as sucrose, elicit appetitive behavioral responses and dopamine release in the ventral striatum. We previously found that, in animals where functional sweet-taste transduction is absent, sucrose solutions can nevertheless produce the same behavioral and neurochemical effects. While these effects were shown to be dependent on calorie content, the underlying mechanisms remain unknown. To clarify the contribution of circulating glucose to the taste-independent effects of sucrose, we performed endovenous administration of glucose solutions simultaneous to oral consumption of water in a two-bottle paradigm. Glucose administered in the jugular vein conditioned robust side-biases in this paradigm. However, this only occurred at concentrations that also resulted in peripheral glycemia significantly higher than what was observed when side-biases were conditioned with oral administration of glucose. Furthermore, glucose administered directly in the hepatic portal vein conditioned robust side-biases at much lower concentrations, where peripheral glycemia was similar to that observed with oral glucose. In fact, in anesthetized rats, the changes in glycemia measured in portal vein blood after administration of glucose solutions orally or in the jugular or portal veins were consistent with the behavioral effects described previously. The same was not true for changes in glycemia measured in tail vein blood. Moreover, glucose administered in the portal but not jugular vein increased the relative frequency of spontaneous dopamine release events in the

ventral striatum of anesthetized rats, as measured by voltammetry. We conclude that glycemia levels in the hepatic portal venous system are more relevant for reward-related responses than systemic glycemia.

Disclosures: A.J. Oliveira-Maia, None; **C.D. Roberts**, None; **Q.D. Walker**, None; **C. Kuhn**, None; **S.A. Simon**, None; **M.A.L. Nicolelis**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.2

Topic: F.03. Motivation and Emotion

Support: James S. McDonnell Foundation

Title: Serotonin transporter genotype modulates extinction retention in humans

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Abstract: Prominent theories of the development of anxiety disorders have long considered fear conditioning to be a central underlying mechanism. In a Pavlovian fear conditioning paradigm, a neutral stimulus comes to elicit a fear response after being paired with an aversive stimulus. Extinction refers to the gradual decrease in fear expression that occurs when the conditioned stimulus is presented repeatedly without the aversive reinforcer. Extinguished fear responses may reemerge after a delay, a phenomenon known as spontaneous recovery. The failure to retain extinction learning over time is associated with pathological anxiety. Thus, factors predictive of individual variation in fear recovery may help to clarify what renders a person resilient against or at risk of developing fear-related anxiety disorders.
A recent study reporting that serotonin transporter (5-HTT) knockout mice show elevated levels of spontaneous recovery (Wellman et al., 2007) suggests that genetic differences in 5-HTT expression may similarly modulate human fear learning. In this study, we explore whether two polymorphisms associated with variation in 5-HTT expression are associated with individual differences in the retention of extinction. We used a two-day partial-reinforcement fear conditioning paradigm, in which visual stimuli were paired with mild electric shock. On day one, participants underwent acquisition followed immediately by the initial extinction phase. On day two, a second extinction phase enabled evaluation of whether participants retained initial

extinction learning.
Results show that a polyadenylation polymorphism in the serotonin transporter (rs3813034) is associated with individual variation in extinction retention. Participants carrying a greater number of the allele associated with reduced 5-HTT expression exhibit increased levels spontaneous recovery. Furthermore, this allele is associated with elevated trait anxiety as well as depressive symptoms in a dose-dependent manner. In contrast, the serotonin transporter-linked promoter region polymorphism (5HTTLPR) was not associated with any of these measures. These results identify a behavioral phenotype associated with the rs3813034 polymorphism and suggest that genetically-mediated differences in extinction retention may confer an increased risk of developing anxiety disorders.

Disclosures: C. Hartley, None; **E.A. Phelps**, None; **B.J. Casey**, None; **C.E. Glatt**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.3

Topic: F.03. Motivation and Emotion

Support: James S McDonnell Foundation Award Ref No. R7270-1

Title: Place your bet: The neural correlates of risk-sensitive decision-making

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Abstract: Economic models of decision-making propose that humans integrate the information about the magnitude and probabilities of the outcomes, in order to select the option with the highest utility. In this study we used functional magnetic resonance imaging to investigate the brain network supporting the selection of risky options in a novel gambling task. We tested how behavioural responses and neural activity were differentially affected by a) the requirement to make an active choice, b) the odds of winning and c) the bet magnitude.
Healthy university students (n=18) completed the Roulette Betting Task. Two trial types were contrasted: “active-choice trials”, in which the subject was required to select a bet, and fixed-bet “no-choice trials” (matched for monetary gain). Odds of winning and bet magnitude were manipulated across both trial types. Functional images were acquired (3-T, TR= 2s) using a standard echo-planar imaging sequence with an event-related design. Data analysis was

conducted using Statistical Parametric Mapping (v5) software, with a canonical hemodynamic response function modeled to the onsets of the selection phase. Whole-brain analyses were thresholded at $p<.001$ uncorrected.
Behaviour was sensitive to the choice requirement: participants were faster to select their bet on active-choice trials than on no-choice trials. Participants were also faster on trials with higher winning chances, showing behavioural sensitivity to the odds of winning. On active-choice trials, participants adjusted their bets to the odds of winning, placing higher bets on trials with higher chances of winning. Active choice trials were associated with an increased activation of the anterior cingulate cortex (BA 32/24), striatum, midbrain, frontal areas (BA 6 & right BA 45) and parietal regions (BA 7). Brain regions sensitive to the odds of winning were the ventromedial prefrontal cortex (BA 10) and left parietal region (BA 39): higher chances of winning were associated with increased activity in these regions during selection. Neural activity during the selection of a risky option was also affected by the magnitude of bets: High bet trials were associated with a greater neural response in medial prefrontal and frontal areas (BA 10 & BA 9), left anterior cingulate cortex (BA 32) and bilateral parietal regions (BA 39 and BA 40) compared to low bet trials.
The results of this study indicate that during risk-sensitive decision-making the requirement for active choice, the chances of winning and the magnitude of potential wins all differentially modulate neural activity in areas involved in conflict, reward processes, reasoning and manipulation/estimation of quantities.

Disclosures: B. Studer: None. **A. Apergis-Schoute:** None. **T. Robbins:** Consultant/Advisory Board; Cambridge Cognition. **L. Clark:** Cambridge Cognition.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.4

Topic: F.03. Motivation and Emotion

Support: NMRC/STaR/0004/2008

DSTA No. POD0713897

Title: Sleep deprivation alters neural mechanisms for economic evaluation of social rewards

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Abstract: Sleep deprivation (SD) has broad and substantial effects on cognitive processing, typically reducing performance across a range of cognitive tasks. Yet, relatively little is known about how it may alter individuals' preferences among different outcomes; i.e., does SD only impair cognitive abilities, or does it change the very values we ascribe to different rewards? We investigated these questions in experiments that examined anticipation, receipt, and exchanges between monetary and social rewards.

Twenty-two male participants were scanned using functional magnetic resonance imaging (fMRI) after a normal night of sleep (rested wakefulness state, RW) and after 22 hours of SD while performing a modified monetary incentive delay (MID) task. Participants made speeded responses to cues that signal potential monetary or social rewards. Successful responses to reward cues resulted in winnings of $1, $5 or $10 or in the display of a female face rated from 1 to 5 stars. Post-scan, participants performed an economic exchange task, in which they could trade some of their accumulated earnings for opportunities to view more attractive faces. Participants then rated the attractiveness of all viewed faces.

SD elicited variable but robust individual differences in economic preferences - with some participants exhibiting increased social value and others reduced social value - that correlated strongly across participants with their state-related change in attractiveness rating. Hence, a participant who showed a greater tendency to exchange money for attractive faces when SD was more likely to rate faces as more attractive under the same condition. This suggests state-driven changes in the valuation of social or monetary rewards or both.

These SD-related changes in exchange rate were associated with SD-related changes in individuals' fMRI activation to the social stimuli within the ventromedial prefrontal cortex and amygdala, regions previously shown to respond to experienced value of social rewards. Notably, SD did not affect activation in the nucleus accumbens or ventral tegmental area, nor did it affect activation associated with the experienced value of monetary rewards.

Thus, SD has distinct effects on the decision value and experienced value of social rewards, those effects can be observed even in the absence of any effects associated with monetary rewards, and those effects do not require SD-related changes in expected value signals.

Disclosures: C. Libedinsky, None; **P. Namburi**, None; **V. Chen**, None; **C.S. Teng**, None; **M.W.L. Chee**, None; **D. Smith**, None; **S. Huettel**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.5

Topic: F.03. Motivation and Emotion

Support: NIH R01 DA023176

Title: Willingness to wait is associated with neural coupling between dorsolateral prefrontal cortex and episodic imagery network

Authors: *S. LUO[1], G. AINSLIE[5], L. GIRAGOSIAN[1], D. POLLINI[2], J. MONTEROSSO[1,3,4]; [1]Psychology, [2]Econ., [3]Neurosci. Grad. Program, [4]Brain and Creativity Inst., USC, Los Angeles, CA; [5]Coatesville Veterans Affairs Med. Ctr., Coatesville, PA

Abstract: Background: "Delay discounting" can be measured with monetary intertemporal choice tasks in which participants choose between smaller sooner (SS) and larger later (LL) options. When the delay-discounted value of an SS and LL are nearly equal, participants will exhibit variability, choosing the SS on some trials and LL on others. We investigated neural correlates related to intertemporal choice by specifically tracking this variability and relating it to functional Magnetic Resonance Imaging (fMRI) signal.
Method: Thirty-one participants completed an intertemporal monetary choice task. The procedure was individualized so that for each participant, pairs of alternatives varied from those in which the SS was just sufficiently large enough to attract 100% preference, to those in which the LL was just sufficiently large to attract 100% preference. A logit model was used to characterize behavior and the trial-by-trial residual from this model provided an index of intertrial variance in delay discounting.
Results: Controlling for value and reaction time, several regions were more active on trials the LL alternatives was chosen relative to SS, including bilateral dorsolateral prefrontal cortex / frontal pole, the ventromedial prefrontal cortex (vmPFC), left hippocampus / parahippocampal gyrus, right central operculum, right medial/ superior temporal gyrus, posterior cingulate cortex, precuneus / cuneal cortex, and lateral occipital cortex. Among SS (but not LL) choices, residuals tracking within-participant variance further predicted signal in these regions; signal was lowest in these same regions when the SS choice was indicative of unusually steep discounting for the participant, given her overall behavior. A connectivity analysis (psychophysiological interaction (PPI)) in which the hippocampus cluster was used as the seed indicated greater correlation between this region and the activity in left dlPFC and paracingulate gyrus/ superior frontal gyrus when participants chose the LL alternative.
Conclusion: Activity associated with choice of LL substantially overlapped the network linked to episodic imagery (Schacter et al., 2007). The fact that those regions associated with LL choices evidenced lowest signal during the most short-sighted choices is consistent with the hypothesis that they contribute causally to intertrial variance in discounting. Results from the PPI analysis provide support for an emerging model of performance on this task, which holds that one determinant of intertemporal choice involves functional coupling between neural systems related to 1) episodic imagery and 2) cognitive control.

Disclosures: S. Luo, None; **G. Ainslie**, None; **L. Giragosian**, None; **D. Pollini**, None; **J. Monterosso**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.6

Topic: F.03. Motivation and Emotion

Support: CIHR Grant 361-370

Title: Reinforcement learning algorithms predict changes in activity within the superior colliculus in response to changes in saccade value

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Abstract: Both probability and magnitude of reward have previously been shown to influence neural activity associated with selecting and preparing simple motor actions. How these variables are weighted and combined to compute the estimated value of impending motor actions in real time at the neuronal level is unclear. Dopamine-related signals in the midbrain and basal ganglia track reward prediction error and the estimated value of potential actions. We hypothesize that when faced with uncertainty, preparatory and sensory signals in pre-motor regions will also reflect the current estimated value of actions. To address this, we recorded activity from neurons in the intermediate and deep layers of the superior colliculus (SC) in three rhesus monkeys while they performed a simple saccadic task. This task required subjects to look to a visual target that could be presented either within or opposite the neuron's response field relative to a central fixation point on each trial. There was a short warning period of 400 ms in which the fixation point was removed before target onset to facilitate advanced saccadic preparation. Saccade value was manipulated by changing the probability of a target being presented to the left or right and the magnitude of liquid reward associated with each. Approximately every 100 trials, unsignaled block transitions occurred in which saccade value was altered by changing these two variables. SC activity both prior to target onset (preparatory) and activity time-locked to target appearance (sensory) reflected changes in estimated value, as indexed by changes in saccade reaction time (SRT). In particular, we focused on changes in neural activity on a trial by trial basis, both within each value block and during the unsignaled block transitions. Trial by trial changes in both preparatory and sensory activity, as well as changes in SRTs were well described by reinforcement learning algorithms. Together, our findings demonstrate that the estimated value of potential actions is updated based on reinforcement learning rules so that preparatory and sensory processes can be efficiently allocated when faced with uncertainty.

Disclosures: D.M. Milstein, None; **R. Webb**, None; **M.C. Dorris**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.7

Topic: F.03. Motivation and Emotion

Title: The effect of contextual framing on the aesthetic appraisal of visual artworks

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Abstract: An artwork in gallery presented with other semantically related artworks in order to convey a specific theme. The appreciating process of an artwork is modulated by the contextual influence of what was previously presented. However, little is known about how exposed artworks influence on the different aesthetic appraisal to the identical artwork. To address this question, we investigated appreciators' perceptual changes and neural systems supporting this modulation by presenting an artwork after different exhibition contexts while acquiring eye movement of 80 and fMRI data of 47 undergraduates participants. Subjects were asked to appraise the aesthetic value and emotional responses to artworks during appreciation. The exhibition which consisted of 6 artworks was designed to evoke different level of pleasantness during observation of an identical artwork which was presented at last (pleasant or unpleasant). Subjects' aesthetic appraisals and emotional valence were significantly higher when stimuli viewed after pleasant than unpleasant contextual composition. Moreover, we also showed the significant changes in time of gazing specific areas on the artworks where expected to evoke the different pleasantness at the outset, indicating that significant perceptual change in attention was facilitated depends on the context. In pleasant context, the contextual modulation was recruited by activity in brain regions involved in goal directed perceptual changes, hedonic experience processing and affective changes including the striatum, the prefrontal cortex, the medial orbitofrontal cortex and insular, whereas in unpleasant context, the negative appraisal was derived by the sub-cortical and the motor cortex and occipital areas. The association between activities in those brain areas and negative responses in aesthetic judgment were consistently reported by previous neuroaesthetic studies. Our results indicate that the high aesthetic appraisals to an artwork by contextual influence are modulated by activities in prefrontal cortex, when it was significantly biased by pleasant context that forms subjects' prior expectations to the

hedonic value. Low appraisals according to the unpleasant context, on the other hand, are attributed to the instant negative emotional responses by activation in motor cortex.

Disclosures: S. Park, None; **Y. Lee**, None; **C. Chung**, None; **J. Jeong**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.8

Topic: F.03. Motivation and Emotion

Support: NEI EY010536-13A2

Title: Well-trained rhesus monkeys are risk averse for fluid rewards

Authors: *H. YAMADA, P. W. GLIMCHER;
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Abstract: Normative economic theory indicates that both human and animal choosers are, in principle, risk-averse in their choices as the law of diminishing marginal utility. Literally hundreds of studies in humans and several other species have validated this normative prediction. Technically, risk aversion (or risk seeking) is defined as negative (or positive) curvature of the utility function observed under conditions in which choice behavior is complete, transitive, and (for some definitions) obeys the Independence Axiom of Expected Utility Theory. A recent study of rhesus monkeys has, however, suggested that these subjects may be risk-seeking for fluid rewards (McCoy et al). They drew that conclusion under conditions in which choice behavior was unlikely to be either transitive or sensitive to the Independence Axiom - a condition under which the label 'risk-seeking' would not be theoretically applied.
To measure the risk attitudes of rhesus monkeys as a function of training, we taught two monkeys to engage in a visual gambling task during which they chose between a risky or a certain option that varied systematically. While fixating a central point, the monkeys received visual cues indicating the payoff volume of fluid reward offered by two options located on the left and right sides which varied from trial-to-trial with a particular option ranging from 0 ml to 0.60ml in 0.06ml increments. The probability that the risky option would yield a reward was fixed at 50% across lotteries. After monkeys showed stable choice a total 7115 and 3843 choices were recorded in each of the two monkeys during 17 days of data collection. To assess risk attitudes we fit a power utility function and a logit noise term to the choices of the animals: Utility = (water amount)$^{\alpha}$. Estimated under these conditions both monkeys showed significant

negative utility function curvature (alpha = 0.929 ± 0.001 and 0.928 ± 0.001). In other words, these monkeys were unambiguously risk averse for fluid rewards. Moreover, we found that early in training these animals were risk-seeking but that this shifted to risk-aversion as the animals became familiar with a task that incentivized maximization behavior. Finally, our data suggested that monkeys were significantly more risk averse during first half trials presented on each day (0.887 and 0.908) when they were presumably more thirsty, compared to the second half trials gathered on each day (0.968 and 0.971). These results imply that subjective values of fluid rewards may be controlled as a function of energy budget and hydration state.

Disclosures: **H. Yamada**, THE UEHARA MEMORIAL FOUNDATION Research fellow, Other Research Support; **P.W. Glimcher**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.9

Topic: F.03. Motivation and Emotion

Support: NIH DK071082

NIH DK047341

Title: Is altered taste sensitivity and food reward processing cause or consequence of obesity?

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Abstract: Obesity clearly is a multifactorial disease with a number of potential causes, but the involvement of recent environmental changes including the easy availability of palatable and energy-dense food is undeniable. Although recent human neuroimaging studies in lean and obese subjects revealed differences in the response to food and food cues in brain areas involved in reward, cognition, and emotion, it is not clear whether they are preexisting (genetic, epigenetic, and/or early life programming) and contribute to the development of obesity, or whether they are maladaptive effects of repeated exposure to palatable foods (as in food addiction) and/or secondary to hormonal, biochemical, and inflammatory dysregulation associated with the obese state. Here we analyze food reward behaviors in the lean, obese, and weight-reduced state in two models of obesity, the high-fat (HF) diet-induced obese Sprague-Dawley (SD) rats and the

selected lines of obesity-prone (OP) and resistant (OR) rats. HF-induced obesity (16 weeks) in SD rats shifted sucrose and corn oil 'liking' (number of positive orofacial hedonic reactions in the taste reactivity test and number of licks/10 s in the brief access test) from low to high concentrations compared with lean rats. This right-shift of the concentration-response curve is already present in young, naive OP rats and is aggravated by obesity, while 'liking' is little affected in OR rats. 'Wanting' as measured in the food-reinforced incentive runway was paradoxically decreased in the obese vs. lean SD rats, as previously reported using progressive ratio lever pressing. Young, naïve OP rats had already greatly reduced 'wanting' compared with both SD and OR rats that was further reduced by HF feeding. All obesity-induced changes in 'liking' and 'wanting' in SD rats were completely reversed after food restriction or gastric bypass-induced weight loss and leptin treatment of weight-reduced SD rats partially reinstated obesity-induced changes towards 'liking' of higher concentrations of sucrose and corn oil. The results suggest that food reward behaviors can be determined by both preexisting differences and by secondary, fully reversible effects of obesity. Predisposition to obesity and obesity by itself appear to shift 'liking' and eventually preference and intake to sweeter and fattier foods. The findings also support the idea that obesity is associated with paradoxically reduced motivation ('wanting') for sweet and oily stimuli when the effort is high to obtain them, but is normal or even increased when the food is readily available.

Disclosures: A.C. Shin, None; **H. Berthoud**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.10

Topic: F.03. Motivation and Emotion

Support: NIH Grant MH57075

NIH Grant T32 MH020002-04

NSF Learning Center Grant SBE-0542013

UCSD Academic Senate

Title: Expected value of information overlaps with reward circuits in humans

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Abstract: The ability to identify useful information, and selectively extract it, is critical for perception and cognition alike. For instance, to discriminate between object categories, the visual system must direct the eyes' gaze to useful features. What are the neural substrates of assessment of features' relative expected information value?
We trained 10 subjects on a probabilistic categorization task in which simulated plankton stimuli are classified as either species A or B, using experience-based learning. Subjects classified randomly sampled specimens, and received immediate feedback. Each plankton specimen has two two-state features (open or connected claw; dotted or non-dotted eye) that probabilistically predict the species. The more- and less- useful features, if viewed individually, lead to 85% and 60% accuracy, respectively. Since all combinations of features occur in both species, 100% accuracy is not possible.
Following behavioral training, subjects participated in an event-related fMRI experiment in which they were cued to anticipate more- or less-useful information. Anticipation of information was induced by first presenting an obscured version of the high- or low-usefulness feature, such that subjects could not yet categorize the stimulus or prepare a specific motor response. The specific form of the feature (e.g. dotted eye) was then revealed, allowing categorization. Finally, subjects received feedback on whether their classification was accurate, which was probabilistic according to environmental probabilities.
During the obscured feature presentation stage, subjects knew whether the subsequently obtained information would lead to 85% or 60% classification accuracy. In this stage presentation of the more-useful obscured feature, versus the less-useful obscured feature, led to greater activation in the ventral striatum (nucleus accumbens), amygdala/hippocampus, and cerebellar vermis.
Positive versus negative feedback led to greater activation in the ventral striatum, orbitofrontal cortex/ventromedial prefrontal cortex, bilateral posterior cingulate cortex, bilateral intraparietal sulcus, and right posterior insula. Negative versus positive feedback led to greater activation in the anterior insula, anterior cingulate cortex, and dorsolateral prefrontal cortex.
These results show that the expectation of information leading probabilistically to classification accuracy, in addition to food and money, activates brain structures that are part of the reward system.

Disclosures: F. Filimon, None; **J.D. Nelson**, None; **T.J. Sejnowski**, None; **M.I. Sereno**, None; **G.W. Cottrell**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.11

Topic: F.03. Motivation and Emotion

Support: DK26687

Title: Sipometer: Validation of a new device for measuring food reward in humans

Authors: *H. R. KISSILEFF[1], K. AGENOR[2], A. SCLAFANI[3];
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Abstract: A new device that measures negative sipping pressure was tested to determine whether humans would work longer for a sweet than a nonsweet milkshake. The device consists of a hard plastic straw connected to a reservoir through a computer-controlled pinch valve, thereby modeling a reinforced licking paradigm that demonstrated increased responding for increasing sweet taste intensity in rats (Sclafani & Acroff, 2003). Six women and five men of normal weight and without eating disorders were tested four times after a screening procedure in which they had to rate liking, on a 9-point scale, at least two units more for a sweet than a non-sweet, chocolate milkshake. The conditions for each trial were either continuous access (CA) or progressively increasing (PR) time (in 2 or 5 sec increments) for 2-sec access to the sweet (S) or non-sweet (N) shake. Participants were tested 3 hr after a 300 kcal breakfast and instructed to consume as much as they wanted as though it was their normal lunch. After they were finished, they marked responses to various questions on 150 mm lines anchored by "not at all" to "extremely". Among them was the question "How much did you enjoy what you've eaten?" (ENJOY). Participants consumed significantly more (190 g ± 56 SE, $p = 0.002$) of the sweet (644 g) than non-sweet shake (450 g) on CA, but there was no significant difference (58 g ± 56, $p = 0.31$) between sweet (129 g) and non-sweet (74 g) in intakes on PR. The time spent sipping (i.e. effort) was no different between the two shakes on either schedule (CAS = 3.8 min, CAN = 4.4 min, PRS = 9.1 min, PRN 9.2 min). However, the difference in time spent sipping between the sweet and non-sweet milkshake was significantly ($p = 0.02$) correlated ($r = 0.66$) with the ENJOY rating on PR, but only marginally significant on CA. Because effort expended, as measured by the time spent sipping, depended significantly on the pleasure derived, as measured by ENJOY rating, the method can be considered a proof of principle that the device can be used to measure reward value in normal weight humans. Further work with other reinforcers and populations will be needed to generalize the paradigm.

Disclosures: H.R. Kissileff, None; **K. Agenor**, None; **A. Sclafani**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.12

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01NS054775

NYU CoE Addiction grant #61

Title: Comparing apples and oranges: evidence for a unified subjective value representation in the brain

Authors: *D. LEVY, P. W. GLIMCHER;
NYU, NEW YORK, NY

Abstract: According to economic theory, a rational chooser compares the expected utility (EU) of different options and then chooses the option with the highest EU. Comparing between apples and oranges logically requires a computation of the EU of each option within it's own domain and then transformation of the different EU's to a common scale for a direct comparison. Our aim was to identify both the brain areas that represent the expected subjective values (ESV - the neuronal correlate of behavioral EU) for specific reward types and brain areas that represent a unified ESV irrelevant of the reward type.
Human subjects fasted for four hours and then asked to choose between monetary and food rewards while inside the magnetic resonance imaging scanner. In the SAME lotteries, subjects had to choose between a certain small reward and a stated probability of either winning a larger amount of the same reward or getting nothing. In the MIXED lotteries, subjects had to choose between a sure win of a small amount of money and a stated probability of winning a fixed amount of food or getting nothing. At the end of the experiment, one SAME trial of each reward type and one MIXED trial were randomly selected and played for real money and real food. Subjects then had to stay in the lab for two hours without access to other food.
We computed, for each subject, the EUs of all of the values of money and food encountered based on their choices. We then looked for brain areas that track the ESV for money and food. We found that different subregions in the ventromedial prefrontal cortex (vmPFC) and striatum track the ESV of money and food. The posterior parietal cortex tracked only the ESV for money and the hypothalamus tracked only the ESV for food suggesting that there is, to some extent, a distinct valuation network for each reward type. However, in both the vmPFC and striatum there was a common area representing the ESV of both reward types.
To test the hypothesis that activity in these common areas could account for choices between apples and oranges, we used the data from the MIXED trials to behaviorally determine the relative pricing between money and food for each subject. This relative pricing scaled the EU of money and food to a unified EU. We found that the relative scaling of food and money ESV activations in the common areas of the vmPFC and striatum correlated significantly with the

relative EU scaling for food and money. This suggests that these brain areas represent ESV irrelevant of the reward type. Comparison of brain activity in these overlapping regions would allow one to compare apples and oranges, so to speak.

Disclosures: D. Levy, None; **P.W. Glimcher**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.13

Topic: F.03. Motivation and Emotion

Support: Wellcome Trust

Science Foundation Ireland

Irish Research Council for Science, Engineering, and Technology

Title: Neural representations of reward in interpersonal attraction

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TCIN, Trinity Col. Dublin, Dublin 2, Ireland

Abstract: Humans, like other animals, are motivated to seek social and sexual partners, and acceptance or rejection by potential partners are some of the strongest positive and negative reinforcements in daily life. Elucidating the neural mechanisms underlying these reinforcements is crucial both for understanding general neural systems for valuation and for understanding the specific neural computations that mediate social value. Previous neuroimaging studies of social feedback have generally used fictional partners in economic or hypothetical interactions, making it difficult to know whether feedback was purely social or non-specifically positive or negative. Here, we used a paradigm in which heterosexual human volunteers underwent real interactions with a large number of potential partners through a speed-dating forum. 151 individuals participated in speed-dating events with 15-22 participants of each sex. At the events, each participant met every opposite-sex participant for a five-minute "date," at the end of which participants were asked to make a "Yes" or "No" decision about whether they would want to see that date partner again. After the events, pairs of participants who gave each other a Yes were given each other's contact information. A subset of these participants (20 men and 18 women) were scanned within a few days of their events with event-related FMRI. During scanning, each

participant saw photos of each of his or her speed-dating partners and was told for the first time of that partner's "yes" or "no" decision. Several regions related to reward were activated by receiving a "yes," including ventromedial prefrontal cortex (VMPFC), ventral striatum, medial parietal cortex, and posterior cingulate. Several regions (including VMPFC and posterior cingulate) also encoded anticipated and experienced social feedback value, as revealed by the participant's own response to the partner. These regions were more active both during anticipation of the decision, and after getting a "yes," for partners that were especially liked. The results suggest that neural representations of acceptance and rejection are strongly modulated by an individual's intrinsic valuation of the partner's suitability.

Disclosures: J.C. Cooper, None; **S. Dunne**, None; **M. Furey**, None; **J.P. O'Doherty**, None.

Nanosymposium

129. Neural Bases of Reward

Location: Room 2

Time: Sunday, November 14, 2010, 8:00 am - 11:30 am

Program Number: 129.14

Topic: F.03. Motivation and Emotion

Support: Howard Hughes Medical Institute

Title: Value representations in the primate orbitofrontal cortex during cost-benefit decision making

Authors: *D. L. KIMMEL[1], A. RANGEL[2], W. T. NEWSOME[1,3];
[1]Neurobio., Stanford Univ. Sch. Med., Stanford, CA; [2]Econ., Caltech, Pasadena, CA; [3]Howard Hughes Med. Inst., Stanford, CA

Abstract: For many decisions, we must explicitly compare the value of two or more goods being offered. Recent experiments in primates have revealed neural correlates of value when animals make choices between two offers. However, many decisions are not between multiple goods, but rather between a single offer and the choice to pass on that offer, such as when deciding to buy a new car, marry a significant other, or read this abstract(!). For these decisions the relevant comparison is between the expected benefit of the offer and its associated cost. An open question is the exact nature of the neural representations of these cost-benefit decisions. For example, do neurons encode only the expected benefit of an option, or also the cost involved in obtaining it? Here we report the results of a first study designed to investigate these issues.
We studied these cost-benefit decisions in the macaque monkey while recording from neurons in the orbitofrontal cortex (OFC), which has been implicated previously in decisions between two

competing goods (Padoa-Schioppa & Assad, 2006). Specifically, we varied the benefit (amount of juice) offered to the animal while requiring a fixed cost (sustained gaze) to accept the offer and obtain the benefit. The animal was sensitive to this balance of cost and benefit. His willingness to accept an offer increased monotonically as we increased the offer (across 5 different magnitudes), and did so through a wide dynamic range, accepting less than 5% of the smallest offers, while accepting nearly 80% of the largest offers.
On average, we found that neurons in the OFC encoded these valuations in a similarly graded manner, with the response to the offer increasing parametrically with increasing offers. We recorded from 165 single units in the medial OFC of one monkey. Of these, 66 were selected on the basis of their isolation quality alone (and therefore represent a random sampling of the OFC population). For nearly 40% of these 66 neurons, the firing rate increased significantly with increasing offer value. However, 15% of the neurons showed the opposite response -- a significant decrease in firing rate with increasing value. The remaining neurons were either unmodulated by value or responded non-monotonically, e.g., firing most for intermediate values. Our results suggest that the OFC carries value signals that could be useful in making cost-benefit decisions. However, this first experiment cannot determine if these neurons are also involved in encoding costs, which will be addressed in future experiments.

Disclosures: D.L. Kimmel, None; **A. Rangel**, None; **W.T. Newsome**, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant 2 R01 NS033123-09A2

Title: Regulatory features of the spinocerebellar ataxia type 2 gene ATXN2 promoter and 3'-UTR

Authors: *D. R. SCOLES, S. T. HANSEN, L. PFLIEGER, S. M. PULST;
Dept. of Neurol., Univ. of Utah, Salt Lake City, UT

Abstract: Spinocerebellar ataxia type 2 (SCA2) is caused by CAG expansion in the *ATXN2* gene resulting in polyglutamine (polyQ) expansion in the SCA2 protein ataxin-2. SCA2 is an untreatable disorder characterized by slow saccades and gait ataxia that always results in death. **Objectives:** to characterize regulation of the *ATXN2* gene by evaluation of features of the

ATXN2 promoter and 3'-UTR; identification of control elements for potential drug design. **Methods:** We constructed an *ATXN2-luc* reporter plasmid possessing 1062 bp of *ATXN2* promoter and the 163 bp 5'-UTR that expresses ataxin-2 up to and including the first polyQ glutamine fused to luciferase (luc). The plasmid also contained the 598 bp *ATXN2* 3'-UTR and another 414 bp of downstream sequence. We modified the *ATXN2-luc* reporter plasmid in three distinct ways including preparation of 1) 12 progressively increasing deletions starting from the upstream-most end of the promoter, 2) 19 overlapping ~100 bp deletions throughout the promoter-5'-UTR region, and 3) 20 overlapping ~60 bp deletions throughout the 3'-UTR. Luciferase assays were used to characterize how the deletions in *ATXN2* altered expression. We also characterized *ATXN2-luc* expression in *ATXN2-luc* transgenic mice. **Results:** Progressive deletion of upstream control regions resulted in progressively increased expression consistent with inhibitory elements throughout the *ATXN2* promoter. A single 100 bp 5'-UTR deletion with reduced expression led to our identifying within it a 26 bp region 75 bp upstream of the start codon that is critical for *ATXN2* expression. Of twenty 3'-UTR overlapping deletions, only one increased expression. This 58 bp 3'-UTR deletion increased expression by 40%. *In vivo* expression of the *ATXN2* reporter construct in transgenic mice directed expression to cerebellar tissues and to a lesser extent to the hemispheres as determined by relative light units (RLU)/mg and by real time PCR. **Conclusions:** SCA2, like other polyglutamine expansion disorders, is likely caused by gain of toxic or gain of normal function. Therefore therapeutic approaches that reduce *ATXN2* expression may be effective. Our study reveals for the first time specific regulatory features of the *ATXN2* promoter that may be exploited therapeutically and provides a reporter mouse useful for testing therapeutics on lowering *ATXN2* expression.

Disclosures: D.R. Scoles, None; **S.T. Hansen**, None; **L. Pflieger**, None; **S.M. Pulst**, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: T32 HD009007

R03-NS52582

Title: Loss of translational fidelity in SCA26

Authors: *K. HEKMAN[1], Y. HU[1], H. ZHU[2], X. DU[2], D. COLLINS[2], C. M. GOMEZ*[2];

[2]Neurol., [1]Univ. of Chicago, Chicago, IL

Abstract: We mapped a novel subtype of autosomal dominant spinocerebellar ataxia (SCA), SCA26, to a 15.5 cM region on chromosome 19p13.3 from a 6-generation kindred of Norwegian ancestry. Sequencing of 10 high priority candidate genes revealed an A->C transversion in the coding region of the gene for eukaryotic elongation factor 2 (eEF2) that co-segregates with the disease phenotype. eEF2 is ubiquitously expressed and fundamental for cellular viability. It facilitates translocation of the deacylated tRNA from the A- to the P-site within the ribosome. This point mutation results in a proline to histidine substitution at residue 596 in the protein. P596 lies in domain IV of the protein, in a region known to play a crucial role in preventing frameshift mutations and ensuring the fidelity of protein translation. We have demonstrated that the P596H variant eEF2 is a stable protein, and SCA26 patient cells heterozygous for the variant demonstrate no evidence of abnormal subcellular localization of the eEF2 protein. In a yeast model system, the equivalent yeast variant, P580H, is sufficient for life and demonstrates grossly normal growth characteristics. Using a dual-luciferase reporter assay, we have demonstrated in our yeast model system that P580H EF2 has an increased rate of shifting into the -1 reading frame of the mRNA transcript, representing a loss of translational fidelity. We hypothesize that this functional deficit will prove to be the molecular cause of SCA26.

Disclosures: K. Hekman, None; **Y. Hu**, None; **H. Zhu**, None; **X. Du**, None; **D. Collins**, None; **C.M. Gomez***, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant AG 16719

Friedreich's Ataxia Research Alliance

Title: Pathophysiology of Friedreich’s ataxia: Microarray and biochemistry of dorsal root ganglion supports a mitochondrial oxidative stress mechanism

Authors: *G. CORTOPASSI[1], Y. SHAN[1], S. SAHDEO[1,3], M. POOK[3], R. SCHOENFELD[2]; [2]VM:Molecular Biosci., [1]UC DAVIS, Davis, CA; [3]Biosci., Brunel Univ., Uxbridge, United Kingdom

Abstract: Friedreich's ataxia is the most common autosomal recessive ataxia, and is thought to result from a primary degeneration of sensory dorsal root ganglion neurons. Our previous microarray and proteomic experiments in cell lines have supported a direct interaction of frataxin with NFS1, the cysteine desulfurase involved in sulfur and thiol metabolism and iron-sulfur cluster synthesis (Shan et al. 2007 Hum. Mol. Genet. 16:929-941). We have carried out microarray of dorsal root ganglion neurons, spinal cord, cerebellum and heart of the expanded human frataxin knock-in YG8 mice that exhibit neurodegenerative and oxidative changes (Al-Mahdawi et al. 2006, Genomics 88:580-590). Frataxin-dependent alterations in gene expression differed substantially between tissues. In microdissected dorsal root ganglion neurons, substantial support arose for differential regulation of genes related to mitochondrial, oxidative and thiol-stress, and also deficiency of myelinating cells. Westerns of extracted dorsal root ganglion proteins from wild-type, homozygous and hemizygous mice supported a frataxin-specific deficiency of NFS1, peroxiredoxins and glutathione-S-transferase. Quantitative analysis of the Western data by Li-Cor in homozygotes and hemizygotes suggested that frataxin expression is more variable in dorsal root ganglion tissue than liver, which is consistent with the increased variability in (GAA)n expansions observed by others in dorsal root ganglion neurons and cerebellum in YG8 mice(Clark et al. 2007, Hum Genet 120:633-640), and could provide an explanation for the selective vulnerability of dorsal root ganglion tissue to the frataxin mutation. In summary, the data support the idea that the dorsal root ganglion environment favors increased GAA expansions, decreasing frataxin expression and causing a consequent deficiency of thiol-based antioxidants, which could be a primary event in the neurodegenerative process of Friedreich's ataxia. Elucidation and confirmation of this mechanism could be relevant for therapeutic strategies.

Disclosures: G. Cortopassi, None; **Y. Shan**, None; **S. Sahdeo**, None; **M. Pook**, None; **R. Schoenfeld**, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MDA Grant

GoFAR Grant

Title: Clinical biomarkers and assays for monitoring HDAC inhibitor treatment in Friedreich's ataxia

Authors: *H. PLASTERER[1], M. BELMONTE[1], S. SHARMA[1], A. COOPER[1], B. SHANDRA[1], A. CHAN[1], J. ESHRAGHI[1], A. MAROLEWSKI[1], S. JONES[1], D. MCCAULEY[1], M. RUGGERI[2], J. FARMER[3], D. LYNCH[4], J. RUSCHE[1];
[1]Repligen Corp., WALTHAM, MA; [2]GoFAR, Turin, Italy; [3]Friedreich's Ataxia Res. Alliance, Exton, PA; [4]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: **Background:** Friedreich's ataxia (FRDA) is an autosomal recessive disease associated with an intronic GAA triplet-repeat expansion in the frataxin gene. The repeat expansion results in down-regulation of transcription, presumably due to hypoacetylation of histones covering the gene. HDAC inhibitors (HDACi) can increase histone acetylation at the frataxin locus, and increase frataxin in patient lymphocytes *in vitro* and in transgenic mice, providing a therapeutic approach through restoring frataxin protein levels in FRDA patients. Previously we described the identification of nucleic acid biomarkers of drug action. We now report the development of additional biomarker measures. A deacetylase enzyme activity assay has been implemented to measure the effect of HDACi on the initial target, deacetylase enzymes, and frataxin protein measures in blood leukocytes and whole blood are also presented.
Methods: Whole blood or peripheral blood mononuclear cells (PBMC) isolated from patients and healthy volunteers was incubated for 24-48 hours with the HDACi RGFP109. Cells were lysed after incubation with either vehicle or HDACi and histone deacetylase activity was determined. Frataxin protein levels were measured in PBMC and whole blood using a lateral-flow immunoassay (MitoSciences).
Results: We report a dose-dependent decrease in histone deacetylase (DAC) activity in whole blood and PBMC following *ex vivo* treatment of cells obtained from control and patient donors. Similarly, a dose-dependent decrease in DAC activity was observed in PBMC and brain tissue collected from Beagle dogs following oral administration of HDAC inhibitor. Pharmacokinetic analysis of these samples indicated that DAC activity in mononuclear blood cells remains inhibited well beyond the presence of free drug in plasma. Previously we reported identification and characterization of four non-frataxin genes that increase in response to HDACi treatment; we have termed these genes 'drug signature genes'. A correlation was observed between DAC inhibition and increase in drug signature genes. Finally, frataxin protein levels were monitored in whole blood in a 15-week study of 5 patients and 5 carriers as well as in *ex vivo* HDACi-treated PBMC isolated from patients. We report that frataxin protein levels were consistent with a CV of 15% in our 15-week study and increased in PBMC in response to HDACi treatment.
Conclusion: The clinical biomarkers that we have developed will be an important aid to HDACi treatment evaluation in FRDA and will enable us to measure the effect of HDACi in Phase I clinical studies, which is usually not feasible in early-stage studies in healthy volunteers.

Disclosures: **H. Plasterer:** Employment; Repligen Corporation. **M. Belmonte:** Repligen Corporation. **S. Sharma:** Repligen Corporation. **A. Cooper:** Repligen Corporation. **B. Shandra:** Repligen Corporation. **A. Chan:** Repligen Corporation. **J. Eshraghi:** Repligen Corporation. **A. Marolewski:** Repligen Corporation. **S. Jones:** Repligen Corporation. **D. McCauley:** Repligen Corporation. **M. Ruggeri:** None. **J. Farmer:** None. **D. Lynch:** None. **J. Rusche:** Repligen Corporation.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS045667

Title: Ataxin-1 phosphorylation by PKA: a potential drug target for SCA1

Authors: *S. LAGALWAR[1], L. A. DUVICK[1], R. D. SINVILLE[2], M. A. WALTERS[2], D. J. HOOK[2], H. Y. ZOGHBI[3], H. T. ORR[1];
[1]Inst. for Translational Neurosci., [2]Inst. for Therapeut. Discovery and Develop., Univ. of Minnesota, Minneapolis, MN; [3]Dept. of Mol. and Human Genet., Baylor Col. of Med., Houston, TX

Abstract: Spinocerebellar ataxia type 1 (SCA1) is a progressive neurodegenerative disorder caused by the insertion of an expanded poly-CAG tract within the ataxin-1 gene. Early SCA1 pathology is characterized by the presence of nuclear inclusions of mutant ataxin-1 containing the expanded polyglutamine tract protein within degenerating Purkinje neurons. However, polyglutamine tract length is not the sole determinant of ataxin-1-induced pathogenesis. Phosphorylation of the serine 776 residue is thought to play an equally crucial role. We now report that a phosphomimetic mutation in wildtype ataxin-1, by replacing serine 776 with an aspartic acid residue, results in the same morphological, biochemical and behavioral features of disease as is typically seen when the expanded tract is present. We recently demonstrated that PKA is the likely candidate kinase of ataxin-1 serine 776 phosphorylation. We present further biochemical support in favor of PKA and demonstrate that PKA preferentially phosphorylates ataxin-1 protein containing the expanded polyglutamine tract compared to wildtype ataxin-1. Additionally, we recognize that serine 776 phosphorylation, being an early event in disease pathogenesis, presents a beneficial drug target. To this end, we have begun a high-throughput screen of 250,000+ small molecule compounds with the aim of finding specific inhibitors of ataxin-1 phosphorylation. Positive hits from the screen will be further tested in biochemical assays and in vivo models.

Disclosures: S. Lagalwar, None; **L.A. Duvick**, None; **R.D. Sinville**, None; **M.A. Walters**, None; **D.J. Hook**, None; **H.Y. Zoghbi**, None; **H.T. Orr**, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Analysis of polymorphisms within ataxin-3 as modifiers of Spinocerebellar ataxia type 3

Authors: *T. SCHMIDT, T. TSOLERIDIS, J. WEBER, H. PÉTURSSON, P. BAUER, O. RIESS;
Med. Genetics, Univ. of Tuebingen, Tuebingen, Germany

Abstract: Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an autosomal-dominantly inherited, neurodegenerative disorder caused by the expansion of a CAG repeat in the *MJD1 gene* resulting in an expanded polyglutamine repeat in the encoded ataxin-3 protein. SCA3/MJD, therefore, belongs to the group of polyglutamine diseases comprised of nine neurodegenerative diseases including five other types of Spinocerebellar ataxias as well as Huntington's disease. Statistically, a correlation between the number of CAG repeats and the age at onset of SCA3 patients exists and patients with a higher number of CAG repeats have first symptoms at earlier ages. However, this correlation is not perfect and the number of CAG repeats contributes only about 55 % to the age at onset. Therefore, the remaining 45 % are influenced by other factors, which we aim to identify in this study.
Ataxin-3 contains three polymorphisms in its coding sequence resulting in amino acid changes. The polymorphism $\underline{A}^{669}TG/\underline{G}^{669}TG$ (Met/Val, rs1048755) is located in exon 8. Exon 10, which also includes the CAG repeat, contains two such polymorphisms. The polymorphism $\underline{C}^{987}GG/\underline{G}^{987}GG$ (Arg/Gly, rs12895357) is situated directly 3' of the CAG repeat and the polymorphism $TA\underline{A}^{1118}/TA\underline{C}^{1118}$ (Stop/Tyr, rs7158733), leading to a premature stop codon, is located 131 bp further downstream. It was demonstrated before that the polymorphism directly 3' of the CAG repeat and certain combinations of additional polymorphism within the *MJD1 gene* are associate with intergenerational instability of the repeat.
Here, we assume that the amino acid changes within ataxin-3 resulting from these polymorphisms influence the function of normal and expanded ataxin-3 and/or its interaction with other proteins and therefore modify the age at onset and disease progression of SCA3 patients. We, therefore, genotyped more than 500 samples of SCA3 patients collected within the EUROSCA consortium for these polymorphisms using allele-specific PCR with fluorescent-labelled primers and fragment analysis after capillary electrophoresis. In addition, this technique allowed us to generate a haplotype comprising the CAG repeat length and the polymorphisms located downstream. We then statistically correlated these results with the clinical data in order

to analyze their impact on the age at onset of the disease. We hope that our results will improve the prediction of clinical symptoms and contribute to the understanding of pathogenic processes in SCA3.

Disclosures: T. Schmidt, None; **T. Tsoleridis**, None; **J. Weber**, None; **H. Pétursson**, None; **P. Bauer**, None; **O. Riess**, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Ciberned

Title: Cell-specific stimulatory effect of IGF-I on frataxin, a protein deficient in Friedreich´s Ataxia

Authors: C. FRANCO, S. FERNANDEZ, *I. TORRES-ALEMAN;
Cajal Inst. CSIC, Madrid, Spain

Abstract: Friedreich´s ataxia (FRDA) is a progressive neurodegenerative disorder that is the most common type of inherited ataxia. FRDA is caused by reduced expression of the mitochondrial protein frataxin (Fxn). Although the biological role of Fxn remains uncertain, its deficiency appears to lead to intracellular oxidative stress.
Insulin-like growth factor I (IGF-I) is a wide-spectrum neuroprotective factor involved in neuronal death by oxidative stress. IGF-I protects against various types of ataxia in experimental animal models and as recently shown, in spinocerebellar ataxia patients.
Since a general therapeutic strategy for neurodegenerative diseases is to potentiate endogenous neuroprotective mechanisms, we have tested IGF-I neuroprotection in Fxn deficient cells. Using the Cre/LoxP system to delete frataxin in cells, we observed that IGF-I increased frataxin levels in frataxin-deficient astrocytes but not in frataxin-deficient neurons. Whereas partially Fxn-deficient neurons died within hours in culture, Fxn-deficient astrocytes were rescued by IGF-I. In astrocyte cultures IGF-I not only restored frataxin levels but also reverted oxidative stress, and normalized mitochondrial function. Furthermore, treatment of co-cultures of wild type astrocytes and Fxn-deficient neurons with IGF-I reduced neuronal death. Stimulatory actions of IGF-I were replicated in wild type astrocytes with normal frataxin levels as well as in cardiomyocytes, while in cerebellar and dorsal root ganglion neurons IGF-I did not modify Fxn content. This suggests

cell-specific regulation of frataxin by IGF-I. Additionally**,** IGF-I increased frataxin protein levels without affecting mRNA synthesis, pointing to a post-translational effect of IGF-I. The stimulatory action of IGF-I on astrocyte frataxin was mediated by the Akt/mTor-dependent protein translation pathway. These results suggest a potential therapeutic efficacy for this pleiotropic growth factor in FRDA.

Disclosures: C. Franco, None; **I. Torres-Aleman**, None; **S. Fernandez**, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant 1K23EY015802

NIH Grant 5T32DC00023

NIH Grant 5T32MH019950

NIH Grant 5T32GM007057

NIH Grant EY01849

Arnold-Chiari Foundation

Robin Zee Fund

Title: Visuospatial deficits correlate with regional cerebellar atrophy in SCA6

Authors: J. L. CUZZOCREO[1], A. X. DU[1], C. H. JUNG[1], Z. Z. GENG[3], S. A. SMITH[4], R. L. MARGOLIS[2], *S. H. YING[5];
[1]Neurol., [2]Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Med., Univ. of Texas Southwestern, Dallas, TX; [4]Radiology & Radiological Sci., Vanderbilt Univ., Nashville, TN; [5]Dept. of Neurol., Johns Hopkins Univ., BALTIMORE, MD

Abstract: Spinocerebellar ataxia type 6 (SCA6) is an excellent candidate disease for studying cerebellar contributions to cognitive dysfunction, as neurodegeneration is largely restricted to the

cerebellum. With neuropsychological testing and structural brain MRI, we compared the cognitive performance and neocerebellar (Crus I, Crus II, and lobule VIIb) regional volumes of SCA6 patients with control participants.
6 SCA6 patients (3M/3F) and 6 controls (3M/3F) matched for age, sex, and education levels were studied. Anatomical scans were acquired on a 3T Philips Achieva magnetic resonance scanner (MPRAGE, 3 acquisitions, TE 6ms, TR 10.33ms, reconstructed 0.83 x 0.83 x 0.75 mm^3). Volumetric data for the total cerebellum, crus I, crus II, and lobule VIIb were obtained via manual delineation. Subjects were given the Rey Auditory Verbal Learning Test, Rey-Osterrieth Complex Figure test, Controlled Oral Word Association test, Trail-Making Tests (TMT A and B), Stroop Color and Color-Word Tasks, and Digit Span Tests (forward and backward). Normalized cumulative distribution function scores were calculated according to age-, education level-, and gender-based normative values. Statistical differences were evaluated with Student's t-tests and Pearson correlation analyses.
Preliminary findings show that total cerebellar ($p<0.004$), crus I ($p<0.03$), and left lobule VIIb ($p<0.03$) volumes were smaller in the SCA6 group compared with controls. The SCA6 group required more time to complete TMT A and B ($p<0.03$), indicating possible deficits in visuospatial, working memory, and set-switching skills, though a lack of similar findings in Stroop and Digit Span tests suggests impairment may be restricted to visuospatial processes. A correlation emerged between crus I volumes and TMT performance in the SCA6 group (TMT A + B $R^2> 0.73$, $p<0.03$). Only left lobule VIIb volumes correlated with disease duration ($R^2 = 0.72$, $p<0.03$).
Given the prominent cerebellar degeneration in SCA6, we expected the volumes of many structures would be smaller in the SCA6 group; however, atrophy was not found in crus II or right lobule VIIb. The region-specific degeneration found in crus I correlated specifically with visuospatial performance in the SCA6 group only, but this measure did not correlate with disease duration. Experienced test administrators anecdotally observed prolonged searching behavior in the SCA6 group during TMT A and B, rather than inhibited motor performance. The visuospatial deficits found in SCA6 patients may reflect disruption of corticocerebellar circuits connecting neocerebellar areas to parietal and frontal lobe regions involved in spatial perception and visual attention.

Disclosures: J.L. Cuzzocreo, None; **A.X. Du**, None; **C.H. Jung**, None; **Z.Z. Geng**, None; **S.A. Smith**, None; **R.L. Margolis**, None; **S.H. Ying**, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NS058500

Title: Expression of a spinocerebellar ataxia type 13 mutant potassium channel subunit in zebrafish impairs execution of the startle response

Authors: *D. M. PAPAZIAN[1], F. A. ISSA[2], C. MAZZOCHI[2], J. L. RICHARDSON[2], J.-Y. HSIEH[2], A. F. MOCK[2];
[1]Geffen Sch. Med. UCLA, LOS ANGELES, CA; [2]Geffen Sch. Med. UCLA, Los angeles, CA

Abstract: We have investigated the usefulness of zebrafish as a model system for studying spinocerebellar ataxia type 13 (SCA13), a human genetic disease caused by mutations in the voltage-gated Kv3.3 K^+ channel. We have identified six Kv3 channel orthologs in zebrafish, including two genes encoding Kv3.3, *kcnc3a* and *kcnc3b*. Like mammalian Kv3.3 channels, zebrafish Kv3.3 channels activate over a depolarized voltage range and deactivate rapidly. These gating properties facilitate high frequency, repetitive firing in neurons. The functional effects of SCA13 mutations are conserved in zebrafish Kv3.3 channels. Mutating the third arginine in S4 to histidine (R3H) generates a dominant negative subunit that suppresses the activity of wild type Kv3.3. Mutating a Kv3-specific phenylalanine in S5 to leucine (F/L) alters channel gating, shifting the voltage dependence of activation in the hyperpolarized direction and dramatically slowing deactivation. Using immunofluorescence, we have characterized Kv3.3 expression in the zebrafish spinal cord, where it is localized to CaP primary motor neurons. CaP motor neurons innervate fast twitch muscle and drive the fastest, largest amplitude movements in the zebrafish repertoire, including the startle response. To investigate whether SCA13 mutations affect the startle response, we injected RNA encoding the R3H dominant negative subunit into single-celled zebrafish embryos. R3H is incapable of forming active K^+ channels on its own and will therefore be functionally silent in cells that do not express endogenous Kv3 subunits. In contrast, in Kv3-expressing cells, R3H will lower current amplitude by a dominant negative mechanism. Expression of R3H reduces the precision and amplitude of the initial C-bend and subsequent counterbend during the startle response. These changes in motor behavior are reminiscent of gait changes seen in human ataxia, which is characterized by increased variability and decreased amplitude of step size. Expression of R3H in fast-spiking CaP motor neurons reduces firing frequency significantly. In parallel, the amplitude of outward K^+ currents is reduced. These results are consistent with decreased Kv3 channel activity, because Kv3 currents increase neuronal excitability by enhancing the recovery of Na^+ channels from inactivation. Decreased firing of CaP motor neurons is sufficient to account for the behavioral changes seen in R3H-expressing animals. Our results demonstrate that zebrafish is an excellent model system for investigating human diseases that affect nervous system function and locomotion.

Disclosures: D.M. Papazian, None; **F.A. Issa**, None; **C. Mazzochi**, None; **J.L. Richardson**, None; **J. Hsieh**, None; **A.F. Mock**, None.

Nanosymposium

13. Hereditary Ataxias

Location: Room 7B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 13.10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The WD-repeat-containing protein WDR81 is essential for Purkinje cell and photoreceptor cell viability in the mouse

Authors: *M. TRAKA, K. J. MILLEN, B. POPKO;
Univ. Chicago, CHICAGO, IL

Abstract: The mechanisms that control the survival of CNS neurons are still poorly understood. To better understand this, we have investigated the mechanism of neurodegeneration in the N-ethyl-N-nitrosourea-induced nur5 line of mutant mice, which we show displays progressive postnatal Purkinje cell and photoreceptor cell death. We demonstrate that the mutant phenotype is caused by a L302P substitution in a novel WD-repeat-containing protein, WDR81, which is expressed in Purkinje cells and photoreceptor cells, as well as other CNS neurons. Yeast two-hybrid screening, confirmed by co-immunoprecipitation studies, reveals that WDR81, which is a predicted Type II membrane-spanning protein, associates with the spectrin-repeat containing protein SYNE1. Interestingly, SYNE1 has recently been shown to be mutated in human patients that suffer from autosomal recessive cerebellar ataxia type-1 (ARCA-1). The association of WDR81 with SYNE1 indicates that these proteins are part of a multimolecular complex that is critical for neuronal survival.

Disclosures: M. Traka, None; **K.J. Millen**, None; **B. Popko**, None.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.1

Topic: C.08. Ischemia

Support: Danish Strategic Research Council-Food & Health 2101-07-006

Title: Testing of plant extracts for neuroprotective effects in hippocampal slice cultures

Authors: *J. ZIMMER[1], J. NORABERG[1], K. B. CHRISTENSEN[2], K. GREVSEN[3], J. SINDBERG[1];
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Abstract: Plants are a major source of known and potentially new health promoting, neuroprotective compounds. Plant compounds with such properties are therefore attractive as natural components or supplements in functional food and as leads for novel drugs in treatment or prevention of neurodegenerative disorders.
Taking advantage of a sensitive, hippocampal slice culture-based model for glutamate receptor mediated, ischemia-induced neurodegeneration, we started to screen extracts of selected plant species for neuroprotective (or neurotoxic) effects, measured against a standardized excitotoxic NMDA lesion. Hippocampal slice cultures derived from 8 day old rat pups (Sprague Dawley strain) were grown for 2-3 weeks before exposed for 24 hrs to 10 μM of the glutamate receptor agonist NMDA, known to induce 50% CA1 pyramidal cell death, with and without addition of plant extracts. Induced neuronal cell death was quantified by recording cellular uptake of the fluorescent dye propidium iodide before and after exposure. Plant extracts examined with positive effect so far include the medicinal plant *Rhodiola rosea* (golden root), the herb *Salvia officinalis* (sage) and the vegetable *Brassica oleracea* var. *italic* (broccoli). Crude methanolic extracts of these plants displayed a significant and dose-dependent protection of hippocampal CA1 pyramidal cells against NMDA excitotoxicity. Neuroprotection by golden root was observed for concentrations ranging from 50-500 μg/ml with maximal reduction in cell death to 47% of lesion-only cultures. Broccoli extracts at 50-100 μg/ml conc. provided a maximal reduction of 37%, while sage extracts in 100-250 μg/ml conc. reduced cell death to 32%. Ongoing experiments, using standards of major metabolites present in the extracts as well as bioassay-guided chromatographic fractionations, aim to identify the bioactive compounds in these extracts. Investigations of mechanisms of action will include test for anti-inflammatory effects, effects on glutamate transporters and selected gene expression analyses by real time PCR of microdissected samples of the CA1 pyramidal cell layer.
We conclude that screening for neuroprotective effects of crude plants extracts in a brain slice culture model of cerebral ischemia is feasible for first level selection of plants species and extracts, in this case golden root, sage and broccoli.

Disclosures: J. Zimmer, None; **J. Noraberg**, None; **K.B. Christensen**, None; **J. Sindberg**, None; **K. Grevsen**, None.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.2

Topic: C.08. Ischemia

Support: NIH Grant NS23002

Title: Neuroprotective effect of LAU-0901, a novel platelet-activating factor receptor antagonist, in focal cerebral ischemia in rats: Characterization by sequential magnetic resonance imaging and behavior

Authors: *L. S. BELAYEV[1], L. KHOUTOROVA[2], T. NIEMOLLER[2], K. ATKINS[2], A. OBENAUS[3], P. HAYES[3], E. TITOVA[3], N. G. BAZAN[2];
[1]Neurosci. Ctr., LSUHSC, NEW ORLEANS, LA; [2]Neurosci. Ctr., LSUHSC, New orleans, LA; [3]Non-Invasive Imaging Lab., Loma Linda Univ., Loma Linda, CA

Abstract: Platelet activating factor (PAF) is a mediator phospholipid with potent pro-inflammatory bioactivity. Under pathological conditions, PAF becomes a neurotoxic agent. We have demonstrated that signaling mediated by PAF is involved in synaptic plasticity, memory, and neuronal protection. Recently we examined the effect of LAU-0901 in models of focal cerebral ischemia in rats and mice. LAU-0901 improved behavioral deficits and reduced infarct volumes when administered at 2 h after MCAo onset. In addition, LAU-0901 conferred enduring neuroprotection in animals allowed to survive for several weeks after stroke. We have used non-invasive magnetic resonance imaging (MRI) in conjunction with behavioral methods to expand our understanding of this novel therapeutic approach.
Male Sprague-Dawley rats (256-310g) were anesthetized with isoflurane/nitrous oxide and mechanically ventilated; rectal and cranial temperatures were regulated at 36-37.5°C. Rats received 2 h middle cerebral artery occlusion (MCAo) by retrograde insertion of an intraluminal suture. Diffusion weighted (DWI) and T2-weighted (T2WI) MRI was carried out on a 4.7T magnet at 24 h, 72 h, and 7 days after MCAo. Animals were treated with LAU-0901 (60mg/kg; n=5) or vehicle (45% cyclodextran; IP; n=4), at 2 h from MCAo onset. Neurological status was evaluated before MCAo and after treatment (at 24 h, 72 h, and 7 days), a grading scale of 0-12 was employed (0=normal and 12=maximal deficit).
All animals showed similar values for rectal and cranial temperatures, arterial blood gases, and plasma glucose during and after MCAo. LAU-0901 treatment significantly improved behavioral scores compared to vehicle on day 1 (5.0±1.0 vs. 10±0.7), day 3 (5.0±0.9 vs. 9.3±0.5) and day 7 (3.0±0.1 vs. 8.5±0.9, respectively; $p<0.01$). On average, T2 values were decreased 8% in LAU-0901-treated animals, consistent with decreased edema formation. In addition, T2WI revealed signficantly decreased cystic lesion development in the LAU-0901 group compared to controls. LAU-0901-treated rats after MCAo did not show the marked decrease in water diffusion that classically occurs early after stroke due to cellular swelling and subsequent cell death, suggesting that this compound is protective. In addition, neuroimaging showed decreased ventricular

enlargement in the LAU-0901 group compared to vehicle treatment, further demonstrating LAU-0901 protective effects.
Magnetic resonance imaging and behavioral testing confirms marked neuroprotective efficacy of LAU-0901, a novel PAF inhibitor, in focal cerebral ischemia and might provide the basis for future therapeutics in patients suffering ischemic stroke.

Disclosures: L.S. Belayev, None; **L. Khoutorova**, None; **T. Niemoller**, None; **K. Atkins**, None; **A. Obenaus**, None; **P. Hayes**, None; **E. Titova**, None; **N.G. Bazan**, None.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.3

Topic: C.08. Ischemia

Support: NIH Grant NS49430

Title: Protection against brain damage following experimental stroke through inhibition of 12/15-lipoxygenase

Authors: *K. VAN LEYEN[1], K. YIGITKANLI[2], A. PEKCEC[2], S. PALLAST[2], T. R. HOLMAN[3], E. H. LO[2];
[1]Massachusetts Gen. Hosp., CHARLESTOWN, MA; [2]Neuroprotection Res. Lab., Massachusetts Gen. Hosp., Charlestown, MA; [3]Chem. and Biochem., Univ. of California, Santa Cruz, MA

Abstract: 12/15-lipoxygenase (12/15-LOX) is a lipid-oxidizing enzyme known to attack mitochondria under oxidative stress. We have here studied the role of 12/15-LOX in ischemic injury following experimental stroke in mice. Levels of the 12/15-LOX metabolite 12(S)-HETE are massively increased on the ischemic side of the brain 24 hours after transient middle cerebral artery occlusion (MCAO). Up-regulation of 12/15-LOX in the ischemic peri-infarct region is followed by increased expression of mitochondrial apoptosis-inducing factor (AIF) and coincides with an oxidative stress marker, suggesting a common cell death mechanism. Administration of the specific 12/15-lipoxygenase inhibitor LOXBlock-1 leads to reduced infarct size at 24 hours after MCAO, and this effect is still apparent two weeks later, demonstrating long-lasting protection against experimental stroke.

Disclosures: K. van Leyen, National Institutes of Health, Research Grant; **K. Yigitkanli**, None; **A. Pekcec**, None; **S. Pallast**, None; **T.R. Holman**, National Institutes of Health, Research

Grant; **E.H. Lo**, National Institutes of Health, Research Grant.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.4

Topic: C.08. Ischemia

Support: Canadian Stroke Network Operating Grant

University of Toronto Surgeon Scientist Program

Title: Treatment of acute ischemic stroke in old world primates with the PSD95 inhibitor NA-1

Authors: *D. J. COOK[1,2], L. M. TEVES[2], M. TYMIANSKI[2];
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Abstract: Background: Neuroprotection following stroke in gyrencephalic species like humans and old world primates has failed in all studies and clinical trials to date. Administration of NA-1, an inhibitor of the interactions of NMDA glutamate receptors with the submembrane scaffolding protein PSD-95, confers neuroprotection in multiple rodent models of middle cerebral artery occlusion (MCAO) over a broad therapeutic window. NA-1 acts on NMDAR(NR2)-PSD95-nNOS protein-protein interactions and preserves normal NMDAR function while markedly decreasing neurotoxic effects of nNOS activation in-vitro and in-vivo. To test whether NA-1 is neuroprotective in acute ischemic stroke in a gyrencephalic species, we performed a blinded, randomized trial of NA-1 versus drug vehicle in a reperfused MCAO model in the cynomolgus macaque with MRI and neurobehavioural outcomes.
Methods: 20 male cynomolgus macaques(2.85-5.05kg) underwent anesthesia with physiologic monitoring and surgical MCAO followed by immediate MRI imaging. NA-1(2.6mg/kg) or drug vehicle were administered in blinded fashion 60-minutes following MCAO. The MCA was reperfused 90 minutes following occlusion. Animals underwent 7-Tesla MRI diffusion-weighted (DWI) and T2 imaging at 4h, 24h and 30d after MCAO. A battery of sensory-motor testing was undertaken serially following MCAO.
Results: Analysis was performed on an intent-to-treat basis. Stroke volume on 24h DWI was reduced from 103mL in placebo treated animals to 58mL in NA-1 treated animals(P=0.039) and this effect was maintained on 30d T2-weighted MRI. There was a significant improvement in Non-Human Primate Stroke Score measured serially from 8h to 30d post-MCAO (P=0.018, Repeated Measures two-way ANOVA). There were also significant improvements in sensory

and motor function as measured by the two-tube, six-well and hill/valley tests.
Conclusions: NA-1 confers both structural and functional improvements in outcome in an NHP model of reperfused MCAO. This study confirms the feasibility of neuroprotection in the gyrencephalic brain and supports further study of NA-1 as a possible neuroprotectant in acute ischemic stroke.

Disclosures: D.J. Cook, None; **M. Tymianski**, None; **L.M. Teves**, None.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.5

Topic: C.08. Ischemia

Support: NIH Grant 5RO1 HL063290

Title: Activated protein C promotes post-ischemic neuronal plasticity and brain repair

Authors: *M. THIYAGARAJAN[1], S. M. LANE[1], T. ALI[2], N. CHOW[2], B. ZLOKOVIC[1,2];
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[2]ZZ Biotech Res. Lab., Rochester, NY

Abstract: Activated protein C (APC) is a serine protease with anticoagulant and direct cytoprotective, angiogenic and neurogenic activities. We investigated whether APC can enhance postischemic neuronal plasticity. In vivo multiphoton imaging of dendritic spines in mice expressing yellow fluorescent protein (YFP) in neurons revealed that multi-dosing therapy with recombinant murine APC [0.2 mg/kg, intraperitoneally (i.p.) beginning day 1 after ministroke and followed by i.p. injections on day 2 and then every second day] increased significantly spine formation in the periphery of infraction after Rose Bengal (RB)-induced photothrombotic ministroke on day 2 and 7 by > 10-fold and 1.8-fold, respectively (two-way ANOVA, $p < 0.001$; Bonferroni post hoc test, $p < 0.001$). Using intrinsic optical signal (IOS) imaging to evaluate the forelimb somatosensary (S1FL) function that was lost to mini-stroke, we show that APC multi-dosing treatment enhances hemodynamic responses to S1FL stimulation. Using cultured mouse neurons, we next demonstrated that APC at low dose (30-300 pM) increased the abundance of pre- and post-synaptic proteins as shown by increased synaptophysin and PSD95 levels, respectively, and the number of electrically excitable synaptophysin^{+}/FM4-64^{+} puncta. Using PAR1-4 receptors specific antibodies and antagonists of sphingosine 1 receptor 1 (S1P1) W146 and VPC23019 we found that PAR1 and S1P1 are required for APC-mediated effects on

synaptophysin and PSD95 levels in cultured neurons. These findings suggest that APC promotes post-ischemic neuronal plasticity and brain repair after mini-stroke possibly by increasing the abundance of synaptic proteins and electrically excitable puncta.

Disclosures: M. Thiyagarajan: None. **S.M. Lane:** None. **T. Ali:** None. **N. Chow:** None. **B. Zlokovic:** Ownership Interest; B.V. Zlokovic is a scientific founder of ZZ Biotech LLC, a startup biotech company with a mission to develop new treatments for the aging brain, stroke, and Alzheimer disease. Other; B.V. Zlokovic is one of the inventors on issued and pending patents related to APC.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.6

Topic: C.08. Ischemia

Support: NIH Grants NS057556, DA024235, DA026108, DA029889, HL101930, RR14075

AHA-09GRNT2060416

Stanley Center for Mental Health/Broad Institute

Title: Diffusion weighted MR imaging reveals neuro-protective efficacy of hydrogen sulfide in a mouse model of cardiac arrest and resuscitation

Authors: H. WANG[1], S. MINAMISHIMA[3], P. K. LIU[1], F. ICHINOSE[3], *C. LIU[2]; [1]Radiology, Massachusetts Gen. Hosp., Charlestown, MA; [2]Massachusetts Gen. Hosp., CHARLESTOWN, MA; [3]Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Global ischemic brain damage is a major cause of morbidity and mortality after sudden cardiac arrest and cardiopulmonary resuscitation (CA/CPR). Although Diffusion-Weighted MRI (DWI) of the brain detects regions of abnormal water diffusion and is often prescribed in comatose post-CA/CPR patients, its prognostic value remains largely unknown. We recently reported that administration of sodium sulfide (Na_2S, hydrogen sulfide donor) at the time of CPR significantly improves neurological function and survival rate 24 hours after CA/CPR in mice. The robust neuro-protective effect of Na_2S was associated with attenuated oxidative stress, caspase 3 activation, and neuronal death. Our laboratories have previously

utilized an in vivo DWI method to delineate brain volumes of metabolic disturbance (VMD) where apoptosis or necrosis eventually occurred in a mouse model of transient global ischemia. In the current study, we aimed to determine the predictive value of the DWI method as an early marker for the neuro-protective effects of Na_2S after CA/CPR. Male C57BL6 mice were subjected to 7.5 min of normothermic CA, treated with Na_2S, (0.55 mg/kg i.v.) or vehicle one minute before CPR, and resuscitated with chest compression and mechanical ventilation. One day after CA/CPR, we observed patchy and globally distributed areas of hyper-intense DWI signals in vehicle-treated mice, most noticeably in the hippocampus and cerebellum. On the other hand, animals that received Na_2S one minute prior to CPR exhibited minimal areas of hyper-intense DWI signal, located mostly in the cortex. Statistical analysis on the aggregated striatal and cortical VMD values from 7 mice in each group revealed that administration of Na_2S markedly decreased VMD compared to vehicle treatment ($p<0.003$, unpaired t-test). Of note, administration of Na_2S significantly improved survival rate 10 days after CA/CPR ($p<0.05$ by Log-rank test). In conclusion, the current results showed that quantification of VMD using DWI allowed early determination of the therapeutic efficacy of Na_2S on the long-term outcome after CA/CPR at acute recovery stage without the need of post-mortem sample collection and behavioral assessment. These results suggest an important prognostic value of DWI in post-CA patients. Our methods may be applicable to screen candidate pharmacological agents in animal models of CA/CPR to facilitate drug development for the treatment of post-CA brain injury.
This project was supported by grants from NIH (NS057556; DA024235, DA026108; DA029889, HL101930 and RR14075), from American Heart Association (09GRNT2060416); the Stanley Center for Mental Health Research at the Broad Institute.

Disclosures: H. Wang, None; **C. Liu**, None; **S. Minamishima**, None; **P.K. Liu**, None; **F. Ichinose**, None.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.7

Topic: C.08. Ischemia

Support: Wyeth Pharmaceuticals

Title: TIQ-A derivatives are potent and selective poly(ADP-ribose) polymerase inhibitors and significantly reduce post-ischemic brain damage in different models of middle cerebral artery occlusion both in male and female rats

Authors: *F. MORONI[1], A. COZZI[1], A. CHIARUGI[1], L. FORMENTINI[1], E. CAMAIONI[2], D. PELLEGRINI-GIAMPIETRO[1], M. ZALESKA[3], C. GONZALES[3], A. WOOD[3], R. PELLICCIARI[2];
[1]Preclinical and Clin. Pharmacol., Univ. Florence, Florence, Italy; [2]Medicinal Chem., Univ. of Perugia, Perugia, Italy; [3]Global Res. and Develop., Neurosci. Res. Pfizer ,, Groton, CT

Abstract: We previously characterized a series of PARP inhibitors with submicromolar affinities for PARP 1 and PARP 2 that were rather potent in reducing post ischemic brain damage in primary neuronal cultures (*JPET 305: 943; 2003*). TIQ-A, the leading compound of the series inhibited PARP1 with an IC_{50} of 500 nM. Among its derivatives, DAM-TIQ had a IC_{50} of 89 and OH-DAM-TIQ of 0.7 nM. When tested against PARP-2, the IC_{50}s were: 600 for TIQ-A; 300 for DAM-TIQ and 0.9 nM for OH-DAM-TIQ. The compounds (up to 100 micromolar) had no inhibitory activity on tankyrase-1 activity.
When TIQ-A (10 mg/kg i.p.), DAM-TIQ (3-10 mg/kg i.p.) or OH-DAM-TIQ (1 mg/kg i.p.) were administered 3 times (30 min, 3,5 and 6,5 h) to male rats with permanent (thread method) middle cerebral artery occlusion (MCAO), brain infarct volumes (evaluated 48 hrs later) were reduced by approximately 40 % . When administered 2h after permanent thread placement, brain infarct volumes were not significantly reduced. When MCA was transiently occluded (2 h) TIQ-A and its derivatives significantly reduced brain infarct volumes even when the beginning of treatment was postponed up to 4 hrs. In male rats with transient (2h) MCAO the administration of OH-DAM-TIQ (0.1; 1 or 10 mg/kg 3 times starting 4 hrs after thread placement) reduced brain infarct volumes (evaluated 48 h later) from 309 $\pm$ 12 in the controls to 233 $\pm$ 7; 130 $\pm$ 18 and 100 $\pm$ 18 mm^3 respectively. The treatment also decreased the per cent loss of body weight in a dose dependent manner and significantly improved the neurological scores (Bederson's test).
It has been suggested that PARP inhibitors do not reduce (or even increase) post-ischemic brain damage in females. We tested the TIQ-A derivatives in tMCAO both in male and in female rats of the same age. Interestingly, brain infarct volumes were 318 $\pm$19 in males and 277 $\pm$ 31 mm^3 in control females. After OH-DAM-TIQ (1 mg/kg 3 times, starting 2 h after thread placement) brain infarct volumes were reduced to 102 + 20 in males and to 183 $\pm$ 20 in females. Thus OH-DAM-TIQ treatment reduces post-ischemic brain damage both in females and in males. In females, however the variability of the infarct volumes is larger and the extent of protection is less impressive (in this experiment the infarct volume decreased by 68 % in males and by 44 %in females).
Taken together, our results shows that TIQ-A derivatives are potent PARP inhibitors that display significant neuroprotective activity both in permanent and in transient models of MCAO. Neuroprotection is robust, it is present both in male and female animals and is associated with improvement of neurological signs.

Disclosures: F. Moroni: Research Grant; Whyeth. **A. Cozzi:** None. **A. Chiarugi:** None. **L. Formentini:** None. **E. Camaioni:** None. **D. Pellegrini-Giampietro:** None. **M. Zaleska:** Employment; Pfizer. **C. Gonzales:** Pfizer. **A. Wood:** Pfizer. **R. Pellicciari:** Research Grant; Wyeth.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.8

Topic: C.08. Ischemia

Support: Spanish Ministry of Education and Science SAF2006-08540 (M.G.L.)

Spanish Ministry of Education and Science SAF2006-03589 (A.G.G.)

Ministry of Health (Instituto de Salud Carlos III) RETICS-RD06/0026/0009 (A.G.G.)

Comunidad Autónoma de Madrid SAL2006/0275 (A.G.G.)

Agencia Laín Entralgo NDG07/9 (A.G.G.)

Fundación Teófilo Hernando

Title: Reduction of infarct volume after focal cerebral ischemia in mice exerted by a new multifunctional compound: ITH12233

Authors: *S. LORRIO[1,2], P. NEGREDO[1,2], M. RODRÍGUEZ-FRANCO[3], S. CONDE[3], M. P. ARCE[3], M. VILLARROYA[1,4], J. M. RODA[2,5], M. G. LÓPEZ[1,4], A. G. GARCÍA[1,4,6];
[1]Inst. Teófilo Hernando, Univ. Autónoma De Madrid, Madrid, Spain; [2]Inst. de investigación del Hosp. Universitario La Paz, Madrid, Spain; [3]Inst. de Química Médica, Consejo Superior de Investigaciones Científicas, Madrid, Spain; [4]Farmacología y Terapéutica, Univ. Autónoma de Madrid, Madrid, Spain; [5]Servicio de Neurocirugía, Hosp. Universitario La Paz, Madrid, Spain; [6]Servicio de Farmacología Clínica, Hosp. Universitario de La Princesa, Madrid, Spain

Abstract: ITH33 is a new L-glutamate acid derivative, chemically synthesized as a possible treatment for Alzheimer and vascular dementia. Three pharmacophores have been merged in the compound: *N*-benzilpiperidine, which binds to the catalytic site and inhibits acetylcholinesterase (AChE); an *N*-benzoyl group which interacts with the peripheral site of AChE, implicated in β-amiloid protein (Aβ) aggregation; and a lipophilic ester to facilitate penetration through the blood-brain barrier (BBB) (Arce MP et al, J Med Chem 2009;52:7249-7257). In human neuroblastoma cell cultures, the compound exerts protection against: calcium overload, free radicals, tau hyperphosphorilation and Aβ 1-42. In rat hippocampal slices subjected to oxygen and glucose deprivation, the compound exerts 55% protection. These “in vitro” results encouraged us to test whether ITH33 would induce neuroprotection using “in vivo” models of brain damage. We performed experiments using photothrombotic focal cerebral ischemia in mice. ITH33 was injected i.p. (1.25, 2.5, 5 and 10 mg/kg) 1 h before ischemia and twice a day

for the two following days. Mice were sacrificed 72 h after ischemia; fresh coronal brain sections were TTC stained for infarct volume measurement. ITH33 was able to reduce infarct volume significantly at 1.25, 2.5 and 5 mg/kg (46, 46 and 45%, respectively). Melatonin was used as a positive control (Zou LY et al, J Pineal Res 2006;41:150-156), which reduced 28% infarct volume. We believe these results show that ITH33 is an interesting compound with an evident neuroprotective therapeutic potential in cerebrovascular disease.

Disclosures: S. Lorrio, None; **P. Negredo**, None; **M. Rodríguez-Franco**, None; **S. Conde**, None; **M.P. Arce**, None; **M. Villarroya**, None; **J.M. Roda**, None; **M.G. López**, None; **A.G. García**, None.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.9

Topic: C.08. Ischemia

Support: Research made possible through the generosity of John A. and Cynthia Fry Gunn

M.G.W. is supported by the Gabilan Stanford Graduate Fellowship

Title: Development of a gene therapy system that targets ischemic stroke using bone marrow-derived immune cells

Authors: N. C. MANLEY[1], J. R. CASO[1], M. G. WORKS[1], G. SUN[3], I. ZEMLYAK[1], A. B. CUTLER[1], S. F. SORRELLS[2], F. V. ERMINI[1], S. CHANG[4], G. K. STEINBERG[3], *R. M. SAPOLSKY[2];
[1]Biol., [2]Stanford Univ., Stanford, CA; [3]Neurosurg., Stanford Univ. Sch. of Med., Stanford, CA; [4]Duke Univ. Sch. of Med., Durham, NC

Abstract: Introduction: As the leading cause of severe long-term disability, stroke is an important focus for biomedical research. Although a wide range of neuroprotective agents have been identified and tested in animal models of stroke, they have not yet translated to novel clinical treatments. A major obstacle for neuroprotective therapies is how best to access the brain, as this requires a delivery system that can bypass the blood-brain barrier and selectively target the injury site. To address this need, we developed a gene therapy system using bone marrow-derived cells as gene transporters, based on the hypothesis that these immune cells could achieve injury-specific targeting in the brain.

Results: We first demonstrated retrovirally-mediated gene transfer and secretion of gene cargo in bone-marrow derived cell cultures. In a rat stroke model, bone marrow-derived cells were then characterized for their injury-homing capacity, using bioluminescent and fluorescent tracking. Further profiling was performed on the cultured and injury-localized cell populations by flow cytometry and immunohistochemistry, respectively. We find that the proposed gene transport system can be constructed, that bone marrow-derived cells can rapidly migrate from the bloodstream to a stroke injury, and that delivery of transgene cargo using such a system can prove neuroprotective.

Disclosures: N.C. Manley, None; **J.R. Caso**, None; **M.G. Works**, None; **G. Sun**, None; **I. Zemlyak**, None; **A.B. Cutler**, None; **S.F. Sorrells**, None; **F.V. Ermini**, None; **G.K. Steinberg**, None; **R.M. Sapolsky**, None; **S. Chang**, None.

Nanosymposium

14. Ischemia: Therapeutic Approaches

Location: Room 24A

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 14.10

Topic: C.08. Ischemia

Support: NIH-NINDS NS-43165

NIH-NINDS NS-48350

NIH-NINDS NS-055832

Title: Mild sensory stimulation completely protects the adult rodent cortex from ischemic stroke

Authors: *C. C. LAY, M. DAVIS, C. CHEN-BEE, R. FROSTIG;
Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Mild sensory stimulation following permanent occlusion of the middle cerebral artery (pMCAO) has been shown to be completely neuroprotective 24 hours after ischemic onset in an animal model of stroke (Lay et al. 2010), but precisely when functional recovery took place during the post-occlusion 24hr period remained unclear. Here, we quantify the return of cortical function immediately following ischemic onset using intrinsic signal optical imaging, electrophysiological recording, laser speckle imaging of blood flow, and histological analysis in order to assess the relationship between protective sensory stimulation, recovery of cortical function, and reperfusion within the ischemic territory. By examining cortical function (i.e. the

spatial extent and amplitude of whisker functional representation) within the somatosensory cortex, we determined that 90 minutes of intermittent whisker stimulation delivered within 2 hours following pMCAO resulted in a return of function to pre-occlusion levels, and was accompanied by a significant reperfusion of the ischemic region via collateral vessels. In contrast, animals which received the identical whisker stimulation three hours after pMCAO never regained cortical function and sustained a major cortical infarct. Therefore, complete protection from impending ischemic stroke is possible within two hours of stroke onset whereas irreversible damage occurs when whisker stimulation is initiated three hours post-pMCAO. In summary, we found that the return of cortical function to pre-pMCAO levels by single whisker stimulation occurred 90 minutes following pMCAO, and was accompanied by a reperfusion of the imperiled region. These findings demonstrate the existence of a stimulus-induced neurovascular plasticity that confers complete protection of the ischemic territory; and that sensory induced protection is bounded within the first two hours (acute phase) of ischemia.

Disclosures: C.C. Lay: None. **M. Davis:** None. **C. Chen-Bee:** None. **R. Frostig:** None.

Nanosymposium

15. Apoptosis and Caspases

Location: Room 32B

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 15.1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS Postdoctoral Fellowship for Research Abroad

NIH grant R01 NS37878

NIH grant P01 HD29587

NIH grant R01 EY09024

NIH grant R01 EY05477

NIH grant R01 NS41207

NIH grant P30 NS057096

Title: Transnitrosylation of XIAP regulates caspase-dependent neuronal cell death

Authors: *T. NAKAMURA[1], L. WANG[2], C. C. L. WONG[3], F. L. SCOTT[1], B. P. ECKELMAN[1], X. HAN[3], C. TZITZILONIS[2], F. MENG[1], Z. GU[1], E. A. HOLLAND[1], A. T. CLEMENTE[1], S.-I. OKAMOTO[1], G. S. SALVESEN[1], R. RIEK[2], J. R. YATES[3], S. A. LIPTON[1];
[1]Sanford-Burnham Med. Res. Inst., LA JOLLA, CA; [2]The Salk Inst. for Biol. Studies, La Jolla, CA; [3]The Scripps Res. Inst., La Jolla, CA

Abstract: X-linked inhibitor of apoptosis (XIAP) is a potent antagonist of caspase apoptotic activity. XIAP also functions as an E3 ubiquitin ligase, targeting caspases for degradation. However, molecular pathways controlling XIAP activities remain unclear. Here we report that nitric oxide (NO) reacts with XIAP by S-nitrosylating its RING domain (forming SNO-XIAP), thereby inhibiting E3 ligase and antiapoptotic activity. NO-mediated neurotoxicity and caspase activation have been linked to several neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's diseases. We find significant SNO-XIAP formation in brains of patients with these diseases, implicating this reaction in the etiology of neuronal damage. Conversely, S-nitrosylation of caspases is known to inhibit apoptotic activity. Unexpectedly, we find that SNO-caspase transnitrosylates (transfers its NO group) to XIAP, forming SNO-XIAP, and thus promotes cell injury and death. These findings provide unique insights into the regulation of caspase activation in neurodegenerative disorders mediated, at least in part, by nitrosative stress.

Disclosures: T. Nakamura, None; **L. Wang**, None; **C.C.L. Wong**, None; **F.L. Scott**, None; **B.P. Eckelman**, None; **X. Han**, None; **C. Tzitzilonis**, None; **F. Meng**, None; **Z. Gu**, None; **E.A. Holland**, None; **A.T. Clemente**, None; **S. Okamoto**, None; **G.S. Salvesen**, None; **R. Riek**, None; **J.R. Yates**, None; **S.A. Lipton**, None.

Nanosymposium

15. Apoptosis and Caspases

Location: Room 32B

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 15.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Institutes of Health (NIH) Fogarty International Center (Grant R01TW008040).

National Research Foundation, South Africa

Title: Maternal separation increases markers of apoptosis in the striatum and frontal cortex

Authors: *W. M. DANIELS[1], L. MARAIS[2], D. J. STEIN[3];
[1]Univ. of Kwazulu-Natal, Durban, South Africa; [2]Univ. of Stellenbosch, Tygerberg, South Africa; [3]Univ. of Cape Town, Cape Town, South Africa

Abstract: Several studies have now shown that adverse events early in life can predispose humans to develop psychiatric disorders later in life. In studying this phenomenon we have shown that maternal separation in rats can lead to these animals displaying anxiety-like and depression-like behavior at a later stage. These behavioural abnormalities were associated with alterations in the neurochemistry of certain brain structures such as changes in neurotrophin expression in the hippocampus, striatum and frontal cortex. The aim of this study was to determine if maternal separation also affects markers of apoptosis in the hippocampus, striatum and frontal cortex. Rats were separated from their dams on postnatal day 2-14 for 3 h per day. Subsequent to MS, the rats were subjected to acute restraint stress or no stress, and levels of Bcl-2, p-Bad and caspase-3/cleaved caspase-3 were measured by Western blots. No differences were found in the hippocampus, but altered levels of the apoptotic markers were seen in the striatum and frontal cortex of separated animals. For instance in the striatum of non-stressed animals, the levels of Bcl-2 were increased while the levels of p-Bad were decreased. Animals that were subjected to subsequent restraint stress showed increases in p-Bad, casp-3 and cleaved casp-3 levels. These results were indicative of an increased risk for apoptosis in the striatum of separated rats.

Disclosures: W.M. Daniels, None; **L. Marais**, None; **D.J. Stein**, None.

Nanosymposium

15. Apoptosis and Caspases

Location: Room 32B

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 15.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: PKSFI Contract No. LEQSF (2007-12)-ENH-PKSFI-PRS-04

NSF Award # 0701491

Title: Modulation of apoptosis and cell growth in brain tumor glia and normal astrocytes by excitatory and inflammatory stimuli

Authors: *M. A. DECOSTER, K. COTTON, B. KARUMURI, H. ALSHAKHOURI, J.

LOPEZ, J. MCNAMARA;
Louisiana Tech. Univ., Ruston, LA

Abstract: Glutamate (Glu) is the main excitatory neurotransmitter for the mammalian brain, and has many functions in healthy, as well as pathological states. We have previously shown that Glu stimulation of normal astrocytes and glioma cells results in a marked delay in the effect of the apoptotic agent staurosporine (STS). It has also been reported that autocrine Glu signaling enhances glioma cell invasion, which suggests our findings may play a role in glioma invasion as well. Additionally, inflammatory factors such as the secreted form of phospholipase A_2 ($sPLA_2$) have been reported to be elevated in cancer. Here we examined STS induced apoptosis in the brain tumor CRL-2199 and CRL-2303 cell lines from American Type Culture Collection, rat hippocampal astrocytes (Lonza), and primary cultured adult rat cortical astrocytes, with modulation by Glu and $sPLA_2$. Using a protein extraction technique and specific colorimetric substrate for Caspase 3, cell lysates were tested for Caspase 3 activity, with Caspase 3 specific inhibitor, and Human Caspase 3 active enzyme providing specificity and experimental control benchmarks. Our findings show an inhibition and delay of apoptosis induced by STS due to pretreatment with 1 mM Glu. While cells treated with 0.4- 1 μM STS showed 50% or more increase in Caspase 3 activity by 12 hours, cells pretreated with glutamate did not, but did demonstrate a Caspase 3 peak at 24 hours. We had previously shown that $sPLA_2$ in combination with Glu has synergistic excitotoxic effects on neurons. Here we showed for the first time that $sPLA_2$ alone reduced cell death in brain tumor cells, while the combination of $sPLA_2$ and Glu was toxic to cells. In measurements of Glu in brain tumor cell cultures, we found that in the condition of KCl-mediated Glu release and elevated Glu due to exogenous addition, both treatments maintained elevated extracellular Glu concentrations for at least 24 hours, consistent with the lack of excitatory amino acid transporters in glioma cells. Numerical analysis and mathematical techniques were utilized to identify intracellular calcium concentration ($[Ca^{2+}]_i$) oscillations in mixed primary cultures of cortical neurons and astrocytes. We found that stimulation of cells with 50 mM KCl followed by Glu induced synchronized and non-synchronized $[Ca^{2+}]_i$ oscillations. We postulate that release of glutamate from glioma cells in brain tumors may alter neuronal network communication and lead to more Glu release, exacerbating neuronal injury and favoring glioma proliferation. Further, inflammatory mediators such as $sPLA_2$ which have been shown to injure neurons, which may also promote brain tumor survival under these conditions.

Disclosures: M.A. DeCoster, None; **K. Cotton**, None; **B. Karumuri**, None; **H. Alshakhouri**, None; **J. Lopez**, None; **J. McNamara**, None.

Nanosymposium

15. Apoptosis and Caspases

Location: Room 32B

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 15.4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: MH38752

Title: Glycogen synthase kinse-3 regulates ER stress-induced CHOP expression in neuronal cells

Authors: *M. A. MINES, G. P. MEARES, E. BEUREL, T.-Y. EOM, L. SONG, A. A. ZMIJEWSKA, R. S. JOPE;
Psychiatry & Behavioral Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Endoplasmic reticulum (ER) stress, often resulting from cellular accumulation of misfolded proteins, occurs in many diseases, particularly neurodegenerative disorders because of the relatively long lifetime of neurons. Excessive accumulation of misfolded proteins activates the unfolded protein response (UPR) that dampens protein synthesis and promotes removal of misfolded proteins to support survival of ER-stressed cells, but the UPR also initiates apoptotic signaling to kill cells if recovery is not achieved. Thus, there is much interest in identifying determinants of the life-death switch and interventions that promote recovery and survival. ER stress-induced apoptosis is promoted by glycogen synthase kinase-3 (GSK3) and is counteracted by GSK3 inhibitors. By examining where GSK3 intercedes in the ER stress-induced signaling pathway, we found that GSK3 promotes expression of the UPR-activated death-inducing transcription factor C/EBP homologous protein (CHOP/GADD153). This action, however, appears to be restricted to cells of neuronal lineage, as ER stress-induced CHOP activation is independent of GSK3 in fibroblasts and primary astrocytes. Facilitation of CHOP expression by GSK3 was not due to regulation of two major transcription factors, ATF4 and ATF6, which activate CHOP expression following ER stress. The accumulation of β-catenin that follows GSK3 inhibition was discovered to inhibit CHOP promoter activation. Collectively, one mechanism by which GSK3 promotes apoptotic signaling is by removing β-catenin-dependent repression of CHOP expression, thereby shifting cell fate towards termination.

Disclosures: M.A. Mines, None; **G.P. Meares**, None; **E. Beurel**, None; **T. Eom**, None; **L. Song**, None; **A.A. Zmijewska**, None; **R.S. Jope**, None.

Nanosymposium

15. Apoptosis and Caspases

Location: Room 32B

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 15.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: the Carolinas Healthcare Foundation

Title: Relationship of early reactive oxygen species (ROS) production to induction of apoptosis by 2,2'-azobis-2-methyl-propanimidamide (AAPH) , hydrogen peroxide (H_2O_2) and homocysteine (Hcy) in a differentiated motor neuron-neuroblastoma hybrid cell line, NSC-34_D

Authors: R. A. HEMENDINGER, E. J. ARMSTRONG, III, *B. R. BROOKS;
Neurol., Carolinas Med. Ctr., Charlotte, NC

Abstract: Oxidative stress has been implicated as a playing a role in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Hydrogen peroxide (H_2O_2) is commonly used to study cell death as a generalized oxidative stressor and is effective in NSC-34_D. The water-soluble azo compound, 2,2'-azobis-2-methyl-propanimidamide (AAPH) is a more specific free radical generator which is capable of generating peroxyl radicals and molecular nitrogen (N_2). The toxicity of AAPH, H_2O_2 and homocysteine (Hcy) was examined in NSC-34_D cells after a 24h exposure. Doses ranged from 0 to 5mM for AAPH, 0 to 100 μM for H_2O_2 and 0 to 500mM for Hcy depending on the assay being performed. Toxicity was assessed using nuclear staining, metabolic activity and ROS production. Analysis of variance and Fisher's LSD test were used to analyze the data at a p value of 0.05. Cell death increased in a dose-dependent manner with AAPH, reaching 46% at 3.0mM and increasing to 84% at 5.0mM ($p<0.05$). The mode of cell death was primarily via necrosis with AAPH. In contrast, Hcy induced primarily apoptotic cell death and H_2O_2 induced a combination of both necrosis and apoptosis. Metabolic activity assays demonstrated a significant dose-dependent reduction in metabolic activity at 24h with AAPH and H_2O_2 ($p<0.05$) while Hcy had no effect on metabolic activity. Preliminary studies examining the role of ROS in the cell death induced by these agents were performed. A dose-response induction of ROS during a 1h exposure to AAPH was observed in NSC-34_D cells. In contrast, Hcy failed to induce ROS up to 500mM dose while H_2O_2 only produced a slight induction of ROS in this assay with. Our data suggests that AAPH induces necrotic cell death and ROS generation in a dose-dependent fashion in the NSC-34_D cells. In contrast, both Hcy and H_2O_2 induce apoptotic cell death in NSC-34_D cells without substantial ROS generation during a 1h exposure. Oxidative stress induced by three different compounds leads to necrosis, apoptosis or both and may be related to the early rate of ROS production in these cells. Understanding how AAPH, H_2O_2 and Hcy modulate molecular targets may allow us to understand the processes involved in neuronal cell death.

Disclosures: R.A. Hemendinger, None; **B.R. Brooks**, None; **E.J. Armstrong**, None.

Nanosymposium

15. Apoptosis and Caspases

Location: Room 32B

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 15.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Molecular cascades activated by silencing of network activity, leading to neuronal death

Authors: *E. SCHONFELD-DADO, M. SEGAL;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Most neuronal networks burst spontaneously, both in-vivo and in-vitro. This activity appears to be essential for the survival of the network, as its chronic blockade leads to a slow death of the activity-deprived neurons. We are studying this type of neuronal death by exposing primary neuronal cultures to the sodium channel blocker, Tetrodotoxin (TTX), and monitoring the neurons over a period of up to two weeks. Already after 3-4 days of exposure to TTX, well before the neurons die, they begin to express markers that predict their eventual death, 10-14 days later. We found an elevation in the expression of the neuronal repressor, REST/NRSF (repressor element 1 silencing transcription factor/neuron restrictive silencer factor), a major regulator of neuronal properties that is also implicated in the neuronal differentiation of embryonic stem cells and in neurogenesis. REST is known to be regulated by all trans Retinoic Acid (ATRA), and indeed we found an elevation in the expression level of one of the enzymes involved in ATRA metabolism (Retinal dehydrogenase (RALDH)). Moreover, the reduction in neuronal activity and the subsequent neuronal death were correlated with the reduction in the expression of the activity-regulated transcription factor MEF2, which was shown to mediate neuronal survival and plasticity. The neuronal activity-deprived death was accompanied by enhanced glial transcription (as was evident by acetylated histone 3 staining) which could account for the elevation in RALDH expression and for the induction of the protease tissue plasminogen activator (tPA) which was apparent during the death process. Downstream to REST activation we detected a reduction in GluR2 expression and a persistent increase in intracellular calcium concentration, leading to the activation of the calcium-dependent protease, calpain, and of the apoptotic mediator, caspase 3. Inhibition of either calpain or caspase 3 activity protected neurons from the toxic action of TTX, indicating that they play a role in the slow death process triggered by the blockade of spontaneous activity of neuronal networks.

Disclosures: E. Schonfeld-Dado, None; **M. Segal**, None.

Nanosymposium

15. Apoptosis and Caspases

Location: Room 32B

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 15.7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Oriental Medicine R&D Project, Ministry of Health, Welfare & Family Affairs, Republic of Korea (B080027)

Title: Protective effects of cryptotanshinone against sodium nitroprusside-induced apoptosis by modulating scavenging markers in neuro2a cells

Authors: *M. RAMALINGAM[1], T. OH[2], J. JUNG[2], Y.-K. PARK[2];
[1]Oriental Med. Res. Institute, Col. of Oriental Medicin, [2]Dongguk Univ., Gyeongju, Korea, Republic of

Abstract: Cryptotanshinone (CRY), a diterpenoid tanshinone identified as major component derivative from *Salvia miltiorrhiza* Bunge, exhibiting a variety of biological activities. In the present study, we describe the neuroprotective potential of cryptotanshinone against sodium nitroprusside (SNP) toxicity for the first time. In cultured mouse neuro2a (N2a) neuroblastoma cells, higher than 400 μM SNP significantly decreased the cell viability, on the other hand, CRY treatment (1-50 μM) to normal cells did not induce any significant changes. For 20 h intoxication of SNP (500 μM) decreased the cell viability, increased the LDH release with intracellular ROS formation, when pre-treatment of 10 and 20 μM of CRY for 20 h dose-dependently reversed the changes. For mitochondrial membrane potential (ΔΨm), healthy normal and CRY treated cells forms complexes known as JC-1 aggregates and SNP induced apoptotic and unhealthy cells express low ΔΨm remains in the monomeric form and evidenced by fluorescence microscopy. We also demonstrated that SNP with or without CRY treatment modulates mRNA levels of antioxidants such as catalase (CAT), glutathione peroxidase (GPx1) and glutamate-cysteine ligase catalytic subunit (Gclc) but not in copper-zinc superoxide dismutase (SOD1). CRY enhanced the activities of SOD and GPx not in CAT. Meanwhile, CRY diminished the lipid peroxidation, protein carbonyls, reduced glutathione and oxidized glutathione contents increased by SNP toxicity as dose-dependent manner. Although both SNP and CRY treatment stimulates the phosphorylation of Akt and CREB signaling pathways. The results suggest that CRY possesses abilities to inhibit SNP-produced toxic concentrations of oxidative molecules through decreased risk of apoptotic symptoms and phosphorylation of Akt and CREB signaling pathway expressions in neuronal cells.

Disclosures: M. Ramalingam, None; **T. Oh**, None; **J. Jung**, None; **Y. Park**, None.

Nanosymposium

16. Neurodevelopmental Disorders I

Location: Room 10

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 16.1

Topic: C.06. Developmental Disorders

Support: Clinical and Translational Science Institute (CTSI) at the University of Florida

Title: Examination of the dopamine, adenosine, and glutamate striatopallidal heteromeric complexes in a mouse model of restricted repetitive behavior

Authors: *A. M. VAN MATRE[1], M. H. LEWIS[2];
[1]Psychology, [2]Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: Restricted repetitive behaviors are extremely common in many neurodevelopmental disorders and are one of the three diagnostic criteria for autism. The deer mouse (*Peromyscus maniculatus*) model of repetitive behavior is a particularly useful model because the repetitive motor behavior develops early, persists through much of their lifetime, and occurs spontaneously (i.e. without pharmacological or environmental challenge). Preliminary evidence from our lab indicates that the repetitive motor behavior exhibited by these mice is a result of an imbalance of activation between the direct and indirect pathways of the basal ganglia. The direct and indirect basal ganglia pathways work in an antagonistic fashion; activation of the direct pathway enhances basal ganglia output and promotes motor behavior whereas activation of the indirect pathway reduces basal ganglia output and inhibits movement. The imbalance between the direct and indirect pathways in the deer mice seems to be caused by decreased activation of the indirect pathway that allows direct pathway activation to over-excite the cortex. On neurons of the direct and indirect pathways there are heteromeric complexes of receptors that exhibit further antagonistic relationships. These receptor complexes include dopamine D2, adenosine A2A, and glutamate mGluR5 receptors on indirect pathway neurons. Activation of A2A and/or mGluR5 receptors reduces the functioning of D2 receptors. Since D2 receptors are negatively associated with adenylyl cyclase, we hypothesized that activation of A2A and mGluR5 receptors and antagonism of D2 receptors could reduce the expression of repetitive behavior by enhancing the functioning of the indirect striatopallidal neurons. In a series of pharmacological studies we examined the efficacy of a D2 receptor antagonist, an A2A receptor agonist, and a positive allosteric modulator of the mGluR5 at reducing repetitive behavior. Individually these drugs were each ineffective. However, combinations of these drugs (in cocktails of either two or three drugs) significantly lessened the expression of repetitive behavior. We are continuing our analyses of these heteromeric receptor complexes by examining membrane and intracellular levels of these receptors in mice that exhibit low and high rates of repetitive behavior. These data further suggest that decreased indirect pathway activation may mediate the expression of repetitive behavior and that targeting the heteromeric receptor complexes on the indirect pathway neurons may offer pharmacotherapeutic benefit for individuals with neurodevelopmental

disorders who exhibit restrictive repetitive behavior.

Disclosures: A.M. Van Matre: None. **M.H. Lewis:** None.

Nanosymposium

16. Neurodevelopmental Disorders I

Location: Room 10

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 16.2

Topic: C.06. Developmental Disorders

Support: Nancy Lurie Marks Family Foundation

Simons Foundation

Autism Consortium

Title: Reversal of a social behavioral deficit in a Pten haploinsufficiency mouse model of autism by modulation of serotonin receptor 2c

Authors: *D. T. PAGE[1,3], O. J. KUTI[1], A. FULLER[2], B. KARKI[1], M. SUR[1];
[1]Picower Inst. Learn, Memory, [2]Dept. of Biol., MIT, CAMBRIDGE, MA; [3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The PI3K and serotonin pathways have been implicated in the pathogenesis of the neuropsychiatric disorders autism and schizophrenia, both of which feature deficits in reciprocal social interaction. *PTEN* encodes a protein and lipid phosphatase that acts as a negative regulator of both PI3K and the serotonin receptor 2c (5-HT_{2c}R). Haploinsufficiency for *PTEN* is a risk factor for autism. Here we present evidence that 5-HT_{2c}R is hyperactive in the brain of *Pten* haploinsufficient mice and that activation of 5-HT_{2c}R via agonist is sufficient to reduce social approach behavior in wild type mice. We demonstrate that antagonism of 5-HT_{2c}R can rescue deficits in social approach behavior present in *Pten* haploinsufficient mice, and that administration of oxytocin, which is regulated by 5-HT_{2c}R, has a similar effect. These results indicate that dysregulation of 5-HT_{2c}R contributes to a neuropsychiatric-relevant phenotype in a mouse model of *Pten* haploinsufficiency. Furthermore, they show that treatment with a 5-HT_{2c}R antagonist can reverse this phenotype.

Disclosures: D.T. Page: None. **O.J. Kuti:** None. **A. Fuller:** None. **B. Karki:** None. **M. Sur:** None.

Nanosymposium

16. Neurodevelopmental Disorders I

Location: Room 10

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 16.3

Topic: C.06. Developmental Disorders

Support: C. W. Post Faculty Research Committee Award

Title: Eight brief seizures during early development cause long term impairment in hearing, learning and behavior

Authors: *J. C. NEILL, E. PAWUL;
Psychology, Long Island Univ., GREENVALE, NY

Abstract: Brief serial seizures in premature infants may have long term effects on learning, auditory discrimination, avoidance and exploratory behavior. 40 male Sprague Dawley rats were used in the study, 20 of which were treated with 8 brief flurothyl seizures on postnatal days (P) 6-9, simulating the effects of serial seizures during human prematurity. On P46, animals were tested on the elevated plus maze to measure their avoidance and exploration behavior. Seizure animals spent more time in the closed arms; made fewer head pokes and entries into the center compartment, displaying avoidance behavior and minimal exploration. Once the animals reached adulthood, they were trained on an auditory quality discrimination, that is, to discriminate between two sounds (S+ = white noise and S- = 2 kHz). A response during S+ led to food reinforcement, and responses during S- had no effect. Seizure animals required more time to earn 10 reinforcers on a VI 23 sec schedule of reinforcement during preliminary training. After sound discrimination training began, seizure animals acquired the sound quality discrimination slower, consistently responding more frequently during extinction and overgeneralizing during S- trials compared to non-seizure animals. Brief serial seizures early in life may go unnoticed and undocumented, however, they may cause a long term elevation in responding during extinction and overgeneralization during non-reinforced stimuli and abnormal avoidance behavior. Just eight brief seizures in premature infants may be responsible for retardation of the acquisition of language, emotional regulation and social skills.

Disclosures: J.C. Neill: None. **E. Pawul:** None.

Nanosymposium

16. Neurodevelopmental Disorders I

Location: Room 10

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 16.4

Topic: C.06. Developmental Disorders

Support: Division of Intramural Research, NIDCR, NIH.

Title: Loss of neuronal tissue-type plasminogen activator expression does not fully ameliorate lethal phenotype of forebrain-specific Cdk5 conditional knockout mice

Authors: *A. B. KULKARNI[1], S. TAKAHASHI[2], E. UTRERAS[1];
[1]Functional Genomics Section, LCDB, NIDCR, NIH, BETHESDA, MD; [2]Dept. of Pediatrics, Asahikawa Med. Col., Hokkido, Japan

Abstract: An essential role of cyclin-dependent kinase 5 (Cdk5) in brain development has been well demonstrated in Cdk5-/- mice, which show neuronal migration defects, neurodegeneration and perinatal lethality. We had recently reported on effects of Cdk5 deficiency in the postnatal brain in Cdk5 conditional knockout (Cdk5 cKO) mice, in which Cdk5 was selectively eliminated from neurons in the developing forebrain (Takahashi et al., Am. J. Pathol 176: 320-9, 2010). These Cdk5 cKO mice were viable but exhibited complex neurological deficits including seizures, tremors, growth retardation associated with the disruption of neuronal layering, and neurodegenerative changes in the forebrain accompanied by upregulation of the neuronal tissue-type plasminogen activator (tPA), a serine protease known to mediate microglial activation. In the current study, we have sought to identify the precise role of tPA in the Cdk5 cKO phenotype by generating and characterizing Cdk5; tPA cKO (2cKO) mice. These 2cKO mice exhibit a partial rescue in body weight gain and gliosis, but they still die about 3 weeks of age, similar to Cdk5 cKO mice. 2cKO mice also had increased locomotor activity with a distinct pattern that overlapped with Cdk5 cKO mice, and they showed decreased expression of neuronal NeuN protein. Thus, our results suggest that the increased expression of tPA observed in Cdk5 cKO mice does not contribute significantly to pathological processes leading to neurodegeneration. Supported by the Division of Intramural Research, NIDCR, NIH.

Disclosures: A.B. Kulkarni: None. **S. Takahashi:** None. **E. Utreras:** None.

Nanosymposium

16. Neurodevelopmental Disorders I

Location: Room 10

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 16.5

Topic: C.06. Developmental Disorders

Support: CIHR

FRSQ

FRMI

Title: Region specific-alteration of blood-brain barrier development caused by prenatal exposure to inflammation

Authors: *S. GIRARD, L. TREMBLAY, M. LEPAGE, G. SEBIRE;
Univ. of Sherbrooke, Sherbrooke, QC, Canada

Abstract: Objectives: Perinatal inflammation affects brain development and has long-term consequences. The permeability of the developing blood-brain barrier (BBB) of neonates has not been characterized such that treatment of brain damage using anti-inflammatory drugs is limited. So far, most of the animal studies testing the efficiency of neuroprotective drugs used invasive (e.g., intracerebral) injection. Transfer of treatment options to the clinic first requires a better understanding of the BBB development and of its permeability. We therefore undertook this study to evaluate postnatally the developing variations of BBB permeability and the modulation by prenatal exposure to inflammation.
Model: Rat pups were prenatally exposed either to saline (Ctrl) or to a bacterial component (lipopolysaccharide, LPS, 200 μg/kg/12h at the end of gestation, from G18 until birth). Anesthetized animals (isoflurane 1%) were imaged using a small-animal 7T MRI scanner from postnatal day 1 (P1) up to P30. A bolus of contrast agent (Gd-DTPA) was injected i.p. with simultaneous and continuous monitoring by T1-weighted images. Brains were harvested at the same time points as the scan, to correlate the in vivo data with histology and protein extracts. The transfer across the BBB of an anti-inflammatory treatment (recombinant human antagonist of IL-1 (IL-1Ra), 10 mg/kg, i.p.) was also correlated at each time point.
Results: We first studied the kinetic of BBB developmental permeability in Ctrl animals. Differences in permeability were detected between different regions of the brain at the earliest time point (P3). By P28 all regions were impermeable to the contrast agent. Prenatal exposure to LPS altered the BBB permeability in a region-specific manner. At P3, the cortex and hippocampus showed a more pronounced signal enhancement than other regions and this regionally increased level of permeability was higher than in Ctrl animals. The same experiments repeated later during development revealed a reduced BBB permeability. Those results were correlated with histology using markers of BBB permeability, glial cells development and also by studying the transfer of an anti-inflammatory drug across the BBB at the same time points.

Conclusion: We showed a window of increased susceptibility of the developing brain that was exposed prenatally to inflammation, due to region-specific increased in the permeability of the BBB. Such region-specific modulation of BBB permeability provides new insights into the mechanisms explaining the vulnerability to inflammation/aggressions in newborns leading to brain damage

Disclosures: S. Girard: None. **L. Tremblay:** None. **M. Lepage:** None. **G. Sebire:** None.

Nanosymposium

16. Neurodevelopmental Disorders I

Location: Room 10

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 16.6

Topic: C.06. Developmental Disorders

Support: NIMH Grant 1P50MH077248

Title: White matter differences in boys and girls with attention-deficit hyperactivity disorder

Authors: *S. SHEMMASSIAN[1], J. A. BROWN[2], A. GALVAN[1], R. POLDRACK[3], S. S. LEE[1], D. SHIRINYAN[1], E. MILLER[1], J. COHEN[1], R. BILDER[2], J. MCGOUGH[2], S. BOOKHEIMER[2], J. T. MCCRACKEN[2];
[1]Psychology, [2]Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA; [3]Psychology, The Univ. of Texas at Austin, Austin, TX

Abstract: Attention-deficit Hyperactivity Disorder (ADHD) is the most commonly diagnosed childhood neurobiological disorder and occurs in 3-8% of school-aged children. Although it is 3-5 times more prevalent in boys, ADHD in boys and girls is equally associated with impairment across academic, emotional, and social domains. However, some but not all data suggest that among children with ADHD, boys are more likely to be rated as more hyperactive than girls, and girls are rated as more inattentive than boys (Gershon, 2005). Using diffusion tensor imaging (DTI), Pavuluri et al. (2009) observed decreased structural connectivity in the anterior corona radiata, anterior limb of the internal capsule, and superior region of the internal capsule in ADHD compared to controls. Moreover, children with ADHD exhibit less structural connectivity in the right premotor, right striatal, right cerebral peduncle, left middle cerebellar peduncle, left cerebellum, and left parieto-occipital areas (Ashtari et al., 2005). Nonetheless neuroimaging studies examining sex differences in ADHD neuroanatomy are scarce. Yang et al. (2008) reported smaller left posterior cingulum and right precuneus in girls, but a non-significant interaction effect of diagnosis and sex on gray matter and white matter volume. However, there

are no published studies of sex differences in white matter integrity in children with ADHD. Thus, we used DTI to analyze potential sex differences in 37 boys and 16 girls with ADHD. Girls with ADHD had significantly lower fractional anisotropy (FA) in the left precuneus compared to boys ($p < .01$). However, relative to girls with ADHD, lower FA was observed in the left superior frontal gyrus, right posterior cingulate gyrus, right superior parietal lobule, left caudate, left pallidum, and right amygdala ($p < .01$) in boys with ADHD. These white matter differences may relate to core problems in behavioral and cognitive inhibitory control in ADHD, and the heterogeneity of FA findings between boys and girls could help elucidate sex differences in these domains. Specifically, lower FA observed in the left caudate and pallidum and right posterior cingulate gryus may be related to increased hyperactive and impulsive behaviors in boys, while lower FA in the left precuneus may contribute to greater inattentive symptoms in girls. Examination of FA values in relation to cognition and symptoms may shed light on the role of white matter alterations in ADHD symptom severity and profile. These data suggest that ADHD symptoms in boys and girls reflect sex-related white matter abnormalities, and call attention to possible differences in neurobiological pathways associated with ADHD between sexes.

Disclosures: S. Shemmassian: None. **J.A. Brown:** None. **A. Galvan:** None. **R. Poldrack:** None. **S.S. Lee:** None. **D. Shirinyan:** None. **E. Miller:** None. **J. Cohen:** None. **R. Bilder:** None. **J. McGough:** Other Research Support; Eli Lilly & Co.. Speakers Bureau/Honoraria; Eli Lilly & Co., Shire Pharmaceuticals. **S. Bookheimer:** None. **J.T. McCracken:** Other; Seaside Therapeutics, Bristol-Myers Squibb, Shionogi.

Nanosymposium

16. Neurodevelopmental Disorders I

Location: Room 10

Time: Saturday, November 13, 2010, 1:00 pm - 2:45 pm

Program Number: 16.7

Topic: C.06. Developmental Disorders

Support: RO1 NS038461

Title: Vestibulo-cerebellar phenotypes in Gbx2-CKO mutant mice revealed by contrast-enhanced micro-MRI

Authors: *K. U. SZULC[1], E. J. HOUSTON[1], R. V. SILLITOE[2], B. J. NIEMAN[3], A. L. JOYNER[4], D. H. TURNBULL[1];
[1]NYU Sch. of Med., New York, NY; [2]Neurosci., Albert Einstein Col. of Med., New York, NY; [3]Hosp. for Sick Children, Toronto, ON, Canada; [4]Developmental Biol., Sloan-Kettering Inst.,

New York, NY

Abstract: The cerebellum (Cb) is a brain structure characterized by complex, foliated morphology, and is best known for its essential roles in motor coordination, vestibular function and integration of sensory information. Numerous mouse models have been produced through gene targeting to elucidate the role of genetic and molecular factors in Cb development, including cerebellum-specific *Gbx2* conditional knockout (*Gbx2*-CKO) mice. *Gbx2*-CKO mice have variable deletions of vermis, the central region of the Cb most commonly affected in human cerebellar hypoplasias. Lacking in past studies of mouse Cb development and mutant phenotypes has been effective methods for analyzing three-dimensional (3D) morphology. The goal of this project was to develop and apply magnetic resonance micro-imaging (micro-MRI) approaches to analyze early postnatal CB development in normal and *Gbx2*-CKO mice. Using 3D image acquisition and volumetric analysis, we have quantified the vermis defects in individual *Gbx2*-CKO mutants, and have also discovered novel phenotypes in the vestibulo-cerebella of these mice. For *in vivo* imaging, we used 3D manganese (Mn)-enhanced MRI (MEMRI) at 100-µm isotropic (equal in 3 directions) resolution to visualize the Cb in individual mouse pups, between postnatal day (P)3 to P11, when the Cb undergoes dramatic changes in morphology to achieve the final foliation pattern. The results highlighted the timing of normal Cb patterning, and demonstrated a range of foliation defects in *Gbx2*-CKO mice, as well as changes in adjacent midbrain structures and a reduced volume of the flocculus-paraflocculus (FL-PFL) complex, the most lateral region of Cb. To visualize the Cb at higher resolution, *ex vivo* 3D micro-MRI was performed in overnight scans after perfusion-fixation of a chelated gadolinium (Gd) contrast agent, providing images of the Cb and surrounding structures at 50-µm isotropic resolution. Interestingly, the *ex vivo* approach allowed for effective visualization of the vestibulo-cochlear organ (VCO), the sensory organ critical for maintaining balance and spatial orientation, and that projects to the FL-PFL. *Ex vivo* micro-MRI demonstrated anatomical phenotypes in both the FL-PFL and the VCO of *Gbx2*-CKO mice. In summary, the combination of *in vivo* and *ex vivo* contrast micro-MRI methods provided 3D analysis of the developing Cb in mice, and has uncovered novel phenotypes in the vestibulo-cerebellar system in *Gbx2*-CKO mice. These findings underscore the potential role for micro-MRI in studies of the complex interplay between brain and sensory organs during development that are difficult to investigate using traditional methods.

Disclosures: K.U. Szulc: None. **E.J. Houston:** None. **R.V. Sillitoe:** None. **B.J. Nieman:** None. **A.L. Joyner:** None. **D.H. Turnbull:** None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.1

Topic: F.03. Motivation and Emotion

Support: HFSC

Title: A Drosophila model for alcohol reward

Authors: *K. KAUN[1], R. AZANCHI[1], J. HIRSH[2], U. HEBERLEIN[1];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]Univ. of Virginia, Charlottesville, VA

Abstract: The rewarding properties of drugs are essential to the development of abuse, dependence and addiction. However, much of the genetic and molecular mechanisms underlying the motivational properties of drug reward are poorly understood. Here we present a new paradigm for the incentive-motivational properties of alcohol in the genetically tractable model organism, Drosophila melanogaster. We show that opponent processes mediate the motivational properties of ethanol: flies are repelled from olfactory cues associated with ethanol intoxication in the short term but are attracted to these cues in the long-term, even when challenged with an aversive barrier to these cues. We demonstrate the importance of dopaminergic systems in maintaining this conditioned preference for ethanol, and reveal the brain regions that mediate this response. We are currently screening a large collection of mutants to isolate genes that prevent development of conditioned preference. Our results thus establish Drosophila as an attractive organism to study the genetic and neural mechanisms underling the motivational properties of ethanol.

Disclosures: K. Kaun, None; **R. Azanchi**, None; **J. Hirsh**, None; **U. Heberlein**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.2

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA013522

NIH Grant AA007611

NIH Grant AA007462

Title: The effects of ethanol intake by alcohol-preferring (P) rats using a drinking-in-the-dark_multiple-scheduled-access (DID-MSA) procedure

Authors: *R. L. BELL[1], Z. A. RODD[2], R. J. SMITH[2], K. K. MCCONNELL[2], J. E. TOALSTON[2], W. J. MCBRIDE[2];
[1]Psychiatry, Indiana Univ. Sch. of Med., INDIANAPOLIS, IN; [2]Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: The drinking-in-the-dark_multiple-scheduled-access (DID-MSA) protocol induces binge-like ethanol (E) intake in mice and rats. Here we examined the amounts ingested by alcohol-preferring (P) rats during this binge-like drinking and its consequential blood alcohol concentrations (BACs) and a measure of intoxication. P rats had access to 15% and 30% E concurrent with water and food 5 or 7 days a week, with three or four 1-hr sessions across the dark cycle. The 1st hr of E access was the 1st hr of dark, and each subsequent hr of E access was separated by 2 hrs of E deprivation. Adolescent [post-natal day (PND) 30] and adult (PND 90) female P rats will consume 3.4 ± 0.2 g/kg and 1.6 ± 0.06 (mean ± SEM) g/kg of E, respectively, during the 1st hr of E access (averaged across the first 5 days). Adolescent and adult male P rats will consume 3.5 ± 0.2 g/kg and 1.1 ± 0.07 g/kg of E, respectively, during this same period. In general, adult intakes increase to ~2.0 g/kg/hr and adolescent intakes decrease to ~2.5 g/kg/hr across 6 weeks of E access. In a separate group of adolescent male P rats, BACs were assessed in 15-min intervals across the 3rd hr of access on the 11th or 15th day of DID-MSA access 7-days a week. The results indicate pharmacologically relevant BACs are readily achieved (E, g/kg; BAC, mg%): 15-min 1.7 ± 0.2 g/kg; 57 ± 10 mg%, 30-min 2.1 ± 0.2; 92 ± 12, 45-min 2.3 ± 0.2; 76 ± 10, 60-min 2.7 ± 0.3; 100 ± 14. When adult female P rats were assessed for intoxication/motor impairment after the 1st hr of the 1st day of the 4th week of E access, using an Oscillating bar (O-Bar) apparatus, intake was 2.9 ± 0.1 g/kg/hr and E and water control rats had 102 ± 5 vs. 119 ± 1 sec latencies to fall off of the O-Bar (120 sec cut-off). These results indicate that the DID-MSA procedure induces binge-like E drinking by both adolescent and adult P rats of both sexes, which result in intoxication and BACs exceeding NIAAA's definition of binge-like drinking (i.e., > 80 mg%). We are presently using this model to assess the long-term consequences of binge-like E drinking in adult and adolescent rats.

Disclosures: R.L. Bell: Research Grant; NIAAA AA013522. **Z.A. Rodd:** None. **R.J. Smith:** None. **K.K. McConnell:** None. **J.E. Toalston:** None. **W.J. McBride:** None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.3

Topic: F.03. Motivation and Emotion

Support: DFG HE2597/4 - 3

DFG HE2597/7 - 3

BMBF grant 01GS08159

BMBF grant 01GQ0411

Title: Prefrontal cortex fails to learn from reward prediction errors in alcohol dependence

Authors: *S. Q. PARK[1,2,3], T. KAHNT[4], A. BECK[3], M. X. COHEN[5], R. J. DOLAN[6], J. WRASE[3], A. HEINZ[3];
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Abstract: Patients suffering from substance dependence fail to use the consequences of their actions to adjust their decision-making behavior. In other words, they seem to ignore behavioral consequences when making choices. Two hypotheses might account for the neural mechanisms underlying this impairment. First, updating the value of behavioral options could be impaired due to disrupted computation of reward prediction errors in the ventral striatum. Second, propagation of valuation signals from the striatum to the executive control regions in the prefrontal cortex could be impaired due to diminished functional coupling. Many theories in decision making have suggested these two possibilities, yet, no single study to date has provided empirical evidence that could adjudicate between these possibilities. Distinguishing between these hypotheses is crucial since therapeutical implications differ substantially depending on what system is impaired. Here we acquired fMRI data from abstinent alcohol dependent subjects and healthy controls during a reward-guided decision making task. We provide novel evidence that learning impairments in these patients are due to diminished functional connectivity between ventral striatum and dorsolateral prefrontal cortex, supporting the second of the hypotheses outlined above. In contrast, neural coding of prediction errors in patients ventral striatum remains intact. The importance of the deficit we highlight is reinforced by our observation that there is a dependent relationship between connectivity and learning performance and between connectivity and patients' ability to control their drug-craving.

Disclosures: S.Q. Park, None; **T. Kahnt**, None; **A. Beck**, None; **M.X. Cohen**, None; **R.J. Dolan**, None; **J. Wrase**, None; **A. Heinz**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.4

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Grant AA016648

INIA Consortium

Title: Ethanol-sensitive synaptic protein-protein interactions in mouse cortex

Authors: *G. GORINI[1], O. PONOMAREVA[1], M. D. PERSON[2], A. J. ROBERTS[3], R. D. MAYFIELD[1];
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Abstract: The development of ethanol dependence arises from a summation of effects on a number of brain targets. The Integrative Neuroscience Initiative on Alcoholism (INIA) consortium has recently investigated ethanol addiction by profiling gene expression through microarray screens: The identified targets include genes involved in neurotransmitter release/recycle machinery and vesicle fusion. Changes in synaptic transmission may therefore strongly contribute to ethanol addiction. Taking the information provided by gene expression work and expanding it to the translational level is the next necessary step in alcohol research. Indeed, the synaptic proteins encoded by these candidate genes cooperate with other proteins to form complexes and networks to accomplish the cellular function. The goal of the present study is to define these synaptic protein-protein interactions and to determine if these complexes can be modified as a result of ethanol excessive consumption.
We used interaction proteomics, which included co-immunoprecipitation, immunoblotting, and LC-MS/MS mass-spectrometry, to identify novel protein interactions in cortical membranes prepared from alcohol-naïve C57BL/6J mice using calcium-activated potassium channel (BKCa), dynamin-1, syntaxin-1A, synaptosomal associated protein of 25 kDa (SNAP-25), and synaptobrevin-2 (VAMP-2) as bait proteins.
Our results highlight novel important interactions among synaptic proteins, including the dynamin associations with BKCa and with VAMP-2. Plus, we found that BKCa, SNAP-25 and VAMP-2 share many interacting partners. We also report additional interacting proteins with diverse cellular functions, including trafficking, cytoskeletal, and ion channel-related proteins.
To evaluate the effect of excessive ethanol consumption on the identified interacting complexes of synaptic proteins, we are currently processing cortices from mice subjected to Withdrawal Induced Drinking, 2 bottle choice (WID-2BC) protocol. Using the same bait proteins for

immunoprecipitation followed by a semiquantitative mass spectrometric analysis (Isotope Tagging for Relative and Absolute Quantification, iTRAQ), we are testing if the interactions with the identified synaptic protein partners can be changed in response to the development of alcohol dependence.
Such an altered protein complexes scenario would likely affect trafficking, targeting, and synaptic function, and may underlie ethanol-sensitive phenotypes. Our data will contribute to the understanding of how ethanol excessive consumption alters cellular function and they could eventually help to define new molecular sites for drug treatment.

Disclosures: G. Gorini, None; **R.D. Mayfield**, None; **O. Ponomareva**, None; **M.D. Person**, None; **A.J. Roberts**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.5

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant K01 AA016849

Title: Patterns of concurrent alcohol and nicotine self-administration provide insights to co-abuse liability

Authors: *M. M. FORD;
Dept Behavioral Neurosci, Oregon Hlth. & Sci. Univ., PORTLAND, OR

Abstract: An estimated 90% of alcoholics use nicotine-containing products and smoking rates among alcoholics have remained elevated over the past few decades despite a decline in the general population. Current animal models of alcohol and nicotine co-abuse suffer from the inability to consistently demonstrate a nicotine-elicited potentiation of alcohol self-administration, complications associated with indwelling intravenous catheters for nicotine delivery, and an experimental design that fails to permit an animal to consume the drugs at a dose and rate that is self-regulated. The purpose of this work was to circumvent the concerns raised with prior animal models and to determine whether the consumption patterns for alcohol and nicotine can predict a propensity for future co-abuse. Thirty-two (four groups; n=8/group) male C57BL/6 mice were housed in lickometer chambers and provided daily 2-hr access to a sweetened ethanol solution (10% w/v sucrose + 10% v/v ethanol; 10S/10E) and one of four sweetened nicotine solutions as follows: 10S only (control group), 10S + 25 µg/ml nicotine (25N

group), 10S + 75 µg/ml nicotine (75N group), and 10S + 125 µg/ml nicotine (125N group). After 2-weeks of exposure the 25N, 75N, and 125N groups exhibited potentiated g/kg ethanol intakes of 22%, 41%, and 42%, respectively, when compared to the control group (at 4.0 ± 0.8 g/kg). During the third week nicotine-elicited increases in 10E were further potentiated in the 25N and 75N groups by 30% and 74% versus the control group (at 3.4 ± 0.7 g/kg). Nicotine-containing solutions were always approached first and consumed at a rapid rate over the initial 5-10 min of access. However, two prominent nicotine consumption phenotypes emerged: 1) mice that pre-loaded with nicotine and then dedicated the remainder of the session to ethanol intake and 2) mice that continuously consumed nicotine concurrently with ethanol throughout the session. These nicotine phenotypes exhibited a profound impact on 10S/10E drinking outcomes. For example, half of the mice in the 75N group were nicotine pre-loaders that consumed 1.8 mg/kg nicotine and 6.7 g/kg ethanol in 2-hrs whereas the other half were continuous nicotine drinkers that self-administered 5.5 mg/kg nicotine and 3.7 g/kg ethanol. Ongoing work with this model will be assessing the effects of nicotine withdrawal, a step-wise removal of the sweetener, and co-administration under a continuous access condition. An oral consumption model of concurrent alcohol and nicotine in mice reveals dynamic and interactive patterns of drug self-administration that will further our understanding of their co-abuse liability.
Supported by KO1 AA16849.

Disclosures: M.M. Ford: Research Grant; K01 AA016849.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant 5 R01 NS033123-10

Title: Differential response to ethanol induced ataxia between mouse strains

Authors: *S. T. HANSEN, S. M. PULST;
Neurol., Univ. of Utah, Salt Lake City, UT

Abstract: The cerebellum is the locus of control for motor movement and disturbance in cerebellar signaling results in abnormal motor ability. A hallmark feature of cerebellar dysfunction is uneven -ataxic- gait. Ethanol (EtOH) has been widely reported to depress normal cerebellar activity. EtOH induced ataxia can therefore be used to study ataxic motor dysfunction

in mice. However, using this approach to model ataxia in mice must take into account strain differences, as not all mouse strains exhibit the same physiological or behavioral response when challenged with EtOH.
Using the Digigait analysis system two common strains of mice were tested for gait abnormality under varying doses of EtOH intoxication. The Digigait apparatus consists of a clear plastic treadmill with high-speed under mounted camera for imaging paw prints. Age and weight matched mice from the C57Bl/6J (B6; n = 16) and 129SV/J (129; n = 17) strains were treated with three different doses of EtOH (1.75, 2.25 and 2.75 mg/kg EtOH). Testing began after 15 min but no later than 20 min from the time of EtOH administration.
No differences in gait were detected between genders; data were therefore collapsed by strain. Measures of ataxia indicated that the two strains of mice varied significantly from each other and as a result of increasing EtOH dose. Mice of the 129 strain showed a significant decrease in stride frequency and both strains of mice exhibited significant, yet opposite dose response slope changes in paw angle -B6 mice increased their paw angle; whereas, 129 mice decreased their paw angle- as a function of increasing EtOH dose. Both B6 and 129 mice increased time spent in hind-limb shared stance with higher doses of EtOH. On measures of stride length and stride frequency significant differences were detected only in the 129 strain.
These data highlight both similarities and surprising disparities in treadmill gait performance between B6 and 129 strains of mice on measures of EtOH induced ataxia. Although B6 mice often behaved similar to mice of the 129 strain, they rarely displayed differences that rose to the level of statistical significance. This was most likely not due to the doses of EtOH selected; rather these data should be weighed against the strain characteristics as evidenced by changes in paw angle placement between the two groups. These data are in general agreement with other motor assays e.g., rotarod, reporting B6 v 129 strain differences in response to EtOH. Although the Digigait was able to detect subtle differences of gait dysfunction between strains, caution should be exercised when testing mixed backgrounds on this instrument in lieu of data presented here.

Disclosures: S.T. Hansen, None; **S.M. Pulst**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.7

Topic: F.03. Motivation and Emotion

Support: NIAAA Grant F32AA018252

NIAAA Grant R01AA011852.

Title: Topographic pattern of ethanol-evoked dopamine release in striatal subregions

Authors: R. A. MANGIERI[1], *R. A. GONZALES[2];
[1]Pharmacol., The Univ. of Texas at Austin, Austin, TX; [2]Pharmacol., Univ. of Texas, AUSTIN, TX

Abstract: Acute intravenous (i.v.) infusion of ethanol has been shown to rapidly increase dopamine in ventral subregions of the striatum (nucleus accumbens core and shell) of male Long-Evans rats (Howard et al., 2008), but the effects on the dorsal striatum are unknown. To further investigate the effects of acute i.v. ethanol on dopamine concentrations in different striatal subregions, we performed in vivo microdialysis in male Long-Evans rats. Animals were implanted with microdialysis probes through guide cannulae aimed at either the dorsolateral (AP, ML, DV coordinates in mm relative to bregma: 0.0, 3.6, -1.0), dorsomedial (1.2, 1.8, -1.0), or ventral (2.2, 0.9, -3.8) striatum. We sampled from both the dorsolateral (n=6) and the dorsomedial striatum (n=9) because evidence suggests these two subregions play different functional roles in habit-related and goal-directed learning that could be relevant to ethanol self-administration. We sampled from the ventral striatum (n=4) as a positive control. We collected 5-min microdialysis samples for 20 min prior to and for 40 min after saline (1 ml/kg, i.v.) and ethanol (1 g/kg, i.v.) infusions. Each sample was analyzed for dopamine and ethanol concentrations. The time courses of ethanol concentrations were similar between all 3 subregions, as were peak dialysate concentrations (mean ± SEM: lateral, 4.5 ± 0.2 mM; medial, 5.0 ± 0.5 mM; ventral, 5.7 ± 0.4 mM). Basal levels of dopamine were comparable across subregions (mean ± SEM: lateral, 1.3 ± 0.1 nM; medial, 1.7 ± 0.1 nM; ventral, 1.3± 0.3 nM). However, we did find differential effects of the ethanol infusion on dopamine concentrations. The ethanol infusion was followed by elevated concentrations of dopamine in the ventral and dorsomedial, but not dorsolateral striatum. Interestingly, the time courses for the increases in dopamine concentrations were strikingly different between the ventral and dorsomedial subregions. In the ventral striatum, concentrations of dopamine peaked in the first 5-min following ethanol infusion. In contrast, dopamine in the dorsomedial striatum was not elevated within 5 min of the ethanol infusion. Rather, dopamine in this subregion was not significantly increased until 10-15 min after the infusion, and it remained elevated even after 40 min. Our findings are consistent with other studies (Imperato & DiChiara, 1986; Melendez et al., 2003), which have found discrepant ethanol-stimulated dopamine responses between striatal subregions. Furthermore, the divergent effects of ethanol may be reflective of the different putative roles of these areas in goal-directed or habitual ethanol self-administration behaviors.

Disclosures: R.A. Mangieri, None; **R.A. Gonzales**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.8

Topic: C.17. Drugs of Abuse and Addiction

Support: CAPES

CNPq

FAPESP

FAPESC

AFIP

Title: Plasticity of the dopaminergic mesocorticolimbic pathway underlies the increased resistance to ethanol addictive properties in cellular prion protein (PrP^C) null-mice

Authors: *D. RIAL[1], P. PANDOLFO[2], R. M. BITENCOURT[2], F. A. PAMPLONA[2], E. L. G. MOREIRA[2], K. M. MOREIRA[3], D. HIPOLIDE[3], P. A. DOMBROWSKI[4], C. DA CUNHA[4], R. WALZ[2], V. R. MARTINS[5], R. N. TAKAHASHI[2], R. D. S. PREDIGER[2];
[1]Univ. Federal De Santa Catarina, Florianópolis, SC, Brazil; [2]Univ. Federal De Santa Catarina, Florianópolis, Brazil; [3]Univ. Federal de São Paulo, São Paulo, Brazil; [4]Univ. Federal do Paraná, Curitiba, Brazil; [5]Inst. Ludwig de pesquisa para o câncer, São Paulo, Brazil

Abstract: Ethanol addiction has enormous individual and social importance and the responses related to this phenomenon mainly involve plasticity processes at the mesocorticolimbic system. Here we investigated the role of the cellular prion protein (PrP^C) in ethanol induced addictive phenotypes, the dopaminergic (DA) content and DA receptors density. Wild-type ($Prnp^{+/+}$) and PrP^C-null female mice ($Prnp^{0/0}$) were submitted to the following behavioral paradigms: climbing behavior, spontaneous locomotor activity (SLA), rapid tolerance (RT), conditioned place preference (CPP) and oral self-administration. DA content was evaluated by HPLC in the olfactory bulb (OB), prefrontal cortex (PFC), striatum (STR) and hippocampus (HIP). STR D1-receptor density was measured by autoradiography method. Ethanol pharmacokinetic was controlled by blood ethanol concentration and hepatic histology. C57BL/6 mice were evaluated in ethanol induced-SLA and in ethanol oral self-administration after i.c.v. treatment with anti-prion protein antibody. The absence of PrP^C significantly increased the climbing behavior, which was blocked when $Prnp^{0/0}$ mice were treated (i.p.) with SCH-23390 (D1-antagonist) or sulpiride (D2-antagonist). Alterations of DA levels (but not of norepinephrine and serotonin) were observed in the OB and PFC of $Prnp^{0/0}$ mice, but not in the STR and HIP, when compared to wild-type mice. $Prnp^{0/0}$ mice also showed increased SLA after 1, 7, 14 and 21 days receiving ethanol. The impact of PrP^C on ethanol SLA alteration was confirmed by the blockade of PrP^C after i.c.v. infusion of anti-prion protein antibody in C57BL/6 mice. $Prnp^{0/0}$ mice were not

susceptible to acquire rapid tolerance. In CPP the lowest ethanol dose tested (0.5 g/kg, i.p.) induced rewarding effects only in $Prnp^{0/0}$ mice, while the highest ethanol dose tested (2 g/kg, i.p.) induced rewarding effects only in $Prnp^{+/+}$ mice. $Prnp^{0/0}$ mice showed decreased ethanol (10 and 20% solutions) consumption when compared to control group. Treatment with SCH-23390, but not with sulpiride, blocked the ethanol intake in $Prnp^{0/0}$ mice. Pretreatment with anti-prion protein antibody also reduced ethanol intake in C57BL/6 mice. The autoradiography for D1 receptors showed diminished receptor density in the STR of $Prnp^{0/0}$ mice when compared to $Prnp^{+/+}$. Our results indicate the participation of PrP^{C} in ethanol addictive properties, probably related with the blockade of plasticity processes and the concomitant DA modulation at the mesocorticolimbic pathway. The suitability of the manipulation of this protein as a therapeutic strategy against alcoholism is advocated.

Disclosures: D. Rial, None; **P. Pandolfo**, None; **R.M. Bitencourt**, None; **F.A. Pamplona**, None; **E.L.G. Moreira**, None; **K.M. Moreira**, None; **D. Hipolide**, None; **P.A. Dombrowski**, None; **C. Da Cunha**, None; **R. Walz**, None; **V.R. Martins**, None; **R.N. Takahashi**, None; **R.D.S. Prediger**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.9

Topic: C.17. Drugs of Abuse and Addiction

Support: AA012355

AA005965

AA013521-INIA

AA011147

Title: The effects of an aldehyde dehydrogenase activator on blood ethanol levels in mice and brain ethanol kinetics in rats

Authors: *N. M. ZAHR[1,4], C.-H. CHEN[2], D. MAYER[3,4], D. MOCHLY-ROSEN[2], E. V. SULLIVAN[1], A. PFEFFERBAUM[1,4];
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Abstract: The first two steps in the body's breakdown of ethanol (EtOH) are carried out predominately by two key liver enzymes: alcohol dehydrogenase (ADH) converts EtOH to acetaldehyde, which subsequently is converted to acetate via aldehyde dehydrogenase (ALDH2). Acetaldehyde, a toxic and highly reactive molecule, has been implicated in both hepatotoxic and neurotoxic effects of EtOH. ALDH2 is polymorphic with two alleles encoding active (ALDH2*1) and inactive (ALDH2*2) enzymes. Individuals heterozygous and homozygous for ALDH2*2 exhibit significantly higher blood acetaldehyde levels following low to moderate alcohol consumption than individuals with the active isozyme, have a higher risk for cirrhosis, and an increased risk for Alzheimer's disease. A compound that can reduce acetaldehyde accumulation following EtOH intoxication could potentially slow down the progress of liver and brain pathology in chronic alcoholism and could also be used to counteract acute EtOH poisoning in emergency settings. Initial studies were carried out using agent A, a novel compound that promotes acetaldehyde clearance by activating ALDH2 to determine its effects on in vivo EtOH metabolism. The effects of agent A on blood alcohol levels (BALs) were explored in 30 mice. Mice (n=15) were first given a dose of agent A (80mg/kg) with a related molecule, agent A1 (38mg/kg) followed by a dose of EtOH (3g/kg), while 15 mice received 3g/kg EtOH alone, all by oral gavage. From blood collected 45min after the EtOH dose, BALs revealed a significant effect of agent A and agent A1 with BALs being reduced from 300±19 to 221±19mg/dL ($t(28)=2.9$, $p=.0075$) suggesting accelerated EtOH clearance. In a second experiment, in vivo EtOH kinetics were monitored in the rat brain using magnetic resonance spectroscopy (MRS) with a temporal resolution of 4s. The time course of EtOH brain concentration followed a consistent pattern characterized by rapid absorption, intermediate distribution, and linear clearance in 2 rats given 2.25g/kg EtOH I.V. In 3 rats given 100mg/kg agent A by oral gavage 15min before 2.25g/kg EtOH I.V., the time course of brain EtOH concentration was modified such that peak concentration was delayed and attenuated. These initial results support a role for agent A as an ALDH2 activator and its potential usefulness in the treatment of the pathological consequences of chronic alcoholism and acute EtOH poisoning.

Disclosures: N.M. Zahr, None; **C. Chen**, None; **D. Mayer**, None; **D. Mochly-Rosen**, None; **E.V. Sullivan**, None; **A. Pfefferbaum**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.10

Topic: C.17. Drugs of Abuse and Addiction

Support: USPHS Grant AA12882

Title: Role of melanin-concentrating hormone in hypothalamic control of ethanol consumption

Authors: *I. YAROSLAVSKY, G. CHANG, J. R. BARSON, S. F. LEIBOWITZ;
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Abstract: Recent reports support the involvement of hypothalamic orexigenic peptides in stimulating ethanol intake. The peptide melanin-concentrating hormone (MCH), produced mainly by cells in the lateral hypothalamus (LH), perifornical area (PF) and zona incerta (ZI), is suggested to have a role in the consumption of food and also of other rewarding substances, such as ethanol, sucrose and palatable diets. However, there is limited information on how and where these substances may themselves modulate the expression of endogenous MCH and also on the specific brain sites where MCH peptide administration acts to stimulate their consumption. Focusing on ethanol intake in Sprague-Dawley rats, the current investigation examined the impact of acute oral ethanol on MCH gene expression in the LH and ZI and also the effects on ethanol drinking behavior of MCH injections into different hypothalamic areas. In Experiments 1 and 2, quantitative real-time polymerase chain reaction (qRT-PCR) and *in situ* hybridization with a radiolabeled or digoxigenin-labeled probe were used to measure MCH expression in response to ethanol. Acute oral ethanol administration (0.70 - 2.7 g/kg) significantly stimulated MCH gene expression, and this occurred specifically in the LH but not the dorsal ZI region**.** In Experiment 3, the effect of MCH injection in brain-cannulated Sprague-Dawley rats on 6% ethanol consumption was examined. Compared to saline, MCH injected in the paraventricular nucleus (PVN) selectively stimulated ethanol consumption, without affecting food or water intake, but it reduced ethanol intake when administered into the LH while having no effect in the ZI. These results demonstrate that a brief exposure to ethanol stimulates MCH-expressing neurons specifically in the LH region and also suggest that MCH peptide, in turn, promotes further ethanol drinking behavior through projections to the PVN, a nucleus known to control consummatory behavior.

Disclosures: I. Yaroslavsky, None; **G. Chang**, None; **J.R. Barson**, None; **S.F. Leibowitz**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.11

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA # AA015434

NIAAA # AA016789

NIAAA # AA07462

Title: Alterations in home-cage locomotor activity induced by binge-like ethanol intake

Authors: *D. N. LINSENBARDT, S. L. BOEHM, II;
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Abstract: A recent rodent model of binge-like alcohol (ethanol) intake referred to as 'Drinking-in the Dark' (DID) was developed wherein C57BL/6J (B6) mice voluntarily consume large quantities of 20% unsweetened ethanol solution when given limited access during peak arousal. Acutely, these high ethanol intakes elicit BEC's ≥100 mg/dl and lead to significant behavioral ataxia as indexed by the balance beam (Moore et al., 2007) and rotarod tests (Rhodes et al., 2005; 2007). However, within-session DID-induced locomotor alterations and/or progressive changes in any such locomotor effects following repeated DID binge drinking behavior have not been examined. To evaluate this directly, we gave 2 weeks of ethanol (EE group) or water (WW group) access to male B6 mice using standard DID procedures and recorded home cage locomotor activity during the first (day1) and last (day14) access session. To further evaluate the observed alterations in locomotor activity in the ethanol consuming animals, we conducted a follow-up experiment with a group of animals that received water access for the first 13 days (days1-13) and then a single ethanol access session on the final day (day14; WE group). Home-cage locomotor activity from the first experiment indicated that DID ethanol access significantly increased locomotion on day 1(stimulation) and significantly decreased locomotion on day 14 (sedation) compared to water drinking controls. Results of the follow-up experiment indicated that the stimulant response on the first day of access in the ethanol consuming animals was likely the result of an interaction with the novelty of the DID tube and/or procedures as this stimulation was absent in the WE on day 14 following 13 days of exposure to DID procedures. These data suggest that 1) high ethanol intake using DID procedures leads to significant within-session motor effects and 2) that novelty of the DID procedures might fundamentally alter the relative 'experience' of intoxication.

Disclosures: D.N. Linsenbardt, None; **S.L. Boehm**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.12

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01AA017656

NIH Grant F31AA018915

NIH Grant R01NS030243

Title: Activation of alpha4* nAChRs is necessary and sufficient for varenicline-induced reduction of alcohol consumption

Authors: *L. M. HENDRICKSON[1,2], R. ZHAO-SHEA[2], P. D. GARDNER[1,2], A. R. TAPPER[1,2];
[1]Grad. Program in Neurosci., Umass Med. Sch., WORCESTER, MA; [2]Brudnick Neuropsychiatric Res. Inst., Umass Med. Sch., Worcester, MA

Abstract: Nicotine and alcohol are two of the most co-abused psychoactive drugs in the world leading to serious health consequences. Recently, the smoking cessation therapeutic varenicline, a nicotinic acetylcholine receptor (nAChR) partial agonist, has been shown to reduce ethanol induced DA release in the nucleus accumbens (NAc) and consumption in alcohol preferring rats. However, the mechanism by which varenicline reduces alcohol consumption and the nAChR subtype(s) involved are unknown. The goal of the present study was to localize and identify nicotinic receptor subtypes expressed in the ventral tegmental area (VTA) that may be involved in the response to alcohol and to determine if they play a role in the molecular mechanism by which varenicline reduces alcohol consumption. Here, we demonstrate that varenicline and alcohol exposure, either alone or in combination, selectively activates dopaminergic (DAergic) neurons within the posterior, but not the anterior, VTA. To gain insight into which nAChR subtypes may be involved in the response to alcohol, we analyzed nAChR subunit gene expression in posterior VTA DAergic neurons. Ethanol-activated DAergic neurons expressed higher levels of α4, α6, and β3 subunit genes compared to non-activated neurons. To examine the role of nicotinic receptors containing the α4 subunit (α4* nAChRs) in varenicline-induced reduction of alcohol consumption, we examined the effect of the drug in two complementary mouse models, a knockout line that does not express the α4 subunit (α4 KO) and another line that expresses α4* nAChRs hypersensitive to agonist (Leu9'Ala). While varenicline reduced alcohol consumption in wildtype (WT) mice, the drug did not significantly reduce consumption in α4 KO animals. Conversely, low doses of varenicline that had little effect in WT mice dramatically reduced ethanol intake in Leu9'Ala mice. Together, our data indicate that activation of α4* nAChRs is necessary and sufficient for varenicline reduction of alcohol consumption.

Disclosures: L.M. Hendrickson, None; **P.D. Gardner**, None; **A.R. Tapper**, None; **R. Zhao-Shea**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.13

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA07611

NIH Grant AA010721

NIH Grant AA019366

NIH Grant AA010717

Title: Chronic ethanol drinking by alcohol preferring P rats increased basal glutamate neurotransmission in the ventral tegmental area (VTA)

Authors: *Z. DING[1], S. HALL[2], E. ENGLEMAN[2], G. DEEHAN[2], Z. GU[2], J. WILDEN[3], W. MCBRIDE[2], Z. RODD[2];
[1]IPR, Sch. Med. IU, INDIANAPOLIS, IN; [2]IPR, Sch. Med. IU, Indianapolis, IN; [3]Dept. of Neurosurg., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Glutamate transmission in the mesolimbic system is involved in mediating the neurochemical and behavioral effects of ethanol. Acute administration of ethanol altered extracellular glutamate levels in the mesolimbic system and repeated ethanol exposure increased basal extracellular glutamate concentrations in the nucleus accumbens (NAc) in rodents. The current study tested the hypothesis that chronic ethanol exposure would increase basal glutamate neurotransmission in the VTA of rats. Alcohol preferring P rats were drinking alcohol for 8 weeks under a two-bottle choice (water vs 15% ethanol) paradigm. Wistar rats received repeated intraperitoneal injections of 1g/kg ethanol once a day for 7 days as a replacement of alcohol drinking due to the difficulty of Wistar rats to voluntarily drink alcohol. This procedure has been shown to increase extracellular glutamate concentrations in the NAc. On the day when rats received the last episode of alcohol exposure, microdialysis probes were inserted in the VTA. Glutamate no-net-flux microdialysis was conducted 16-18 hr thereafter. During microdialysis, four concentrations of glutamate (1, 5, 10 and 20 μM) were randomly perfused into the VTA and glutamate samples were collected and analyzed with HPLC-EC technique. At the end of the drinking session, P rats consumed approximately 5.9 ± 1.0 g/kg/day of ethanol. Chronic alcohol drinking by P rats significantly increased basal extracellular glutamate concentrations in the VTA compared to water drinking (8.1 ± 1.7 μM vs 5.6 ± 0.9 μM, $p < 0.01$, n = 7-10 / group)

without significantly altering the extraction fractions (22.1 ± 2.5% vs 22.4 ± 1.5%, $p = 0.89$). However, repeated ethanol injections in Wistar rats did not significantly alter extracellular glutamate concentrations (7.5 ± 1.7 uM vs 7.1 ± 2.6 μM, $p = 0.60$, n = 4-6 / group) or extraction fractions (19.0 ± 1.7% vs 21.6 ± 1.8%, $p = 0.33$) compared to saline injections. In addition, alcohol-naïve Wistar rats exhibited a trend of higher basal extracellular glutamate concentrations in the VTA than alcohol-naïve P rats (7.5 ± 1.7 μM vs 5.6 ± 0.9 μM, $p = 0.08$). Overall, the results suggest that increased basal glutamate transmission in the VTA may contribute to the maintenance of alcohol drinking of P rats.

Disclosures: Z. Ding, None; **S. Hall**, None; **E. Engleman**, None; **G. Deehan**, None; **Z. Gu**, None; **J. Wilden**, None; **W. McBride**, None; **Z. Rodd**, None.

Nanosymposium

17. Alcohol and Reward

Location: Room 2

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 17.14

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH-NIAAA P50 AA017072 (D.R.),

the State of California for Medical Research on Alcohol and Substance Abuse through the University of California, San Francisco (D.R.)

Title: The mammalian target of rapamycin: A new target for alcohol abuse disorders

Authors: J. NEASTA, S. BEN HAMIDA, Q. YOWELL, S. CARNICELLA, *D. RON;
Gallo Clin. & Res. Ctr., EMERYVILLE, CA

Abstract: Alcohol addiction is a chronically relapsing psychiatric disease, which is thought to be a maladaptive form of learning and memory. Long-lasting forms of synaptic plasticity and memory depend on new protein synthesis. The serine and threonine kinase, mammalian target of rapamycin (mTOR), has been implicated in synaptic plasticity, learning and memory by controlling protein translation *via* the phosphorylation of p70 ribosomal S6 kinase (S6K) and the eukaryotic translation initiation factor-4E binding protein (4E-BP) proteins. We found that excessive voluntary consumption of alcohol in mice and rats induces a prolonged activation of the mTOR-mediated signaling pathway in nucleus accumbens (NAc), a key component of the mesolimbic reward pathway that underlies the reinforcing actions of all drugs of abuse and alcohol. Accordingly, we found that certain synaptic proteins whose translation is regulated

through the mTOR signaling pathway are increased upon alcohol exposure in rat NAc. Furthermore, intra-NAc infusion of the FDA-approved inhibitor of mTOR, rapamycin, decreases binge, as well as sustained excessive, consumption of alcohol in mice and rats without affecting water intake. We further show that systemic administration of rapamycin to rats attenuates operant self-administration of alcohol. Self-administration of sucrose was not altered in response to rapamycin suggesting that the effect of the drug is selective for alcohol and does not reflect a general attenuation of motivation to obtain a reward. Finally, we provide data suggesting that rapamycin decreases alcohol seeking. Together, our results imply that mTOR within the mesolimbic reward pathway is a novel contributor to molecular mechanisms underlying alcohol-drinking behaviors. Importantly, these data put forward the possibility that targeting the mTOR signaling cascade is an innovative and valuable strategy for the treatment of alcohol use and abuse disorders.

Disclosures: J. Neasta, None; **D. Ron**, None; **S. Ben Hamida**, None; **Q. Yowell**, None; **S. Carnicella**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.1

Topic: D.02. Auditory

Support: Wellcome Trust (TDG, SB, CIP)

Newcastle University Faculty of Medicine (CIP)

MRC (Medical Research Council in the U.K., Strategic Life Skills Award, CIP)

Title: Cortical regions sensitive to sound movement in the monkey brain

Authors: *T. D. GRIFFITHS, S. BAUMANN, C. POIRIER, C. I. PETKOV;
Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Human auditory cortical regions to sound movement have been identified in a number of fMRI experiments that have measured the blood-oxygen-level dependent (BOLD) response (e.g., [1,2]). These experiments have implicated the non-primary auditory cortex for sound movement. We assessed the regional representation of sound movement using BOLD fMRI in a primate model: the macaque (*Macaca mulatta*). These species allow auditory fMRI activity to be

related to a detailed organisation of auditory cortical areas from which electrophysiological recordings are possible.
Using a primate-dedicated vertical MRI scanner (Bruker 4.7 Tesla), we recorded the BOLD activity response from two awake macaques during an auditory sparse-imaging design that was based on three randomly presented conditions: 1) silence; 2) sound movement, and 3) a stationary spatial control. Virtual-acoustic space (VAS) stimuli were created for fMRI by recording the pressure waveform within the ear canals of each individual, during the presentation of broadband noise (amplitude modulated at a rate of 80 Hz, at a distance of 0.5 m from the head) from different spatial locations in azimuth (-50 to +50 degrees). To confirm that the monkey could identify the spatial locations of the VAS stimuli, we played them back over headphones and observed that the monkey visually tracked the simulated sound locations. For the sound movement stimuli, the recorded sounds were combined to simulate a sound moving from left-to-right or right-to-left in front of the monkey's head. The stationary spatial control was based on the simultaneous presentation of the stationary sound locations between -50 and +50 degrees azimuth. The principal contrasts were between: 1) silence and the two sound conditions, and 2) between the sound movement and stationary sound conditions. Activity was related to detailed maps of the auditory cortical areas obtained using previously defined methods [3].
The contrast between silence and sound produced activation in auditory 'core' and 'belt' areas. The contrast between the moving and stationary spatial conditions resulted in increased activation posterior to A1 on the superior temporal plane, in the caudal belt. Our results in monkeys are consistent with those that have been obtained in humans, and suggest that primates possess regions sensitive to spatial movement in the posterior auditory cortical regions.
[1] Pavani F, Macaluso E, Warren JD, Driver J and Griffiths TD. (2002). Curr Biol *12*, 1584-1590.
[2] Warren JD, Zielinski BA, Green GG, Rauschecker JP, and Griffiths TD. (2002). Neuron *34*, 139-148.
[3] Baumann S, Griffiths TD, Rees A, Hunter D, Sun L, Thiele A. (2010) Neuroimage *50,* 1099-1108.

Disclosures: T.D. Griffiths, None; **S. Baumann**, None; **C. Poirier**, None; **C.I. Petkov**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.2

Topic: D.02. Auditory

Support: Wellcome Trust

Title: Anterior auditory core areas in macaques are specifically responsive to regular interval noise (RIN) at rates associated with human pitch perception

Authors: *S. BAUMANN, S. KUMAR, L. SUN, A. THIELE, T. D. GRIFFITHS;
Inst. of Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Pitch is a fundamental percept with a complex relationship to the spectral and temporal properties of the stimulus. It therefore is difficult to make inference about the neural correlates of pitch from simple sensory maps of stimulus properties such as frequency. Studies of ferrets, marmosets and humans [1,2,3] suggest representations that correspond to the pitch percept in auditory cortex but are not congruent with respect to the detailed organisation of such representations across areas. In this study we examined pitch-associated activity in a species with a similar hearing range to humans and multiple auditory areas organised within a superior temporal plane. We examined responses to RIN with rates below and above the lower limit of pitch in humans [4].
BOLD responses were recorded from the ascending auditory pathway and auditory cortex using a Bruker 4.7 T vertical scanner during fixation of a visual stimulus. Three auditory stimuli were presented at fixed rms level in a sparse imaging design: 1) fixed-amplitude random-phase broadband noise; 2) 8-Hz RIN and 3) 256-Hz RIN. In humans the 8 Hz is associated with a timbre change in the noise and the 256 Hz with a clear pitch. The RIN was based on 32 iterations of a delay-and add algorithm [4] to produce a highly regular stimulus associated with a strong pitch in humans.
Robust, bilateral BOLD increases for each sound were demonstrated in the inferior colliculus, the medial geniculate body and auditory core and belt areas in the cortex. A clear maximum in the response to all stimuli was recorded in the core field A1. The contrast between RIN at a rate of 256 Hz and control noise showed a highly significant increase of the BOLD response in the anterior core areas R (max: $t = 8.8$) and RT (max: $t = 8.0$) with a maximum in R. The same contrast was less significant in A1 (max: $t = 6.6$) and not significant in any subcortical structure. The contrast between RIN at a rate of 256 Hz and 8 Hz, showed an almost identical pattern of activity.
The data suggest a prominent role for the anterior auditory core areas R and RT in pitch analysis that is not explained by the stimulus manipulation used to produce RIN.
[1] Bizley JK, Walker KMM, Silverman BW, King AJ , Schnupp JWH. *J. Neurosci.* 29, 2064-2075 (2009).
[2] Bendor D, Wang X. *Nature* 436, 1161-1165 (2005).
[3] Patterson RD, Uppenkamp S, Johnsrude IS , Griffiths TD. *Neuron* 36, 767-776 (2002).
[4] Griffiths TD, Kumar K, Sedley W, Nourski K, Kawasaki H, Oya H, Patterson RD, Brugge JF, Howard MA. *Current Biology* (in press).

Disclosures: S. Baumann, None; **S. Kumar**, None; **L. Sun**, None; **A. Thiele**, None; **T.D. Griffiths**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.3

Topic: D.02. Auditory

Support: NIDCD: 1R01DC004290

National Center for Research Resources (NCRR): Grant Number UL1RR024979

Title: Comparison of intracranial electrophysiologic and BOLD signal responses recorded from human auditory cortex: A case report

Authors: *H. OYA[1], K. V. NOURSKI[2], J. XIONG[3], H. KAWASAKI[2], R. A. REALE[2,4], T. D. GRIFFITHS[5], J. F. BRUGGE[2,4], M. A. HOWARD, III[2];
[1]Neurosurg., Univ. of Iowa, IOWA CITY, IA; [2]Neurosurg., [3]Radiology, Univ. of Iowa, Iowa city, IA; [4]Psychology, Univ. of Wisconsin - Madison, Madison, WI; [5]Neurol., Univ. of Newcastle, Newcastle, United Kingdom

Abstract: Functional MRI (fMRI) is used extensively to study functional organization of human auditory cortex, with the assumption that blood oxygen level-dependent (BOLD) response provides an indirect measure of cortical neural activity. Previous studies performed in experimental animals and human subjects indicate that the relationship between BOLD signal changes and neural activity is complex, varies across cortical regions and may include a hemodynamic component that is not linked to local neural activity (Maier et al, Nat. Neurosci. 11:1193-200, 2008; Sirotin et al, Nature, 457:475-80, 2009). Knowledge of the relationship between human auditory cortex BOLD signal changes and neural activity requires that direct comparisons be made between electrophysiological and BOLD responses recorded from the same auditory cortical sites in the same experimental subject.
This is a case report of a subject with medically intractable epilepsy who underwent placement of intracranial electrodes. Auditory tone mapping fMRI experiments were performed prior to implantation surgery. T2-weighted volumes were acquired (3T Siemens Trio, 10 s TR, 68x68 matrix, 3.0 mm pixel size, 40 slices) using a sparse sampling paradigm. The auditory stimuli consisted of 6.4 s amplitude-modulated tones (500,1000 and 5040 Hz, 5 Hz modulation rate). Following surgery the same auditory stimuli, including scanner noise, were delivered to the awake subject as local field potential (LFP) recordings were obtained from electrode arrays positioned over the left superior temporal gyrus and within Heschl's gyrus (HG). Extensive tonotopic mapping (12 frequencies x 6 intensities) was also performed. LFP were analyzed by decomposing them in the time-frequency domain. A least-squares support-vector regression with linear kernel method was used to examine the correlation between electrophysiological and BOLD responses. Pearson product-moment correlation coefficients between tuning curves of

both modalities were also computed.
At auditory cortex sites, especially within the HG, where robust, tone frequency-tuned LFPs were recorded, strong correlations were consistently observed with stimulus frequency tuning of BOLD responses. Preferred frequencies derived from extensive tonotopic LFP mapping generally coincided with the BOLD response tuning. At other auditory cortex sites where weak, broadly tuned LFP responses were observed, BOLD responses were often robust, frequency tuned, and correlated poorly with the electrophysiological brain response. These findings demonstrate that correlations between LFP and BOLD responses are complex and variable across regions of human auditory cortex.

Disclosures: H. Oya, None; **K.V. Nourski**, None; **J. Xiong**, None; **H. Kawasaki**, None; **R.A. Reale**, None; **T.D. Griffiths**, None; **J.F. Brugge**, None; **M.A. Howard**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.4

Topic: D.02. Auditory

Title: Effects of microstimulation in the primate inferior colliculus on auditory perception: Implications for the auditory midbrain implant

Authors: *D. A. ROSS, J. GROH;
Duke Univ., DURHAM, NC

Abstract: The Inferior Colliculus (IC) has recently been tested as a candidate site for an auditory prosthetic device in several patients with bilateral damage to the auditory nerve (Lim et al. 2007). How best to harness the representation of sound frequency in this structure for maximum benefit to such patients is unclear. The initial group of patients perceived only a limited range of frequencies, all low, in response to stimulation. Unless this problem can be solved, the effectiveness of this prosthesis may be limited.
In this study, we tested the effects of microstimulation in the IC of hearing monkeys in a task in which they compared the stimulation-induced percept to real sounds. Our ultimate goal is to understand the relationship between the frequency-tuning properties at the stimulation site and the perceived frequency of the stimulation-induced percept. Our proximate goal is to establish the behavioral context and stimulation parameters necessary to investigate this question.
Monkeys were trained on a directional frequency discrimination task. The task began with a series of reference tones followed by a series of probe tones alternating with reference tones. The

task was directional because the monkeys were trained to respond one way if the probe tones were higher in frequency than the reference tones and another way if it was lower in frequency. The behavioral report consisted of "Go Now" or "Go Later" responses: immediate release or delayed release of a touch sensor.
We report here on the initial results of stimulation applied during presentation of the probe tone on 50% of the trials at 17 sites in the IC of one monkey. We found reliable effects on the psychometric function at about 80% of the sites stimulated with 20 microamps, and about half of the sites tested with 10 microamps. To date, the majority of sites tested favored low frequency sounds (i.e. below 1000 Hz), and the effect of stimulation was usually also to bias the monkey's choices in favor of low frequency sounds. Sites preferring higher frequency sounds were rare, which may account for the failure of human subjects to perceive high frequencies when receiving IC stimulation.

Disclosures: D.A. Ross, None; **J. Groh**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.5

Topic: D.02. Auditory

Support: National Institute on Deafness and Other Communication Disorders (NIDCD)

Title: Neural influence of vision on illusory filling-in of degraded speech

Authors: *A. J. SHAHIN[1], J. R. KERLIN[2], L. M. MILLER[2];
[1]OSU Eye and Ear Inst., Ohio State Univ., Columbus, OH; [2]Ctr. for Mind and Brain, Univ. of California, Davis, CA

Abstract: Illusory filling-in of degraded speech requires the brain to suppress its usual sensitivity to acoustic changes, such as at the onset and offset of interruptions. Since visual cues behaviorally enhance the auditory filling-in illusion, we hypothesized that neural sensitivity to speech interruptions will be reduced in the presence of congruent lip-movements. Using electroencephalography (EEG), we show that phase-locking of auditory theta band activity is suppressed at the offset, though not at the onset, of interruptions for congruent compared to incongruent conditions. This rendered the offset theta band of the illusory percept similar to that of the physically continuous stimulus. Moreover, increased right lateral-temporal phase-locking of theta and alpha bands followed the onsets of interruptions, except when the stimuli were

audiovisually incongruent, suggesting a higher-level process involved in initiating repair. Thus, vision alters the temporal fidelity of auditory processing during illusory filling-in, but this influence is contingent upon initial evidence of audiovisual incongruency.

Disclosures: **A.J. Shahin:** Employment; The Ohio State University, University of California Davis. Research Grant; National Institute on Deafness and Other Communication Disorders (NIDCD), The Ohio State University New Faculty Award. **J.R. Kerlin:** Employment; University of California. **L.M. Miller:** University of California. Research Grant; National Institute on Deafness and Other Communication Disorders (NIDCD).

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.6

Topic: D.02. Auditory

Support: ESRC/MRC 060250010

Title: Neural processing of social traits from voices: A multivariate searchlight fMRI study

Authors: ***P. MCALEER**[1], M. LATINUS[1], P. E. G. BESTELMEYER[1], A. TODOROV[2], P. BELIN[1,3];
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Abstract: Neural processing of social traits from faces involves two key traits: trustworthiness and dominance (Oosterhof & Todorov, 2008). However, limited work explores the same attributions in voices. Bruckert et al (2010) showed that similar to faces, averaged voices are deemed more attractive. Furthermore, Bestelmeyer et al (2009) found negative correlations between attractiveness ratings and BOLD activity in bilateral STG, i.e. Temporal Voice Areas (Belin et al, 2000). The present study uses a Searchlight analysis (Kriegeskorte et al, 2006) to investigate the neural processing of a range of social traits previously explored in faces. It was expected that the searchlight would show areas of brain activity correlated with behavioural ratings.
Participants (n=20) were scanned (3T Siemens) listening to male (n=32) and female voices (n=32) in a fast event-related design. Gaps in the sequence (TA/TR=1.5/2s) allowed for stimuli

to be presented in silence. Participants performed an orthogonal pure tone detection task to monitor attention. Two separate runs for both male and female voices were used, with a randomised stimulus order. Anatomical scans were also obtained. Analysis was first performed at the single subject level, followed by a group-level random effects analysis to give a beta-value stats map for each stimulus. A searchlight sphere was implemented across the whole brain for all maps (diameter = 7 voxels). Brain activity within each sphere was converted into a Representational Dissimilarity Matrix (RDM) across all voices, indicating the correlation in brain activity for each voice at every location.
A second group of participants (n=20) rated the voices between 0 and 1 using an analogue scale. Seven social traits were selected: attractiveness, competence, dominance, intelligence, prototypicality, trustworthiness and warmth. Ratings were z-scored and converted into RDMs for each trait. PCA analysis of the ratings showed a 1st component of dominance and a 2nd component involving trust, attraction and intelligence. A series of correlations were performed between behavioural and neural RDMs to locate brain areas in which the multi-voxel pattern of neural activity was significantly related to behavioural social ratings.
Preliminary results indicated weak but significant correlations between behavioural and neural RDMs: the strongest (range 0.3-0.35) were between bilateral activation in prefrontal cortex (B.A. 9) and ratings for attractiveness, dominance and warmth. These results validate the use of Searchlight analysis for the neural processing of social traits from voices, and give rise to comparisons with the processing of similar traits from faces.

Disclosures: P. McAleer, None; **M. Latinus**, None; **P.E.G. Bestelmeyer**, None; **A. Todorov**, None; **P. Belin**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.7

Topic: D.02. Auditory

Support: NIH Grant

Deafness Research Grant

Wellcome Trust Grant

Title: Selective responses to salient acoustic stimuli in nucleus basalis of the behaving ferret

Authors: N. D. LEACH[1], V. M. BAJO[1], A. J. KING[1], S. V. DAVID[2], S. A. SHAMMA[2], *J. B. FRITZ[3];
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Abstract: Neurons in the nucleus basalis (NB) are thought to play a key role in attention by modulating cortical receptive fields in sensory cortices to enhance responses to behaviorally-relevant stimuli. Encoding of behavioral salience in NB is likely to be influenced by limbic and para-limbic inputs to NB and also shaped by the projection NB receives from prefrontal cortex (PFC). Since an earlier study of activity in ferret PFC has shown selective encoding of task-relevant information (Fritz et al., 2010), we asked whether NB cells displayed similar responses in the same task paradigm. We initiated neurophysiological studies of NB in the head-fixed, behaving ferret, trained on a simple auditory discrimination task (distinguishing tones from noise bursts) using a conditioned avoidance paradigm (Fritz et al., 2003). Stereotaxic coordinates obtained from neuroanatomical studies (Bajo et al., 2009) were used to position electrodes. Putative electrode location in NB was confirmed by electrical stimulation, which elicited cortical desynchronization in primary auditory cortex (A1) and PFC, and shifted power in the EEG spectrum (decreased for lower frequencies and increased for higher frequencies). As in PFC, some NB neurons showed no response to any acoustic stimuli in a pre-behavior quiescent "non-task" condition but during behavior responded selectively to the functionally equivalent class of behaviorally relevant target tones. These target-selective responses could be excitatory or suppressive, and phasic or tonic. Response latencies varied from 50ms to >500 ms. Other neurons in the same location responded selectively to target tones in pre- and post-task passive conditions as well as during active behavior. These results are consistent with the hypothesis that during auditory task performance top-down signals activate a subset of NB neurons following target onset. Other nearby cells may have a longer-term memory of salient target stimuli that is not behaviorally gated. We conjecture that NB firing leads to broad activation of A1 during the target window by enhancing responses to incoming salient acoustic stimuli. This may reset synaptic weighting for those inputs, and hence re-shape A1 receptive field properties to optimize target stimulus detection. Thus attention may play a dual role, both initiating immediate changes in primary sensory cortices to target signals during task performance, and through the action of persistent cells in PFC and NB, sustaining these adaptive responses after behavior is completed.

Disclosures: N.D. Leach, None; **V.M. Bajo**, None; **A.J. King**, None; **J.B. Fritz**, None; **S.A. Shamma**, None; **S.V. David**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.8

Topic: D.02. Auditory

Support: DFG Collaborative Research Centre 618

Title: Neural adaptation in the auditory system: Competing demands for object recognition and localization

Authors: *K. J. HILDEBRANDT[1], R. M. HENNIG[1], J. BENDA[2];
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Abstract: Because the dynamic range of sensory systems is limited, they have to adapt to changes of the statistics of the relevant stimulus space. Sensory adaptation ensures precise and reliable encoding of natural stimuli despite differences of several orders of magnitude in mean intensity. By removing the mean intensity level, adaptation enables object recognition invariant of the local context. Thus, adaptation removes information from the sensory representation, as for example the mean intensity. However, sensory systems have to represent different features of a stimulus in parallel, and the information that is important for detection of these may differ. Consequently, adaptation should act differently in different parts of a sensory pathway.
We asked how adaptation mechanisms should act in a simple pathway that processes two features in parallel: in the auditory system of vertebrates and invertebrates, amplitude modulations are used for object recognition, while intensity differences between the two ears are informative on object localization. By analytically calculating the optimal response curves for both tasks we investigated how adaptation should ideally affect these response curves and whether adaptation should act primarily peripherally or centrally. The results show that for processing amplitude modulations, a peripheral removal of mean intensity by adaptation is desirable. This has indeed been found both in invertebrate and vertebrate model systems. However, peripheral adaptation removes information that is important for localization. Numerical simulations of a simplified binaural auditory pathway demonstrate that strong adaptation in central units greatly improves computation of locality. The onset of a stimulus is most informative for localization because the peripheral units have not been adapted yet. Later during the stimulus adaptation in the peripheral neurons deteriorates information on localization and adaptation in the central neuron ensures that it does not transmit this. In invertebrates and vertebrates alike there is evidence for such strong adaptation or negative feedback in the central units processing intensity differences.
Our modeling also makes predictions for the localization of stimuli that either slowly increase or decrease in amplitude. We present results from tests of these predictions for localization performance in human psychophysics experiments and behavioral studies in grasshoppers.

Disclosures: K.J. Hildebrandt, None; **R.M. Hennig**, None; **J. Benda**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.9

Topic: D.02. Auditory

Support: Intramural Research Program, NIMH/NIH

Title: Neural encoding of natural sounds in macaque auditory cortex probed with micro-electrocorticographic arrays

Authors: *M. FUKUSHIMA, R. C. SAUNDERS, D. A. LEOPOLD, M. MISHKIN, B. B. AVERBECK;
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Abstract: Primate auditory cortex consists of several highly interconnected sub-regions on the supratemporal plane (STP) and adjacent superior temporal gyrus (STG). The extent to which neural ensembles in these regions differentially encode acoustic information in natural sounds, such as species-specific vocalizations, is not well understood. To examine this issue, we recorded auditory evoked field potentials to macaque vocalizations (MVs) from three chronically implanted micro-electrocorticographic (μECoG) arrays in the left hemisphere of one monkey. Each μECoG array had 26 recording sites each 200 μm in diameter on a 3 x 3 mm grid of polyimide with 600 μm spacing. Two of the arrays were inserted into the lateral sulcus by retracting the banks of the sulcus (the first array on a part of STP estimated to be area A1, and the second one rostral to the first array to cover a part of area R). The third array was positioned lateral to the A1 array on the surface of the caudal STG, so as to cover a part of the parabelt (PB). We simultaneously monitored the surface potential from all electrodes on all three arrays, for a total of 78 sites. We first observed auditory stimuli elicited spatiotemporally propagating waves of negative potentials across the STP and STG, with the dynamics of the waves depending on the acoustic properties of the stimulus. We next focused on the auditory evoked responses to 20 MV stimuli (e.g. coo, grunt) as well as control stimuli in which the spectrum carrier from each MV stimulus was replaced by broadband noise (envelope-preserved "vocalization", EPV). To assess the differential encoding of MVs for individual channels, we used linear discriminant analysis of the evoked waveforms. For each of the 78 sites, a linear classifier was estimated using 4-fold cross validation to compute the percentage correct classification (PCC). The PCC averaged across the 26 channels within each array was 25.6±3.1%, 15.1±1.1%, and 17.0±0.9% (mean±2SEM) for the "A1", "R" and "PB" arrays, respectively (the chance level = 5 %). The average PCC for the EPV set was lower than that for the original MV set in "R" (11.4±0.8%) and "PB" (15.4±0.8%) while the performance was higher in "A1" (32.6±4.1%). These results suggest that complex spectral features of MVs contribute to differential encoding particularly in higher

auditory areas. Interestingly the percentage increase of the average PCC is significantly higher in "R" (33.4%) than in "PB" (10.5%), which suggests differences in processing species-specific vocalizations along caudal-to-rostral and medial-to-lateral axes.

Disclosures: M. Fukushima: Research Grant; Intramural Research Program, NIMH/NIH. **R.C. Saunders:** None. **D.A. Leopold:** None. **M. Mishkin:** None. **B.B. Averbeck:** None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.10

Topic: D.02. Auditory

Support: NIH Grant K99 DC010439

NIH Grant R01 DC005779

Title: Nonlinear temporal processing of natural sounds in auditory cortex

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Abstract: A major goal of sensory neuroscience is to understand how the brain represents and extracts information from complex natural stimuli. General predictive models that map the functional relationship between arbitrary complex stimuli and neural responses provide one approach to this problem. However, this approach has been limited by problems of dimensionality. Models that make few assumptions about mechanism tend to require a large number of parameters. Low-dimensional models that explicitly model important mechanisms in fewer parameters should, in theory, perform well, but knowing a priori which mechanisms are important is not possible. Here we describe an iterative approach that uses complex nonlinear models focused in a limited stimulus domain to identify key mechanisms that can subsequently be tested in a more general model framework.
Previous studies of primary auditory cortex (A1) have suggested that a major limitation of current models is their ability to predict the nonlinear temporal dynamics of neural responses. In recent experiments, we have collected data from A1 using a reduced-dimensionality stimulus composed of band-pass noise modulated by a natural sound envelope. This stimulus permits fitting models that span only a single spectral dimension, leaving more power to model complex

temporal integration. The resulting fits suggest that feedforward synaptic depression and interactions between co-tuned excitatory and inhibitory inputs both play a role in neural response dynamics. Ongoing work is implementing these mechanisms explicitly in more general spectro-temporal models.

Disclosures: S.V. David, None; **S.A. Shamma**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.11

Topic: D.02. Auditory

Support: Deutsche Forschungsgemenschaft, DFG, Grant SO 465/7-1 to ANS

Title: Statistical context other than stimulus range alters magnitude estimates of loudness

Authors: *A. SOKOLOV[1], P. GUARDINI[2,3], M. PAVLOVA[1];
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Abstract: Since the seminal work by Stevens (1956, 1975) on establishing the psychophysical law, sensory attributes of stimuli such as loudness of tones, have most commonly been assessed by magnitude estimation, in which participants directly assign a number to their current sensory impression (Gescheider, 1997). Magnitude estimates of sensations have been argued to be largely robust and resistant to context effects, although the stimulus range was shown to modulate the magnitude estimates of loudness of single tones presented within the context of a low or a high intensity range (Gescheider et al., 1991, 1992). Category judgments of visual stimuli were shown to exhibit frequency and primacy (serial presentation order) effects: For example, greater judgments of visual speed occur for identical ranges of stimulus speeds with on overall frequent low compared to high speeds, and with mainly low compared to mainly high speeds presented on the initial trials at the series outset (Sokolov et al., 2000). Here, we examined if magnitude estimates of loudness would vary as a function of the frequency of occurrence (base rate) and serial order of distinct tones taken from the same intensity range. Fifty six healthy adult participants were assigned to seven separate groups and estimated, without a modulus, a set of five tones (60, 65, 70, 75, and 80 dB SPL, sound pressure level) that were equally frequent and (1) randomized in a standard computer-assisted way, or randomized such that either (2) mainly soft or (3) mainly loud tones occurred at the series outset. With different-

frequent tones (frequency of occurrence, 20-14-8-4-4 or 4-4-8-14-20, soft tones come on the left), the randomization conditions were as follows: on overall frequent soft tones with either (4) mainly soft or (5) mainly loud tones presented at the series outset, and on overall frequent loud tones with either (6) mainly loud or (7) mainly soft tones presented at the series outset. The results indicate abundant primacy effects on magnitude estimates of loudness: Regardless of the overall frequency of occurrence, substantially higher estimates for the same tones occurred with mainly soft rather than mainly loud tones presented at the series outset. For the first time, we show the context (primacy) effects on magnitude estimates of loudness of tones taken from the fixed intensity range. The outcome questions the context invariance of psychophysical scales and the viability of a unique psychophysical law. Further research is required to determine if the neural mechanisms of the primacy effects on magnitude estimates are response bias or sensory dependent.

Disclosures: A. Sokolov, None; **P. Guardini**, None; **M. Pavlova**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.12

Topic: D.02. Auditory

Support: NIH Grant HD2080

Title: Subregional specialization for resolution of spectral and horizontal sound position cues in auditory cortex

Authors: *D. A. STORACE[1], N. C. HIGGINS[1], H. L. READ[1,2];
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Abstract: Accurate orientation to horizontal position of a sound depends heavily upon the interaural level difference (ILD) for sound reaching right and left ears. Conversely, accurate orientation to vertical sound position depends upon spectral distortions in sound caused by reflection off the head and pinna. Previous studies have suggested that many cortical spike rate responses to ILD and spectral cues add linearly to accurately predict the combined horizontal and vertical spatial receptive fields in primary auditory cortical neurons (A1). In the present study we examined the relationship between spectral and ILD tuning across A1, ventral (VAF) and suprarhinal (SRAF) auditory fields of the rat temporal cortex. Spectral response sensitivities were assessed with optical and electrode recording techniques. Spectral frequency response area

(FRA) tuning was determined from spike rate responses to a wide range of tone frequencies and average binaural levels (ABLs). Spike rate tuning to ILD was probed with noise over a wide range of ILDs and ABLs. Corresponding rate level curves were fit with linear, Gaussian and a modified sigmoid function, and "best fits" were determined from bootstrap estimations and comparisons of the corresponding residuals. ILD and FRA tuning widths were estimated and compared for each cortical site. Narrow spectral, ILD and ABL tuning was characteristic of VAF and caudal SRAF (cSRAF), but not A1 and rostral SRAF (rSRAF). These results suggest that ventral core and belt cortices (VAF and cSRAF) are specialized to discriminate horizontal and vertical sound position near the body midline.

Disclosures: D.A. Storace, None; **N.C. Higgins**, None; **H.L. Read**, None.

Nanosymposium

18. Neural Basis of Auditory Perception and Action

Location: Room 33C

Time: Saturday, November 13, 2010, 1:00 pm - 4:15 pm

Program Number: 18.13

Topic: D.02. Auditory

Support: NICHD2080

Office of Sponsored Programs University of Connecticut

Title: Enhanced binaural spike rate tuning to midline sound positions in ventral belt cortex

Authors: *N. C. HIGGINS[1], H. L. READ[1,2], M. A. ESCABI[1,2];
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Abstract: Attention, social interaction, predation and escape from predation are all behaviors that rely on the accuracy of sound localization. One of the primary cues for horizontal position of a sound is the interaural level difference (ILD) for sound reaching right and left ear. Though auditory cortex is needed for mammals to accurately localize sound position it is still unclear how localization cues are functionally organized at this level. Here we measured spike rate responses to monaural and binaural sound presentation with varying level conditions to probe a wide range of ILD cues available to the rat. Spike rate versus ILD level response curves were generated for a fixed range of average binaural level (ABL) conditions. Likewise, spike rate versus ABL level response curves were generated for a fixed range of ILD conditions. Corresponding rate level curves were fit with linear, Gaussian or modified sigmoid functions and the "best fit" was determined with automated bootstrapped predictions and analysis of residuals.

Most rate-ILD and rate-ABL curves were well approximated by some variation of a sigmoid including a peaked function for those sites that were well tuned to ILD. Sites with the narrowest ILD tuning functions had significant binaural facilitation and suppression for contralateral hemifield cues. In all cortices examined, ILD tuning width increased with Best ILD. SRAF had the highest percentage of sites with narrow midline ILD tuning shaped by nonlinear binaural facilitatory and inhibitory interactions. Theoretically, the nonlinear binaural interactions that characterize ventral belt cortex could provide the spatial tuning necessary to generate a spike rate code for 'attention' to sound source location near the body midline.

Disclosures: N.C. Higgins, None; **H.L. Read**, None; **M.A. Escabi**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.1

Topic: D.04. Vision

Title: Pinwheel cartography: A fundamental organizing principle of the human visual system

Authors: *B. BARTON, A. A. BREWER;
Univ. of California, Irvine, IRVINE, CA

Abstract: Introduction: One of the more important larger scale organizing principles of visual cortical organization is the visual field map: neurons whose visual receptive fields lie next to one another in visual space are located next to one another in cortex, forming one complete representation of contralateral visual space. As increasing numbers of visual field maps have been defined in human visual cortex, one question that has arisen is whether there is an organizing principle for the distribution of these maps across visual cortex. A basic approach that has worked for early visual cortex has been to define strings of visual field maps along contiguous strips of occipital cortex, with adjacent portions of the maps representing similar portions of space, but performing different computations. In V1, at an order of magnitude smaller than the visual field map, there exist orientation pinwheels, which are orderly organizations of neurons that have preferred tuning systematically spanning the full range of oriented lines (e.g., Bartfeld & Grinvald, 1992). We investigate the possibility that visual field maps are organized into similar circular clusters, which are replicated across visual cortex, oriented independently to one another, and subserve similar computations within a cluster.
Methods: We measured angular and eccentric retinotopic organization and population receptive fields across visual cortex using fMRI and population receptive field (pRF) modeling (Dumoulin

& Wandell, 2008). We model pRFs as 2-dimensional differences of Gaussians with preferred centers (x, y) and spreads (sigma), convolve the predicted response to the stimuli with the haemodynamic response function, and fit the best population receptive field independently to each voxel via a least-squares method. Retinotopic stimuli consisted of black and white, drifting bar apertures comprised of flickering checkerboards, 11° in radius.
Results/Discussion: We identify 16 new visual field maps in medial, lateral, and ventral occipital cortex, organized with previously defined visual field maps into 6 visual field map clusters: Occipital Pole (OP), V3A/B, Temporal-Occipital (TO), Inferior Temporal-Occipital (ITO), Ventral-Occipital (VO), and posterior Superior Temporal Sulcus (pSTS). We propose that these pinwheel clusters are a fundamental organizing principle of the human visual system, extending from low-level to higher-order visual processing areas.

Disclosures: B. Barton, None; **A.A. Brewer**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.2

Topic: D.04. Vision

Support: NIH R01EY09314

Title: The dynamics of contextual modulations in primary and secondary visual cortex

Authors: *A. M. SCHMID, Q. HU, F. MECHLER, I. E. OHIORHENUAN, K. P. PURPURA, J. D. VICTOR;
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Abstract: The detection of texture borders in the visual cortex likely involves multiple stages of processing. In the primary visual cortex (V1), texture borders may be captured as contextual modulation by processes such as surround suppression and facilitation. Neurons in secondary visual cortex (V2), on the other hand, respond more directly to texture borders. It is as yet unclear how contextual modulation in V1 and direct activation by borders in V2 relate to each other: are they independent of each other and serve different purposes or are they connected and jointly serve to detect texture borders? To gain more insight into this relationship, we investigated the dynamics of both types of nonlinearities.
We recorded from single neurons in V1 and V2 of anesthetized monkeys. The stimulus consisted of a 4 by 5 or 6 by 6 grid of adjacent rectangular regions, covering the classical and non-classical

receptive field. Each region contained sinusoidal gratings with either the preferred orientation or the non-preferred orthogonal orientation controlled by an m-sequence with frame durations of either 20 or 40 ms.
In V1, for frame durations of 20 ms, only positive interactions were observed indicating that the response is larger when neighboring patches have the same orientation, compared to when they differ. For frame durations of 40 ms, however, positive as well as negative interactions were observed in V1. Positive interactions, which indicate preference for continuous orientations across patches, occurred with a shorter latency than negative interactions, which yielded larger responses for orientation texture borders.
In V2, negative interactions, corresponding to larger responses to texture borders, were very robust for transient V2 neurons (Schmid et al., 2009) for both frame durations. Also, these interactions in V2, which are consistent with a spatial differentiation operation, began with the same latency as the contextual interactions in V1.
This study sheds light on the dynamics of contextual modulations in V1 and V2. Very short frame durations of 20 ms do not elicit negative interactions in V1 but do so in V2, suggesting that contextual modulation in V1 may require more temporal integration for texture borders than V2. Longer frame durations of 40 ms however, elicit negative interactions with the same latency in both areas. Thus, we conclude that the fast V2 responses to texture borders arise independently of the slower contextual modulations in V1.

Disclosures: A.M. Schmid, None; **Q. Hu**, None; **F. Mechler**, None; **I.E. Ohiorhenuan**, None; **K.P. Purpura**, None; **J.D. Victor**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.3

Topic: D.04. Vision

Title: V2 activation in response to figure-ground cues defined by orientation and motion

Authors: *M. D. ZARELLA, D. Y. TS'O;
Neurosurg., Upstate Med. Univ., SYRACUSE, NY

Abstract: It’s been shown that many cells in early visual cortical areas respond more strongly to stimuli composed of elements that differ from the background within some feature domain compared to full field homogeneous stimulation. These types of responses are thought to play an important role in scene segmentation and the segregation of figure from ground. In a previous

report, we demonstrated, using optical imaging of intrinsic signals in macaque V1, that the ensemble response had a similar tendency; stimuli with figural regions defined by orientation or motion discontinuities gave rise to stronger optical signals than those without. Here we extend these findings to area V2. We developed a statistical measure that compares the signals obtained from any two stimulus conditions, and find that regions of cortex corresponding retinotopically with the position of the figure are more strongly activated by orientation contrast and motion contrast stimuli than by homogeneous gratings. Beyond the figural region, however, no such enhancement exists. This result is consistent with our observations in V1. The expanse of cortex representing the figural region is much larger in V2 than in V1, and the strength of modulation appears to be greater in V2 as well. Interestingly, figural regions formed by orientation and motion cues traverse all functional stripe types, suggesting the capacity for segmentation throughout V2 in its entirety. The lack of specificity for such response modulation departs from the pattern of orientation columns in V2, implying that unoriented cells can participate in orientation contrast computations. This finding is corroborated by single-unit electrophysiology. These results have broad implications for how we think about the organization of scene segmentation in the cortex and the functional specificity of the feedback connections presumed to underlie it.

Disclosures: M.D. Zarella, None; **D.Y. Ts'o**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.4

Topic: D.04. Vision

Support: CIHR grant MOP-79352

FRSQ grant 13159

NSF grant CRCNS IIS-0904430

Title: Hierarchical processing of complex motion in the primate dorsal visual pathway

Authors: *P. MINEAULT[1], F. A. KHAWAJA[1], D. A. BUTTS[2], C. PACK[1];
[1]Neurobio., Montreal Neurolog. Inst., Montreal, QC, Canada; [2]Biol., Univ. of Maryland, College Park, MD

Abstract: While neuronal computations in early sensory areas have been thoroughly characterized, far less is known about neurons deep within association cortex, where the relationship between neuronal responses and sensory stimulation is necessarily indirect. Addressing this problem requires models that are more complex than those typically used to study low-level sensory areas, and fitting such models requires an ensemble of input stimuli that is rich enough to probe the complexity of the underlying processing.
One well-known example of complex cortical processing is the range of selectivities found in the medial superior temporal (MST) area of the primate visual cortex. Previous work has shown that MST neurons are highly selective for combinations of visual stimuli comprised of motion patterns such as expansion, deformation, translation, and rotation. Although this selectivity has been documented many times over the last 25 years, very little is known about the computations by which it is derived. In this work we show that the response properties of MST neurons can be can be understood based on the known hierarchy of the visual cortex. In particular, we recorded the responses of 61 MST to visual stimuli that were designed to efficiently map the space of first-order optic flow. We then fit the resulting data to various models, each composed of subunits that respond to local motion features.
We find that transforming the visual stimulus with subunits that approximate the behavior of neurons in the middle temporal (MT) area helps to elucidate the processing performed by MST neurons. Specifically it appears that MST neurons respond primarily to fairly simple conjunctions of excitatory and inhibitory inputs. We further show that a mathematically simple and biologically plausible form of nonlinear integration is required to capture the selectivity of MST cells to complex optic flow.
With this modeling framework in place we are able to explore particular hypotheses about the functional utility of MST neurons. By training an optimal linear estimator on the output of the MST population, we find that MST neurons convey highly accurate information about self-motion direction, as suggested by many previous experimental and theoretical studies. However, the more complex computational aspects of the model seem far better suited to recover the trajectories of moving objects, as they provide strong tuning for velocity with little dependence on other stimulus features. As a similar style of computation is found in other brain areas and in other species, we suggest that it represents a fundamental feature of sensory processing.

Disclosures: P. Mineault, None; **F.A. Khawaja**, None; **D.A. Butts**, None; **C. Pack**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.5

Topic: D.04. Vision

Support: NIH Grant EY018897

Title: Modular and sub-modular organizations of areas V2 and V4 revealed by HSL maps of selectivity and response strength

Authors: H. LIM[1], *D. J. FELLEMAN[2];
[1]Neurobio. and Anat., [2]Univ. Texas Med. Sch., HOUSTON, TX

Abstract: Intrinsic optical recording was used to investigate the functional organizations of areas V2 and V4 of macaque monkeys. Single condition responses to luminance contrast gratings, presented at 4-6 orientations, were used to calculate maps of preferred angle, orientation selectivity (orientation vector magnitude), and single condition peak response magnitude. Single condition responses to isoluminant hue stimuli that were evenly distributed around circular color space (4-8 color angles) were used to calculate similar maps of preferred hue, hue selectivity, and hue response magnitude. The three sources of data within each functional map type were then visualized using Hue-Saturation-Lightness (HSL) maps in which the preferred orientation (or hue) is illustrated by hue, the orientation (or color) vector magnitude (selectivity) is illustrated by saturation, and the peak single condition response magnitude is illustrated by pixel brightness. These HSL maps thus provide a powerful visualization tool for the delineation of the complex functional domains in V2 and V4.
HSL visualization of V2 luminance contrast orientation selectivity maps reveals prominent differences between stripe compartments; Thick and thin stripes are readily distinguished by both orientation selectivity and peak response magnitude while Type II and Type I interstripes are clearly distinguished by orientation selectivity. In addition, HSL maps reveal sub-modular organizations within both thin and thick stripes such that regions of high (thick stripes) or low (thin stripes) selectivity can be subdivided according to their peak single condition response magnitudes. While the significance of sub-modules in thick stripes is not fully understood, the sub-modules in thin stripes appear to coincide with hue-selective and luminance change-selective domains.
HSL visualization of V4 orientation maps reveals that the degree of orientation selectivity and single condition peak response magnitude vary across parafoveal V4. In many cases, 1-2 mm wide domains were observed that were both briskly responsive and highly selective for orientation. Other 1-3 mm domains could be distinguished by their brisk responses and weak selectivity, weak responses and high selectivity, or weak responses and selectivity. Ongoing studies will link these functional domains to their specific inputs from V2 Type I and II interstripes and thin stripes.

Disclosures: H. Lim: None. **D.J. Felleman:** Research Grant; NIH EY-018897.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.6

Topic: D.04. Vision

Support: NIH Grant 5T32NS07467

Title: Network reverberation of image sequences in alert monkey V4

Authors: *S. L. ALWIN, V. DRAGOI;
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Abstract: It has been shown that individual neurons in many brain regions display the same spiking pattern of previously evoked activity in the absence of further external stimulation - this is known as replay or reverberation. It has been proposed that neuronal replay is a mechanism of memory consolidation among neural ensembles and might be an inherent property of neurons in many cortical areas. Replay of neuronal activity has been found in the hippocampus and prefrontal cortex, but whether this phenomenon can be found in populations in visual cortex is unclear. Our objective was to induce the replay of neuronal activity in macaque area V4 by exposing multiple neurons to portions of natural images briefly flashed for 120 ms each in a random spatio-temporal sequence (the total stimulus duration was 3s). Each stimulus trial was followed by a blank trial of similar duration (each session had approximately 150 blank and stimulus trials). Additionally, we presented 30 blank trials at the beginning and end of each session to assess differences in activity with stimulation. Since the individual images stimulated the receptive fields of the neurons in a specific temporal pattern, the stimulus caused neurons to fire in a temporal sequence. Analysis of reverberation was performed by examining the neuronal responses across the population of cells in the blank trials that followed each stimulus presentation. Our main goal was to determine whether the temporal population response seen in the blank trials resembled the population response during the stimulus trials. We assessed the degree of similarity between the neuronal responses in the blank and stimulus trials by computing the correlation coefficient (cc) between the two sets of response patterns. Bootstrap analysis revealed significant correlations in 74% of all sessions. Furthermore, ccs between the prestimulus blank period and stimulation period were less than that found between the stimulus and blank periods, indicating a significant change in the population response pattern with stimulation. Additionally, we found when more than one sequence was presented the effect was stimulus specific, the effect was reduced when the population size decreased, and using a similar correlation analysis we determined that reverberation occurs in LFP activity. These findings demonstrate successful induction of reverberation in primate V4 in the absence of visual stimulation.

Disclosures: S.L. Alwin, None; **V. Dragoi**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.7

Topic: D.04. Vision

Support: IUAP P 6/29c

GOA /10/19

GSKE

EF /05/014

FWO

Title: Mapping 3D-shape processing in the macaque monkey

Authors: *I. C. VAN DROMME[1], P. JANSSEN[1], H. KOLSTER[1], W. VANDUFFEL[1,2,3]; [1]Neurofysiology, Kuleuven, Leuven, Belgium; [2]Athinoula A Martinos Ctr. Biomed Imaging, MGH, Charlestown, MA; [3]Radiology, Harvard University, Med. Sch., Boston, MA

Abstract: Higher-order disparity selectivity has been demonstrated in the lower bank of the rostral superior temporal sulcus (STS), called TEs (Janssen et al., 2000), and in a small part of the anterior intraparietal area (AIP) (Srivastava et al., 2009). Compared to the TEs neurons, AIP neurons were less sensitive to disparity discontinuities. Monkey fMRI-studies have revealed a strong activation in AIP to 3D-shapes (Durand et al., 2007). However, fMRI-activations in the inferior temporal cortex (IT) (recorded at1.5T with a single loop coil, Joly et al., 2007) were weak (due to reduced signal-to-noise ratio, SNR) and more posteriorly located than the TES region of single-cell studies. We scanned two macaque monkeys in a 3T Siemens MR scanner with an 8-channel phased-array coil (higher SNR) to visualize higher-order disparity sensitivity and to identify differences between IT and AIP in sensitivity to disparity discontinuities. We presented stereo random-dot stimuli in four different depth conditions using a block design: smoothly curved concave and convex surfaces (second-order disparity), linear and discrete approximations of these surfaces, and flat surfaces at different disparities (zero-order disparity). For every depth condition a control condition with no disparity was presented. The stimuli were displayed through red/green anaglyph glasses. Depth structure sensitivity was assessed by the interaction between the factors binocular disparity (disparity vs. no-disparity control condition) and depth order (zero-order disparity vs. second-order disparity). AIP and an adjoining portion of

LIP were strongly activated by depth structure (Joly et al., 2009; Durand et al., 2007). Furthermore depth-structure sensitivity was also found in CIP, F5, V3, V4t/TEO and the lower bank of the STS in IT. In agreement with Joly et al. (2007), the activations in IT were located more posteriorly than the TEs region (Janssen et al., 2000). We assessed the sensitivity for disparity discontinuities by computing the interaction between the factors binocular disparity (disparity vs. no-disparity) and smoothness (smoothly curved vs. linear approximation, and smoothly curved vs. discrete approximation). AIP and a small region in IT were significantly more activated by smooth surfaces compared to discrete approximations, whereas earlier visual areas (CIP, V4t/TEO and V3) did not show any differential activation. No significant differences were observed for the contrast smooth vs. linear approximations. These results indicate that the network of cortical areas involved in the processing of disparity-defined 3D shape may be more extensive than previously described.

Disclosures: I.C. Van Dromme, None; **P. Janssen**, None; **H. Kolster**, None; **W. Vanduffel**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.8

Topic: D.04. Vision

Support: FWO

GSKE

IUAP

EF

GOA

Title: The retinotopic organization of cortical areas on the inferotemporal gyrus in the macaque monkey

Authors: *H. KOLSTER[1], T. JANSSENS[1], W. VANDUFFEL[1,2];
[1]Lab. Neuro & Psychophysiol, KU Leuven, Leuven, Belgium; [2]Martinos Ctr. for Biomed. Imaging, MGH, Charlestown, MA

Abstract: The organization of visual areas on the posterior portion of the inferotemporal gyrus has been under debate since the original description of areas V4 and V4A (Zeki 1969) in this region. The organization was extended to the V4-complex (Van Essen and Zeki, 1978), and further expanded by area V4B (Pigarev et al, 2002). A recent study (Ungerleider et al., 2008), however, suggests a functional rather than a visuotopic subdivision of V4 in the macaque confining the visuotopy of the V4-complex to a single representation of the visual field in one dorsal and one ventral quarterfield.
Recent fMRI results (Kolster et al., 2009) yielded a field map associated with area PITd that is located ventrally to the MT field map cluster. The posterior limit of PITd was found to be a representation of the upper vertical meridian while the anterior border of dorsal V4, which covers the lower visual field, is a representation of a horizontal meridian. The mismatch between the borders and the observed gap between the two areas hints at the existence of additional retinotopically organized areas at the inferotemporal gyrus.
In order to investigate the detailed retinotopic organization in this region we used functional magnetic resonance imaging (fMRI) in awake macaques at 3T with 1mm isotropic resolution to identify and localize retinotopic areas anterior to areas V4d and V4v. We found field maps for eccentricity and polar angle in three monkeys and have reliably located eight representations of a quarterfield. These quarterfield representations are consistent with an alternating sequence of polar angle representations with matching representations of the meridians at the areal borders. The data supports the existence of retinotopic areas V4A consisting of a dorsal and ventral quarterfield and of hemifield representations in areas V4B, PITd, and PITv. These areas are grouped around a central eccentricity representation that is located on the inferotemporal gyrus and which is separate from the foveal confluence of visual areas V1, V2, V3 and V4. The number of quarterfield representations found between areas V3 and PITd/v equals the number found in the human (Kolster et al., VSS 2010) and the areas are observed in a similar topological relationship anterior and ventral to the MT field-map cluster.

Disclosures: H. Kolster, None; **T. Janssens**, None; **W. Vanduffel**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.9

Topic: D.04. Vision

Support: MEXT 19002010

Takeda Science Foundation

Title: Information flow between single neurons in macaque inferior temporal cortex: Granger causality analysis for spike trains

Authors: *T. HIRABAYASHI, D. TAKEUCHI, K. TAMURA, Y. MIYASHITA;
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Abstract: Functional connectivity between single neurons has been widely investigated using cross-correlation analysis of spike trains. The strength of functional coupling between single neurons in the visual cortex has been shown to change depending on the presented stimulus. However, the dynamics of directionality of the coupling between single neurons is poorly understood. Recently, Granger causality analysis has been utilized to investigate the directionality of information flow between interconnected areas for continuous neuronal signals. Here we conducted a non-parametric Granger causality analysis, which has been shown to directly applicable to spike trains (Nedungadi et al., 2009), to investigate dynamic changes in the directionality of information flow between simultaneously recorded pairs of single neurons in the monkey inferior temporal (IT) cortex. In a visual object discrimination task, spikes from neuron pairs with a displaced peak on the cross-correlogram (CCG) showed Granger causality in the gamma frequency range (30-80 Hz) with the dominance in the direction consistent with the CCG peak (forward direction). Although, in a classical view, displaced CCG peak has been interpreted as an indicative of a pauci-synaptic serial connection between neurons, temporal dynamics of the gamma Granger causality following stimulus onset exhibited more complex triphasic pattern, with a transient forward component followed by slowly developing backward component, and subsequent reappearance of the forward component. This triphasic dynamics of causality was not explained by the firing rate dynamics, and was not observed for cell pairs that exhibited a center peak on the CCG. Furthermore, temporal dynamics of Granger causality depended on the configuration of local features within the presented whole object. Together, these results demonstrate that the classical view of functional connectivity between cortical neurons could be expanded to incorporate more complex forward-and-back dynamics.

Disclosures: T. Hirabayashi, None; **D. Takeuchi**, None; **K. Tamura**, None; **Y. Miyashita**, None.

Nanosymposium

19. Extrastriate Cortex: Organization and Circuitry

Location: Room 1B

Time: Saturday, November 13, 2010, 1:00 pm - 3:30 pm

Program Number: 19.10

Topic: D.04. Vision

Support: NSF 0924636

Title: Modeling the representation of two-dimensional visual space using a neural population code

Authors: *A. B. SERENO[1], S. R. LEHKY[2];
[1]UT Houston Med. Sch., HOUSTON, TX; [2]Salk Inst., La Jolla, CA

Abstract: Although the representation of space is as fundamental to visual processing as the representation of shape, it has received relatively little attention. Here we develop a neural model of two-dimensional space and examine how the representation is affected by the characteristics of the encoding neural population (RF size, distribution of RF centers, degree of overlap, etc.). Spatial responses of the model neurons in the population were defined by overlapping Gaussian receptive fields. Activating the population with a stimulus at a particular location produced a vector of neural responses characteristic for that location. Moving the stimulus to n locations along the frontoparallel plane produced n response vectors. To recover the geometry of the visual space encoded by the neural population, the set of response vectors was analyzed by multidimensional scaling, followed by a Procrustes transform. The veridicality of the recovered neural spatial representation was quantified by calculating the stress, or normalized square error, between physical space and this recovered neural representation. The modeling found that large receptive fields provide more accurate spatial representations, thus undermining the longstanding idea that large receptive fields in higher levels of the ventral visual pathway are needed to establish position invariant responses. Smaller receptive field diameters degrade and distort the spatial representation. In fact, populations with the smallest receptive field sizes, which are present in early visual areas and, at a single cell level, contain the most precise spatial information, are unable to reconstruct even a topologically consistent rendition of space. Development of this neural model provides a general theoretical framework not only for understanding neurophysiological spatial data from dorsal and ventral streams, but also for testing how various neuronal parameters affect spatial representation.

Disclosures: A.B. Sereno, None; **S.R. Lehky**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.1

Topic: D.18. Brain-Machine Interface

Support: NIH Grant EY012978

Title: A retinal prosthetic strategy with the capacity to restore normal vision

Authors: *S. NIRENBERG, C. PANDARINATH;
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Abstract: Retinal prosthetics offer hope for patients with retinal degenerative diseases. There are currently 25 million people worldwide, who are blind or facing blindness due to these diseases, and there are few treatment options. Alternate therapies, such as drug and gene transfer approaches, are able to help some subpopulations - they can slow the degeneration down - but for the large majority of patients, their best hope is through prosthetic devices (reviewed in Chader et al., 2009). Current prosthetics, though, aren't yet able to restore normal vision: for example, they allow for perception of spots and edges, but not yet natural scenes. Efforts to improve prosthetic capabilities have been focusing largely on increasing the resolution of the device's stimulators (either electrodes or optogenetic transducers). Here, we show that a second factor is also critical: driving the stimulators with the retina's neural code. Using the mouse as a model system, we generated a prosthetic system that incorporates the code - this dramatically increased the system's capabilities, well beyond what could be achieved just by increasing resolution. Further, the results show, using 6000 optogenetically stimulated mouse ganglion cell responses, that the combined effect of using the code and a high-resolution stimulator is able to bring prosthetic capabilities out of the realm of simple image detection into the realm of natural sight.

Disclosures: S. Nirenberg: Other; Patent pending. **C. Pandarinath:** None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.2

Topic: D.18. Brain-Machine Interface

Support: NIH

NEI

Title: The effect of electrode-retina distance on threshold in Argus™ II epiretinal prosthesis subjects

Authors: *A. K. AHUJA[1], J. DORN[2], A. CASPI[2], M. MCMAHON[2], G. DAGNELIE[3], M. HUMAYUN[1], R. GREENBERG[2], ARGUS II STUDY GROUP[2];
[1]USC, Los Angeles, CA; [2]Second Sight Med. Products, Sylmar, CA; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: The Argus II epiretinal prosthesis has been developed to provide partial restoration of vision to subjects blinded from outer retinal degenerative disease. To date, the device has been implanted in multiple subjects with profound retinitis pigmentosa (RP) as part of phase I feasibility study (clinicaltrials.gov identifier: NCT00407602; active) at a number of clinical sites worldwide. Determining which factors affect electrical thresholds will inform surgical placement of the multielectrode array. Here we present data from implanted subjects showing a significant positive correlation between electrode-retina distance and electrical threshold of individual electrodes.
Electrode-retina distances were measured using spectral domain optical coherence tomography (SD-OCT). Single electrode thresholds to 250 ms duration, 20 Hz trains of 0.45 ms cathodic-first, biphasic pulses were measured using custom-developed software. The maximum charge density used was 1 mC/cm^2/phase.
Linear regression analysis showed a significant direct relationship between mean threshold and mean electrode-retina distance (R^2=0.50, p=0.0002; n=703 electrodes). Although a square-of-distance function might be expected to provide a better fit, there was no significant difference between R^2 values of linear and square-of-distance fits across individuals (two-tailed paired student's t-test, p>0.15). This linear dependence is consistent with in vitro studies done with similar disc diameters; this implies that large electrodes are less sensitive to retinal distance than smaller electrodes. Across all analyzed subjects (n=22), 90.3% of electrodes placed in direct contact with the retina and positioned within the macula (< 3 mm of fovea) had thresholds below 1 mC/cm^2. Given the fact that the subjects had severe retinal degeneration and no better than bare light perception, and disease genotype was not controlled for, it is encouraging to find such a high percentage of electrodes capable of eliciting electrical percepts. These data support that viability of the epiretinal approach in restoring vision. The significant percentage of electrodes with threshold allows for the consideration of using smaller electrodes in future implants for more focal stimulation of cells.

Disclosures: A.K. Ahuja: Consultant/Advisory Board; Second Sight Medical Products. **J. Dorn:** Employment; Second Sight Medical Products. **A. Caspi:** Second Sight Medical Products. **M. McMahon:** Second Sight Medical Products. **G. Dagnelie:** Other Research Support; Second Sight Medical Products. **M. Humayun:** Consultant/Advisory Board; Second Sight Medical Products. **R. Greenberg:** Employment; Second Sight Medical Products. **. Argus II Study Group:** Other Research Support; Second Sight Medical Products.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.3

Topic: D.18. Brain-Machine Interface

Support: NEI Grant 5R01EY012893-10

Title: The temporal dynamics of percepts generated by the Argus™ II epiretinal prosthesis

Authors: P. CHRISTOPHER[1], M. J. MCMAHON[1], U. PATEL[1], J. WEI[1], R. J. GREENBERG[1], *J. D. DORN[2];
[1]Second Sight Med. Products, Inc., Sylmar, CA; [2]Second Sight Med. Prod, SYLMAR, CA

Abstract: The Argus™ II epiretinal prosthesis provides partial restoration of vision to subjects blinded from outer retinal degenerative disease. The prosthesis, developed by Second Sight Medical Products, has been implanted in multiple subjects with profound retinitis pigmentosa (RP) as (clinicaltrials.gov identifier: NCT00407602; active) at a number of clinical sites worldwide. The system consists of an implanted array of 60 electrodes that electrically stimulates the retina in response to camera or computer control. The temporal features of visual percepts (phosphenes) generated in response to electrical stimulation vary among subjects, with some phosphenes fading before stimulation has ended. Here we present data from 29 implanted subjects characterizing the temporal dynamics of these phosphenes.
Experiments were conducted to investigate the effect of different stimulation parameters on the temporal persistence of phosphenes. On each trial, a group of four electrodes was stimulated with pulse trains consisting of biphasic square-wave pulses; current amplitude, frequency, pulse width, and interphase delay were varied across trials. Subjects were instructed to depress a key on a keypad when they saw a phosphene and hold it down until the phosphene disappeared, and to press the key again if the percept reappeared. The trial ended after the subject had not seen anything for 5 continuous seconds or after 15 seconds if the phosphene did not fade.
We found that the temporal dynamics varied widely across subjects. Some subjects perceived phosphenes that lasted for several seconds while others saw percepts for less than 1 second. Within subjects, we found that decreasing frequency led to longer persistence durations while increased charge had a small effect in increasing persistence. Despite the across-subject variance, persistence of phosphenes did not correlate with any measures of system performance, such as the ability to localize an object, determine the direction of an object's motion, or orientation and mobility when using the system. Subjects who experience short persistence have continued to use their system effectively at home and in the clinic. Previous studies have shown that the fading of phosphenes is the result of adaptation that occurs in the normal retina, so it is perhaps not surprising that our RP subjects experience this phenomenon as well. The variation in degree of this effect may be related to the health of a given subject's retina, but this has yet to be shown.

Disclosures: **P. Christopher:** Employment; Second Sight Medical Products, Inc. **M.J. McMahon:** Second Sight Medical Products, Inc. **U. Patel:** Second Sight Medical Products, Inc. **J. Wei:** Second Sight Medical Products, Inc. **R.J. Greenberg:** Second Sight Medical Products, Inc. **J.D. Dorn:** Second Sight Medical Products, Inc..

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.4

Topic: D.18. Brain-Machine Interface

Support: 5R01EY012893-10

Title: Manipulation of frequency and amplitude have separable effects on the size and brightness of percepts in a retinal prosthesis subject

Authors: ***D. NANDURI**[1], I. FINE[3], R. J. GREENBERG[4], A. HORSAGER[2], J. D. WEILAND[2]; [1]Biomed. Engin., [2]Dept. of Ophthalmology, Doheny Eye Inst., USC, Los Angeles, CA; [3]Dept. of Psychology, Univ. of Washington, Seattle, WA; [4]Second Sight Med. Products Inc., Sylmar, CA

Abstract: Purpose: In an effort to restore functional vision, subjects with severe retinitis pigmentosa have been implanted with an epiretinal prosthesis that elicit percepts, known as phosphenes, using electrical stimulation (Humayun, 1999). For single pulses, both phosphene brightness and apparent size increase with stimulus amplitude (Greenwald, 2009). Here we examine whether, for pulse trains, varying pulse train frequency and amplitude have separable effects on phosphene appearance.
Methods: Experiments were performed on a single subject with severe retinitis pigmentosa (implanted with a 16-channel prosthesis in 2004). Pulse trains were presented on 9 single electrodes, consisted of biphasic pulses and had a duration of 500 ms. We measured the brightness and size of phosphenes when the amount of 'effective charge' within a pulse train was varied by either modulating current amplitude between 1.25 - 6x threshold (while holding frequency constant at 20 Hz) or frequency varied between 13 - 120 Hz (while holding current amplitude constant at 1.25x threshold). Phosphene size was measured by calculating the area of images created from subject tracings of perceived phosphenes on a grid screen. Phosphene brightness was measured using a brightness rating procedure in which the subject compared the brightness of the phosphene to a reference stimulus (Stevens, 1957). Size and brightness judgments were made in different runs. Within a run each frequency/amplitude was presented 5 times in random order.

Results: When current amplitude varied, in all 9 electrodes the apparent size of phosphenes increased as a function of amplitude. However brightness only increased with amplitude in 5 electrodes. In the other 4 electrodes apparent brightness either did not increase with amplitude or increased in brightness as a function of amplitude with very shallow slope. When frequency varied, brightness increased as a function of frequency across all 9 electrodes, but percept size only increased in 2 electrodes. In the other 7 electrodes apparent size either did not vary with frequency or increased with very shallow slope.
Conclusions: Increasing stimulation amplitude always increases the size and often the brightness of elicited phosphenes in a single subject. However increasing frequency increases phosphene brightness, while generally having little effect on phosphene size. These data suggest it may be possible to develop stimulation protocols for encoding visual images that can independently manipulate the size and brightness of phosphenes. For example, frequency coding may provide a means of representing the brightness of visual images while keeping phosphene size constant.

Disclosures: D. Nanduri, None; **I. Fine**, None; **R.J. Greenberg**, Second Sight Medical Products Inc., Ownership Interest; **A. Horsager**, None; **J.D. Weiland**, Second Sight Medical Products Inc., Research Grant.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.5

Topic: D.18. Brain-Machine Interface

Support: UK EPSRC EP/H019472/1

NIH Pioneer Award 1DP1OD006409

NIH-NINDS-CRCNS-RO1

NSF, NDSEG, Stanford Med Scholars, Soros Fndn, HHMI, SGF

CDRF, BWF

DARPA RP2009

Title: A closed-loop human simulator for understanding feedback-control and its relevance for brain-machine interfaces

Authors: *J. P. CUNNINGHAM[1,2], P. NUYUJUKIAN[3,4], V. GILJA[5], C. A. CHESTEK[2], S. I. RYU[6], K. V. SHENOY[2,7];
[1]Engin., Univ. of Cambridge, Cambridge, United Kingdom; [2]Electrical Engin., [3]Bioengineering, [4]Sch. of Med., [5]Computer Sci., [6]Neurosurg., [7]Neurosciences Program, Stanford Univ., Stanford, CA

Abstract: By translating neural activity into control commands, brain-machine interfaces (BMI) seek to improve the lives of severely disabled people. There are many challenges in developing such a system, but all BMI share in common the need for a decode algorithm. Decode algorithms map recorded neural activity into physical commands. These algorithms are typically applied offline to neural activity previously gathered from a healthy animal, and the decoded arm reach is then compared to the true movement that corresponded to the recorded neural activity. However, this offline testing may neglect important features of a real BMI, most notably the critical role of feedback-control, which enables the user to adjust neural activity while using the BMI. We hypothesize that a full understanding of decoder design requires an experimental platform where the human learning machine (the brain and motor plant) is in closed-loop with the various candidate decode algorithms. It remains unexplored the extent to which the subject can, for a particular decode algorithm or parameter choice, engage feedback mechanisms, learning and adaptation, and other control strategies to improve decode performance. Here we ask the previously unaddressed research question: can a healthy human subject, using a closed-loop BMI driven by synthetic neural activity, inform the choices made in prosthetic decode algorithms? We design this Online Prosthesis Simulator (OPS), we use it to ask a key algorithm design question, and we demonstrate a definitive answer. We use the OPS to optimize decode performance based on a key parameter of a current state-of-the-art decode algorithm - the bin width of a Kalman filter. First, we show that offline and online analyses do indeed suggest different parameter choices. Previous literature and offline analyses agree that neural activity should be analyzed in bins of roughly 200ms width. Our OPS analysis, which incorporates feedback-control, disagrees with these findings and suggests that much shorter bin widths (25-50ms) will yield higher decode performance. Second, we confirm this surprising finding with a real BMI system (animal experiments), which suggests that the OPS does provide an accurate proxy to real neural control. We hypothesize that this novel testing approach will allow rapid and lower-cost testing of many algorithmic choices and will be a better proxy to clinical use than offline data analyses. Providing further evidence of the importance of feedback-control and the OPS approach, this finding has critically enabled some of the significant performance gains in our related BMI experiments.

Disclosures: J.P. Cunningham, None; **P. Nuyujukian**, None; **V. Gilja**, None; **C.A. Chestek**, None; **S.I. Ryu**, None; **K.V. Shenoy**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.6

Topic: D.18. Brain-Machine Interface

Support: NSF, NDSEG, Soros Fndn, HHMI, SGF

JHU APL under DARPA RP2009: N66001-06-C-8005

CDRF, BWF, NIH-NINDS-CRCNS-RO1

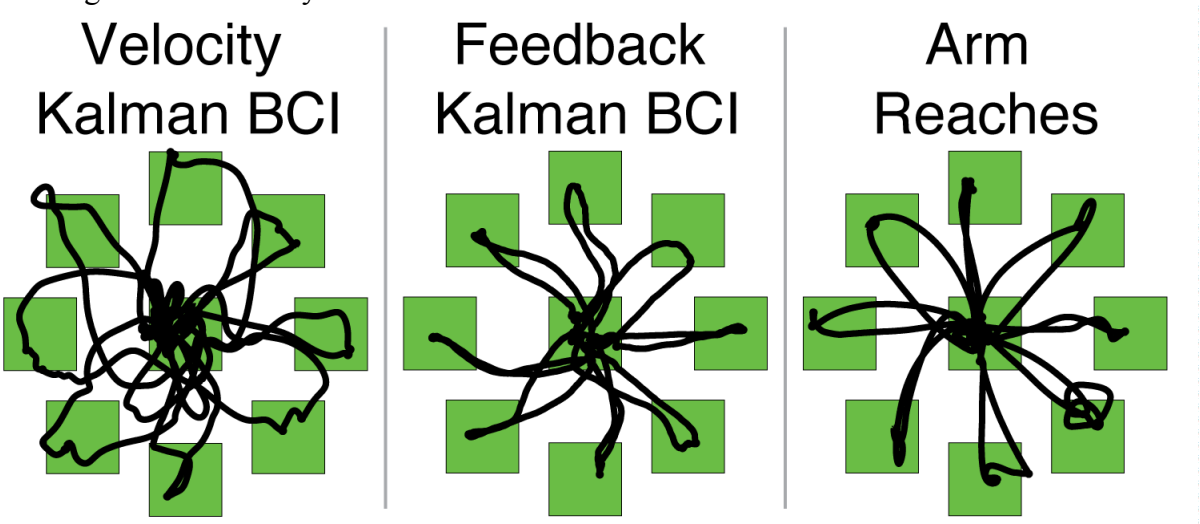
NIH Pioneer Award 1DP1OD006409

Title: A high-performance continuous cortically-controlled prosthesis enabled by feedback control design

Authors: *V. GILJA[1], P. NUYUJUKIAN[2,3], C. A. CHESTEK[4], J. P. CUNNINGHAM[4,6], B. M. YU[4,7,5,8], S. I. RYU[4,9], K. V. SHENOY[4,2,5];
[1]Computer Sci. Dept., [2]Biomed. Engin. Dept., [3]Med. Sch., [4]Electrical Engin. Dept., [5]Neurosci. Program, Stanford Univ., Stanford, CA; [6]Engin. Dept., Cambridge Univ., Cambridge, United Kingdom; [7]ECE Dept. and BME Dept., Carnegie Mellon Univ., Pittsburgh, PA; [8]Gatsby Computat. Neurosci. Unit, Univ. Col. London, London, United Kingdom; [9]Palo Alto Med. Fndn., Palo Alto, CA

Abstract: Neural prostheses, or brain-machine interfaces (BMIs), translate neural signals to guide an actuator or computer cursor. Although current demonstrations provide a compelling proof-of-concept, limited performance impedes clinical viability. BMIs can be viewed from a feedback control perspective (see Cunningham et al, SFN 2010): the brain is the controller of a new plant defined by the BMI. This perspective leads us to two advances that significantly improve qualitative & quantitative performance.
We modify the Kalman filter commonly used in BMI literature (e.g. Kim et al, 2008). In the first advance, during training, we fit neural data to a presumption of the desired volitional control signal, instead of observed or instructed kinematics. In the second advance, we develop the feedback Kalman filter, whose observation model incorporates cursor position as feedback. Performance was tested in closed loop with two rhesus macaques. On each trial, the monkey acquired a 2D target in an allotted time period with a cursor, controlled by the native contralateral limb or BMI. Neural data were recorded from a 96-electrode array (Blackrock) spanning PMd & M1. All experiments used spike counts found by -4.5×RMS threshold detection without spike sorting (see Chestek et al, SFN 2010). Such a system has clinical appeal, particularly for arrays with potentially decreased SNR (monkeys L & J were 19-27 & 4-8 months post-implantation, respectively).
During online tests, the new BMI appears more controllable, producing straighter reaches & crisper stops, as shown in the figure of monkey L's continuous cursor traces. Relative to a

standard Kalman filter, mean time to target (& failure rate) is reduced from 1323 ± 686ms (20%) to 993 ± 364ms (1%) in the same center out & back session. Further bin width & model training optimizations reduce this time to 600-700ms for both L & J, approaching arm reach times of 550-650ms. These innovations, supported by extensive experimental verification & design studies (see Nuyujukian et al, SFN 2010), offer a significant performance advance, thereby increasing clinical viability.



Disclosures: V. Gilja, None; **P. Nuyujukian**, None; **C.A. Chestek**, None; **J.P. Cunningham**, None; **B.M. Yu**, None; **S.I. Ryu**, None; **K.V. Shenoy**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.7

Topic: D.18. Brain-Machine Interface

Support: NSF, NDSEG, Soros Fndn, HHMI, SGF

JHU APL under DARPA RP2009: N66001-06-C-8005

CDRF, BWF, NIH-NINDS-CRCNS-RO1

NIH Pioneer Award 1DP1OD006409

Title: Generalization and robustness of a continuous cortically-controlled prosthesis enabled by feedback control design

Authors: *P. NUYUJUKIAN[1,2], V. GILJA[3], C. A. CHESTEK[4], J. P. CUNNINGHAM[4,6], J. M. FAN[1], B. M. YU[4,7,5,8,9], S. I. RYU[10,4], K. V. SHENOY[4,1,5];
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Abstract: Brain-machine interfaces (BMIs) translate neural signals into useful control signals. Using the algorithmic advances discussed in Gilja et al. (SFN 2010), we describe here our attempt to systematically test the performance and robustness of this BMI on two rhesus macaques, as well as to generalize the behavioral tasks beyond the center-out task. Monkeys were implanted with 96 channel electrode arrays, and experiments were carried out 19-27 (4-8) months post implantation for monkey L (monkey J). To control for inter-day variations in array or monkey performance, experiments were carried out intra-day in an A-B-A block design, thereby directly assessing the difference between various algorithms and modes of control. All decodes were performed with one 96 channel electrode array per animal (PMd in monkey L; M1 in monkey J), using neural threshold crossings set at -4.5×RMS without spike sorting at the beginning of each experimental day.
Using a standard Kalman filter without any of the new algorithmic advances, when monkey L was first switched into BMI control, two weeks of training were required before 4 cm targets could be acquired and held for 500 ms on an 8 cm center-out task. However, using our algorithmic advances, training appears considerably streamlined and high performance was achieved on the first day with monkey J.
Using this BMI on the same center-out task, monkey L had a mean target acquire time (and success rate) of 671 ms (96%) vs 543 ms (100%) for hand. Enforcing a maximum 1000 ms acquire time during BMI control improved mean target acquire time (618 ms) but decreased success rate (87%). This was 86% the performance of hand control and was repeatable with similar results over a span of six months.
On a more generalized "pinball" task, 4 cm targets appeared randomly in a 16x16 cm workspace with a 500 ms hold time. Using the BMI, monkey L achieved 54% the performance of hand control, the BMI at 722 ms (99%) vs hand at 496 ms (98%). This performance could be maintained for nearly two hours under BMI control, matching the duration of the longest hand-controlled sessions.
On a more complex, obstacle avoidance task, monkey J was trained to acquire 6 cm targets while avoiding an intervening visual barrier. Monkey J successfully navigated around the obstacle by instructing a curved trajectory and acquired targets 60% of the time with the BMI vs 62% of the time with hand control. BMI performance was 63% that of hand, the acquire time of BMI at 1219 ms vs hand at 889 ms.
We believe this new feedback-control design substantially increases the performance, robustness, and generalization of BMIs and may help bring BMIs closer to clinical viability.

Disclosures: P. Nuyujukian, None; **V. Gilja**, None; **C.A. Chestek**, None; **J.P. Cunningham**, None; **J.M. Fan**, None; **B.M. Yu**, None; **S.I. Ryu**, None; **K.V. Shenoy**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.8

Topic: D.18. Brain-Machine Interface

Support: NYS Department of Health SCIRBs # C022048

DARPA REPAIR Contract# N66001-10-C-2008

SUNY Downstate Medical School. (Principal Investigator)

Title: Force control in a brain-machine interface

Authors: *P. Y. CHHATBAR[1,2], M. SEMEWORK[1,3], S. XU[1], B. T. MARSH[1,2], J. T. FRANCIS[1,2,3];
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Abstract: To date Brain Machine Interfaces (BMIs) have allowed their users to control kinematic based variables such as position and velocity of computer cursors, or robotic arms, however no force related variables have been included to deal with novel dynamical environments. So far, BMI experimental setups provide a single external dynamic environment and thus performance of the BMI under different dynamic environments is unknown. To illustrate, the performance of the robotic device trained to hold and move an empty glass might be sub-optimal to move a water-filled glass or a glass with different weights. Similarly, different environmental stiffness conditions (air versus water) might lead to poor control of motion. To address the issue of force related control of a BMI, we employed a primate reaching paradigm under different viscous gain field conditions and let the animal perform the brain-controlled movements using predictions of force-related variables. In our experimental setup, a non-human primate (M. radiata) was implanted with multiple microelectrode arrays (10x10 electrode grid, 1.5 mm length, 400 μm inter-electrode spacing at the tip, Blackrock Microsystems) in the shoulder area of the primary motor cortex, areas 1 and 2 of the primary somatosensory cortex and the dorsal premotor cortex contralateral to the right arm. The monkey would sit in a primate

chair with its arm resting on the attached exoskeletal robotic manipulandum (Kinarm, BKin Technologies) and perform the standard center-out reaching task. The viscous gain force field was created using motors attached to the robotic manipulandum. We used a simple Weiner filter as our force/torque prediction algorithm, trained using the neural and behavioral data from such manual tasks. The animal was then given neural control of the forces/torques and allowed to perform the task. On offline analysis, we found that primary motor cortex activity gives better performance of the task and we plan to present the results of online performance in this paper.

Disclosures: P.Y. Chhatbar, None; **M. Semework**, None; **S. Xu**, None; **B.T. Marsh**, None; **J.T. Francis**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.9

Topic: D.18. Brain-Machine Interface

Support: DARPA W911NF-06-1-0053

NIH 5R01NS050256

NIH R25MH054318-15

Title: Toward robust continuous decoding for prosthetic arm control

Authors: *M. VELLISTE[1], Z. ZOHNY[2], S. T. CLANTON[2,3], S. M. JEFFRIES[4], A. J. C. MCMORLAND[1], J.-W. SOHN[1], G. W. FRASER[1], A. B. SCHWARTZ[1,3];
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Abstract: Motor-cortical population activity patterns are well predictive of arm movement and have been used in brain-machine interfaces to control cursors or prosthetic devices in multi-dimensional space. But prosthetic performance has yet to approach that of natural arm control. One limiting factor has been decoder bias, i.e. systematic error in decoder output when real neural activity does not match calibrated model parameters. When the predicted kinematic parameter is velocity, the bias manifests itself in the form of a characteristic “drift”, meaning that the cursor or prosthetic arm veers off in some direction when the subject is not trying to move it. A practical solution has been to add a counter-bias either as a manually specified constant offset

or by using some ad-hoc algorithm.
Here, we take a novel, more theoretically grounded approach by testing the hypothesis that much of the "drift" problem is due to a change in the baseline firing rates when the subject goes from "active" to "idle", i.e. stops being engaged in the task and stops trying to perform any movement. This would produce incorrect decoding because the baseline firing rates would not match the values that were calibrated during active movement.
Previous studies have shown that several behavioral states during different phases of movement are well reflected in both local field potential (LFP) and single-unit activity, and that classifiers can be trained to distinguish between these states. But the specific relationship of the "idle"-state activity to movement-related tuning functions has not been reported.
We present single- and multi-unit firing rates from monkeys during both a prosthetic arm experiment and a natural arm study that validate our hypothesis and show that the population activity during "idle" behavior lies far outside the manifold of movement-related tuning functions. In experiments with real arm movement in the past, these "idle" activity patterns have been overlooked because data between trials (when the "idle" behavior occurs) is typically disregarded or never even recorded. We show that the "idle" state can be accurately decoded from the population activity and present a new method for decoding arm kinematics more robustly, continuously throughout a recording session where previous methods fail during "idle" behavior.

Disclosures: M. Velliste, None; **Z. Zohny**, None; **S.T. Clanton**, None; **S.M. Jeffries**, None; **A.J.C. McMorland**, None; **J. Sohn**, None; **G.W. Fraser**, None; **A.B. Schwartz**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.10

Topic: D.18. Brain-Machine Interface

Support: Land-Sachsen-Anhalt Grant MK48-2009/003

NINDS Grant NS21135

Title: Improving arm movement decoding from the electrocorticogram (ECoG) by brain data fusion and a realistic arm model

Authors: *J. W. RIEGER[1,2], C. REICHERT[2], L. SECUNDO[1], M. KENNEL[3], E. F. CHANG[4], H. KIRSCH[4], N. M. BARBARAO[4], H. HINRICHS[2], R. T. KNIGHT[1];

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Abstract: Decoding arm movements from brain signals is often done by using only one decoder. In the context of BMI, this may lead to unphysiological, jerky prosthesis movements, and impossible constellations of arm joints. We investigated two ways to improve continous control signals derived from a MIMO Wiener Filter (WF) by using a) additional information derived from brain data to constrain the output, and by b) incorporating a mechanically realistic arm model in the control pipeline.
ECoG was recorded from 3 patients with electrode grids covering sensorimotor cortex. Subjects performed a center-out reaching task (six or eight peripheral positions) with 120-180 movements per block (n=4 blocks). A causal WF was trained to predict momentary X/Y-positions, velocity profiles (Vx/Vy), and shoulder and elbow (S/E) angles. Filter evaluation was done on a separate dataset. In addition, we trained support vector machines (SVM) to discriminate movement from non-movement intervals.
The average correlation between actual X/Y-position and Wiener regression predicted arm position were 0.6/0.55, for Vx/Vy correlations were 0.53/0.55, and for S/E-angles correlations were 0.59/0.57. By constraining the WF results with the SVM-derived probability for movement, we improved the correlation of the position estimates moderatly (X: 7%, Y: 3%), the velocity estimates considerably (Vx: 20%, Vy: 21%), and the arm angles moderately (S: 3%, E: 5%). Feeding the S/E signal into a mechanical arm model considerably improved S/E-correlations (S: 11%, E: 14%)
Our results demonstrate that the combination of independent information available in the EcoG measurements and the inclusion of realistic arm models to constrain the predicted output can considerably improve decoded arm dynamics and position. Moreover, the contrast between the marked improvement of velocity decoding compared to position decoding suggests relatively independent coding of position and movement related information in sensorimotor cortex.

Disclosures: J.W. Rieger, None; **C. Reichert**, None; **L. Secundo**, None; **M. Kennel**, None; **E.F. Chang**, None; **H. Kirsch**, None; **N.M. Barbarao**, None; **H. Hinrichs**, None; **R.T. Knight**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.11

Topic: D.18. Brain-Machine Interface

Support: NSF Cyber Enabled Discovery and Innovation Program

Title: A control approach towards closed-loop neural prosthesis

Authors: *G. KUMAR[1], V. AGGARWAL[2], N. V. THAKOR[2], M. H. SCHIEBER[3], M. V. KOTHARE[1];
[1]Chem. Engin., Lehigh Univ., Bethlehem, PA; [2]Biochem. Engin., Johns Hopkins Univ., Baltimore, MD; [3]Dept. of Neurobio. and Anat., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Current applications in neural-based prostheses use "closed-loop" cortical neuronal activities in "open-loop" controller forms to actuate prosthetic devices. Implicit or explicit feedbacks are not formally incorporated in designing the control action. In this presentation, we attempt to address the problem of incorporating feedback in designing closed-loop neural prostheses by studying the closed-loop control problem of a right hand index finger extension. We propose a receding horizon (RHC) based non-linear control problem and estimate optimal timing of spikes (ISIs) to reach the desired angular position in a closed-loop system. Using Model based RHC, we define our objective function $J_p(k)$ as the minimum time required to the desired target from information available at time step k. The choice of a minimum time problem is quite reasonable in cases where a specific task is required to be completed within a limited period of time. We hypothesize that in such cases our brain tries to minimize the total required time with available information at that time. With a time varying prediction horizon as the number of ISIs required in reaching the desired target, we compute the minimum time as the sum of ISIs. The control horizon is considered fixed during the initial period and eventually converges to the prediction horizon as the desired target is approached. The objective function is minimized over the prediction horizon subject to two inequality constraints. The first constraint defines the minimum and maximum limit on a single ISI. The minimum limit on an ISI captures the refractory period and a neuron can not fire another action potential within this time period. The maximum limit defines the smooth behavior of the finger movement and states that the next action potential must be generated before the torque generated at the finger by the previous action potential dies out. The second constraint defines a target space around the desired angular position.
We solve the constrained non-linear optimization problem using the primal-dual interior point method in MATLAB. We show our results by varying the control horizon for a fixed desired angular position and the desired angular position for a fixed control horizon. We compare our theoretical estimations with experimentally recorded closed-loop ISIs of a non-human primate. Our results conclude a possibility of this approach in designing closed-loop neural prostheses by extending it to more complex neural networks. Finally conclusions from our study propose the need for designing experiments that elucidate the difference between open-loop and closed-loop neural prostheses.

Disclosures: G. Kumar, None; **V. Aggarwal**, None; **N.V. Thakor**, None; **M.H. Schieber**, None; **M.V. Kothare**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.12

Topic: D.18. Brain-Machine Interface

Support: Globe Foundation

McCormick Foundation

Title: Learning analysis of novice and experienced users of electromyogram pattern recognition control for multi-functional myoelectric prostheses

Authors: *N. E. BUNDERSON[1], T. A. KUIKEN[2];
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Abstract: As of today, a primary obstacle to realization of multi-functional prostheses is their method of control. Pattern recognition of the electromyogram (EMG) has been demonstrated in the laboratory to be a successful control method for multifunctional prostheses. However, this technology will not become clinically viable until the problems inherent with mutual human machine learning are addressed. The goal of this study was to identify how EMG patterns change as subjects practice EMG pattern recognition control. We propose three learning scenarios, a combination of which may be responsible for reductions in classification error with practice. First, subjects may learn to reduce between-trial variability by becoming more consistent at producing a pattern between subsequent attempts. Second, subjects may learn to produce more distinct patterns by reducing the variability of their EMG signal for a given attempt. Third, subjects may learn to increase between-class distance by learning slight variations in contraction patterns that increase differences between classes. We compared the classification error of novice non-amputee subjects across a practice session and one day after the practice session to determine retention. In addition, we compared the classification error of novice subjects with experienced non-amputee subjects. We found that classification error in novices was reduced after the practice period but not to the level of the experienced subjects. In addition, we found that all three scenarios were involved in learning. Under the practice protocol, novices learned quickly (on the order of 40 minutes) to reduce between- and within-trial pattern variability (scenario 1 and 2) to the level of the experienced subjects. However, an hour and a half was not sufficient to learn to increase inter-pattern distance (scenario 3) to the level of experienced subjects. These differences can be used to guide the development of methods to train subjects to use pattern recognition devices. In particular we recommend training protocols that emphasize increasing the between-class distance.

Disclosures: N.E. Bunderson, None; **T.A. Kuiken**, None.

Nanosymposium

20. Brain Machine Interface: Neuroprosthetics

Location: Room 5B

Time: Saturday, November 13, 2010, 1:00 pm - 4:30 pm

Program Number: 20.13

Topic: D.18. Brain-Machine Interface

Title: Spike acquisition at ultra-low sampling rates for neuroprosthetic devices

Authors: *L. SRINIVASAN[1], L. VARSHNEY[2], J. KUSUMA[3];
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Abstract: For upper-extremity and other neuroprosthetic devices that rely on action potentials, power consumption, bandwidth, and hardware complexity represent fundamental operating constraints. Here, we present the initial development of a spike acquisition technology to achieve these three demands. Drawing on finite rate of innovation signal theory, our process aims to ultimately acquire the precise shape and timing of spikes from electrodes using sampling rates of 1000 Hz or less. The key insight is that action potentials are essentially stereotyped pulses that are generated by neurons at a rate limited by an absolute refractory period. We use this insight to push sampling below the Nyquist rate. Our approach is parametric and distinct from compressed sensing. The preliminary concept is illustrated with a simulated example from the control of upper-extremity neuroprosthetic devices.

Disclosures: L. Srinivasan, None; **L. Varshney**, None; **J. Kusuma**, None.

Nanosymposium

219. Signaling by Neurotrophins and Other Neuromodulators

Location: Room 6E

Time: Sunday, November 14, 2010, 1:00 pm - 2:45 pm

Program Number: 219.1

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: A role for the NAD+ dependent deacetylase Sirt-1 in CREB mediated gene expression and in neurotrophin signaling

Authors: *S. FUSCO[1], C. RIPOLI[2], S. CHIATAMONE RANIERI[1], G. TOIETTA[3], M. MCBURNEY[4], A. RICCIO[5], C. GRASSI[2], T. GALEOTTI[1], G. PANI[1];
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Abstract: The NAD+ dependent Class III hystone deacetylase Sirtuin-1 (Sirt-1) mediates lifespan extension by calorie restriction in lower organisms and promotes neuronal survival and resistance to neurodegenerative disorders in mammals. Molecular interactions involved in neuroprotection by sirtuins are poorly understood and their knowledge may help preventing brain ageing and related diseases.
We found that, in PC12 cells and in primary cortical neurons, the cAMP-responsive Element Binding protein (CREB) transcription factor, a key effector of neurotrophin signaling during neuronal differentiation and brain development, induces the expression of Sirt-1. Notably, the Nerve Growth Factor, a peptide critically reduced in the brain of Alzheimer's patients, induces sirt-1 through CREB. Beside being transcriptionally regulated by CREB, sirt-1 was found to physically associate with the factor to regulate the expression of two CREB target genes, PGC-1 alpha and neuronal Nitric Oxide Synthase (nNOS), directy involved in neuroprotection and cell resistance to oxidative damage. In fact, the expression of both genes is significantly reduced in PC12 cells depleted of sirt-1, and in the brain of Sirt-1 KO mice. In keeping with a major role of sirt-1 in CREB-mediated cell response to Neurotrophins, attenuation of oxidative stress and prevention of apoptosis by NGF was compromised in PC12 cells by sirt-1 knock down, while low concentration of glucose or overexpression of SIRT1 promoted the expression of the survival factors Bcl2 and manganese-dependent superoxide dismutase and increased resistance to H2O2 and staurosporin in cortical neurons. Finally NGF driven PC12 differentiation was severely impaired by genetic or pharmacological inhibition of the deacetylase, but partially rescued by nutrient restriction in the differentiation medium. Taken together, our findings outline a novel molecular circuitry whereby CREB-dependent neuronal cell response to environmental cues is modulated by cellular metabolism through the nutrient-sensitive deacetylase sirt-1. This circuitry represents a potential target for the prevention and the treatment of neurodegenerative diseases.

Disclosures: S. Fusco, None; **C. Ripoli**, None; **S. Chiatamone Ranieri**, None; **G. Toietta**, None; **M. McBurney**, None; **A. Riccio**, None; **C. Grassi**, None; **T. Galeotti**, None; **G. Pani**, None.

Nanosymposium

219. Signaling by Neurotrophins and Other Neuromodulators

Location: Room 6E

Time: Sunday, November 14, 2010, 1:00 pm - 2:45 pm

Program Number: 219.2

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Human Frontiers Science Program

NIH Grant NS 30687

NIH Grant NS21072

NIH Grant HD23315

Title: Proneurotrophin signaling leads to retraction of actin-rich structures in hippocampal neurons

Authors: *K. DEINHARDT[1], B. L. HEMPSTEAD[2], M. V. CHAO[1];
[1]Mol. Neurobio, Skirball Inst., NEW YORK, NY; [2]Dept. of Med., Weill Cornell Med. Col., New York, NY

Abstract: The role of neurotrophins in neuronal survival, differentiation and neurite outgrowth is well established. Neurotrophins are initially synthesized as precursors, or proneurotrophins, that are cleaved intracellularly by furin and proconvertases to release the mature, active proteins. Alternatively, proneurotrophins are released and subsequently processed extracellularly into the mature forms by matrix metalloproteases. Over the past years, it has been recognized that in addition to the mature neurotrophins, these precursors are also biologically active. Interestingly, the proneurotrophins have opposite biological effects to the mature versions, inducing both apoptosis as well as long-term depression at synapses. However, the molecular mechanism underlying these differential effects has not been characterized.
Here, we hypothesized that the negative impact of proneurotrophins on cellular physiology is in part a consequence of the effects on proneurotrophin-induced cytoskeletal remodeling. Using fixed and live cell imaging, we found that both proNGF and proBDNF application induced rearrangement of the actin cytoskeleton, leading to a collapse of actin filaments in young hippocampal neurons. This process was dependent on p75NTR, and triggered by the phosphorylation, and therefore inactivation, of the actin bundling protein fascin. In addition, proneurotrophin addition led to decreased activity of the small GTPase Rac through dissociation of the Rac GEF Trio from the p75NTR. This displaces Trio, and therefore active Rac, from motile actin structures, ultimately leading to their collapse. Finally, we provide evidence that proneurotrophin-induced pruning of processes is indeed occurring in vivo, since mice impaired in proBDNF processing to mature BDNF displayed reduced dendritic complexity in the dentate gyrus.

Together, this provides a mechanism for proneurotrophin-induced pruning of processes, which may underlie the electrophysiological measures of long-term depression in response to proneurotrophin action.

Disclosures: K. Deinhardt, None; **B.L. Hempstead**, None; **M.V. Chao**, None.

Nanosymposium

219. Signaling by Neurotrophins and Other Neuromodulators

Location: Room 6E

Time: Sunday, November 14, 2010, 1:00 pm - 2:45 pm

Program Number: 219.3

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR Grant

Title: Monitoring the retrograde transport of signaling molecules fused to Halotag protein in NGF-dependent sympathetic neurons

Authors: *S. A. MOK, K. LUND, R. B. CAMPENOT;
Cell Biol., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Neurotrophins, such as Nerve Growth Factor (NGF), critically regulate the survival of neurons during development. NGF is secreted by the target tissues of innervating neurons. The survival of these neurons is dependent on signals initiated at their axon terminals by the target-secreted NGF. There is evidence that NGF supplied at distal axons of cultured rat sympathetic neurons promotes neuronal survival by producing pro-survival retrograde signals and suppressing apoptotic retrograde signals within the distal axons.
Survival signals and apoptotic signals initiated in axons by NGF are thought to be retrogradely transported to the cell bodies where they exert their effects on neuronal survival. Although there is evidence that both NGF and TrkA are retrogradely transported and are potential carriers of retrograde survival signals, other reports suggest that alternative carriers of survival signals exist, and no carrier of a retrograde apoptotic signal has been identified thus far.
In an effort to identify retrograde carriers of NGF-dependent survival and apoptotic signals, we have transfected sympathetic neurons in compartmented cultures with plasmids expressing candidate proteins fused to the Halotag protein (Promega). The expressed Halotag fusion protein can be covalently labeled in cultured neurons through the addition of cell-permeable fluorescent or biotin-tagged ligands to the medium. Fusion proteins present in the distal axons of compartmented cultures of sympathetic neurons are selectively labeled then monitored for their movement to the cell bodies under both survival and apoptotic conditions.

Disclosures: S.A. Mok, None; **K. Lund**, None; **R.B. Campenot**, None.

Nanosymposium

219. Signaling by Neurotrophins and Other Neuromodulators

Location: Room 6E

Time: Sunday, November 14, 2010, 1:00 pm - 2:45 pm

Program Number: 219.4

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Akt/mTOR signaling pathway regulates neurite outgrowth in cerebellar granule neurons affected by methylcobalamin

Authors: *K. OKADA[1], H. TANAKA[2], Y. KURODA[1], M. OKAMOTO[1], H. MORITOMO[1], T. MURASE[1], H. YOSHIKAWA[1];
[1]Osaka Univ., Suita, Japan; [2]Osaka Kosei Nenkin Hosp., Osaka, Japan

Abstract: Introduction: Mammalian target of rapamycin (mTOR), a well known Akt substrate, is a serine/threonine protein kinase and regulates multiple cellular functions including neurite outgrowth. In the previous report, we suggested that methylcobalamin (MeCbl), which was one of vitamin B12 analogs, promoted neurite outgrowth with the activation of Akt in cerebellar granule neurons (CGN). In this study, we try to examine our hypothesis that MeCbl increases the mTOR activity through the activation of Akt and promotes neurite outgrowth in CGN.
Materials and methods: CGN culture was prepared from P9 Wistar rats. MeCbl was added in media at the concentration as 10 micro-M. And in the some group, LY294002, an inhibitor of Akt, and rapamycin, an inhibitor of mTOR, were supplemented in the media. Cells were cultured for 72 hours and followed by visualization with immunofluorescent images to measure the axonal length of neurons. For the evaluation of the activities of Akt and mTOR, CGN were cultured for 10 minutes with MeCbl, LY294002, and/or rapamycin. The activity of Akt and mTOR in CGN was detected by Western blotting. To calculate the normalized density, the density of phospho-Erk1/2 or phospho-Akt was divided by the density of Erk1/2 or Akt, respectively, in the same membrane. The significance was determined by one way ANOVA and post hoc every t-test.
Results: MeCbl increased axonal length from 183.9 ± 11.1 micro meters (mean ± SE) to 320.8 ± 16.0 micro meters in CGN. LY294002 inhibited the effect of MeCbl on neurite outgrowth, and rapamycin decreased axonal length to 121.8 ± 5.3 micro meters, which was not affected by addition of MeCbl. As a result of the evaluation of Akt and mTOR activities, MeCbl produced 3.3 ± 0.5 fold stronger activation of Akt and 3.0 ± 0.5 fold stronger activation of mTOR. LY294002 decreased the activities of Akt and mTOR, and MeCbl did not increase the activities

of Akt and mTOR under addition of LY294002. Addition of rapamycin, which did not have an influence on the Akt activity, inhibited the activation of mTOR affected by MeCbl.
Discussion: In our study, we reveal that MeCbl increases Akt and mTOR activities in CGN and promotes neurite outgrowth through the activation of Akt and mTOR.

Disclosures: K. Okada, None; **H. Tanaka**, None; **Y. Kuroda**, None; **M. Okamoto**, None; **H. Moritomo**, None; **T. Murase**, None; **H. Yoshikawa**, None.

Nanosymposium

219. Signaling by Neurotrophins and Other Neuromodulators

Location: Room 6E

Time: Sunday, November 14, 2010, 1:00 pm - 2:45 pm

Program Number: 219.5

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant RO1 HD38679-10

Cleveland Clinic

Title: Supplemental neurotrophins improve functional recovery after simulated childbirth injury

Authors: *C. DISSARANAN[1,2], B. C. GILL[2,3], B. M. BALOG[2], H.-H. JIANG[1,2], H. B. GOLDMAN[1,3], M. S. DAMASER[1,2,3,4];
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Abstract: The pudendal nerve (PN) innervates the external urethral sphincter (EUS) and can be injured during childbirth, leading to stress urinary incontinence (SUI). Peripheral nerve regeneration is facilitated by brain derived neurotrophic factor (BDNF), which is upregulated in muscles following motor nerve injury. PN crush (PNC) produces upregulation of BDNF in the external urethral sphincter (EUS). In contrast, direct sphincteric injury from vaginal distention (VD) reduces BDNF expression in the EUS. Combined PNC/VD, simulating childbirth injury, reduces BDNF expression in the EUS and delays neuroregeneration and functional recovery compared to either PNC or VD alone. The aim of this study is to investigate whether continuous, local supplementation of exogenous BDNF improves PN recovery following simulated childbirth injury.
Female virgin Spraque-Dawley rats (200-225g) were separated into 3 groups: PNC/VD with BDNF treatment (N=11), PNC/VD with sham (saline) treatment (N=9), and sham injury with no

treatment (N=10). VD consisted of placing a balloon into the vagina and inflating it with 3ml of water for 4 hours. PNC was comprised of 2 consecutive, bilateral, 30 second PN crushes using a Castro-Viejo needle holder. Treatment was administered to each PNC site after injury by a catheter run from an ipsilateral subcutaneous mini-osmotic pump.
Outcomes were tested 2 and 3 weeks after injury/treatment and consisted of simultaneous leak point pressure (LPP), EUS electromyography (EMG), and PN electroneurography (ENG) measurements. Briefly, the bladder was filled and intravesical pressure was recorded via a urethral catheter while PN motor branch ENG and EUS EMG were continuously recorded at rest and while the exposed bladder was gradually compressed to induce leakage and the continence reflex.
LPP was significantly reduced in VD/PNC animals treated with saline 2 weeks after injury compared to sham injured animals or those given BDNF. Similarly, EMG amplitude, frequency, and power, were also significantly reduced at rest with saline treatment but not BDNF administration; EMG amplitude and power during the LPP procedure were reduced as well. LPP in VD/PNC animals recovered to that of sham injured rats 3 weeks after injury, yet PN ENG amplitude and frequency at rest were significantly lower than sham-injured animals with saline treatment but not BDNF administration.
These results suggest that continuous, local BDNF treatment accelerates functional recovery of the PN following simulated childbirth injury and may prevent long-term PN dysfunction and SUI. As such, BDNF may hold promise as a preventative treatment for SUI.

Disclosures: C. Dissaranan, None; **B.C. Gill**, None; **B.M. Balog**, None; **H. Jiang**, None; **H.B. Goldman**, None; **M.S. Damaser**, None.

Nanosymposium

219. Signaling by Neurotrophins and Other Neuromodulators

Location: Room 6E

Time: Sunday, November 14, 2010, 1:00 pm - 2:45 pm

Program Number: 219.6

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Kidins220/ARMS mediates BDNF signalling during the development of mouse central and peripheral nervous system

Authors: *F. CESCA[1], A. YABE[2], B. SPENCER-DENE[2], J. SCHOLZ-STARKE[1], P. BALDELLI[1], M. AL-QATARI[3], R. H. ADAMS[4], F. BENFENATI[1], G. SCHIAVO[2];
[1]Italian Inst. of Technol., Genova, Italy; [2]Cancer Res. UK, London, United Kingdom; [3]The Natl. Hosp. for Neurol. and Neurosurg., London, United Kingdom; [4]Max Planck Inst. for Mol. Biomedicine, Münster, Germany

Abstract: Kidins220 (*Ki*nase *D in*teracting *s*ubstrate of *220* KDa) / ARMS (*A*nkyrin Repeat-*r*ich *M*embrane *S*panning), is a conserved integral membrane protein mainly expressed in brain and neuroendocrine cells. It is a substrate for PKD as well as an intracellular target of the signalling cascades initiated by neurotrophins and ephrins. Kidins220 has been implicated in the process of neuronal differentiation, by mediating the sustained activation of the mitogen-activated protein kinase (MAPK) pathway in response to neurotrophic stimuli.
To test the functional role of Kidins220 *in vivo*, we have engineered a construct, based on the Cre/LoxP recombination system, which allows the conditional knockout of Kidins220 in mice. In order to obtain a complete ablation of the protein, we have crossed the Kidins220$^{lox/lox}$ animals to a mouse line bearing the Cre recombinase under the PGK promoter, which is active since the early stages of embryogenesis. Whilst the Kidins220$^{+/-}$ mice are alive and fertile, the Kidins220$^{-/-}$ animals die at late stage of embryonic development. Here, we have conducted an analysis of the phenotypes caused by Kidins220 ablation. By immunohistochemical analysis, we identified distinct areas of cell death in specific brain structures, such as the neuroepithelium, the thalamic and hippocampal regions, as well as defects in the development of sensory ganglia. In addition, Kidins220$^{-/-}$ embryos display striking heart defects. We have further characterised the behaviour of cultured Kidins220$^{-/-}$ hippocampal neurons, focusing on their electrophysiological properties, as well as on their ability to survive and differentiate in response to specific neurotrophic stimuli. Kidins220 ablation results in an impaired response to BDNF signalling, in terms of both the fast electrophysiological response to acute BDNF treatment, and the long-term neurite outgrowth upon chronic BDNF exposure.

Disclosures: F. Cesca, None; **A. Yabe**, None; **B. Spencer-Dene**, None; **J. Scholz-Starke**, None; **P. Baldelli**, None; **M. Al-Qatari**, None; **F. Benfenati**, None; **G. Schiavo**, None; **R.H. Adams**, None.

Nanosymposium

219. Signaling by Neurotrophins and Other Neuromodulators

Location: Room 6E

Time: Sunday, November 14, 2010, 1:00 pm - 2:45 pm

Program Number: 219.7

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: FONDECYT 1085273

MINREB (P07/011-F)

CARE PFB 12/2007

Title: Rab11 monomeric GTPase is a down-stream target of BDNF-TrkB signaling to induce dendritic arborization

Authors: O. M. LAZO[1], *F. C. C. BRONFMAN[1], A. COUVE[2];
[1]Physiol., Pontificia Univ. Catolica De Chile, Santiago, Chile; [2]Physiol. and Biophysics (ICBM), Univ. Chile, Santiago, Chile

Abstract: Brain-derived neurotrophic factor (BDNF) and its receptor TrkB are ubiquitously expressed in the central nervous system and, among several functions, regulate dendritic arborization participating in neuronal plasticity. Some key cellular events tuning receptor signaling are endocytosis and intracellular trafficking. It has been shown that the monomeric GTPase Rab11 controls the recycling pathway in hippocampal neurons, both in soma and dendrites, playing a key role in synaptic plasticity. Taking this together we have hypothesized that Rab11 is regulating BDNF-induced dendritic arborization. To test this possibility we have first set up a model of BDNF-induced dendritic branching, where 7 DIV hippocampal neurons are treated for 48 hours with BDNF increasing up to the double the number of branching points. Then, we performed experiments where 7 DIV hippocampal neurons were infected with Rab11-EGFP WT and the dominant negative mutant (DN) or the constitutively active mutant (CA) forms of Rab11-EGFP and treated or not with BDNF for 48 hours. We have found that expression of Rab11DN mutant inhibits BDNF-induced dendritic branching. Similar results were obtained when 7 DIV neurons were transfected with siRNA against Rab11A and B. On the other hand, the expression of Rab11CA increases dendritic branching by its own and BDNF (100 ng/ml) does not potentiate this effect. However, the expression of Rab11CA re-distributes the TrkB receptor to dendritic filopodia, suggesting that the mechanism of action of the Rab11CA is to sensitize neurons to BDNF/TrkB signaling. All this results suggest that Rab11 is a downstream target of TrkB signaling. Consistently with this idea, BDNF induces an increase of Rab11-EGFP localization in secondary dendrites measured by live-cell imaging. Our results suggest that BDNF increases the activity of Rab11 to induce dendritic ramification, integrating membrane trafficking and regulation of cytoskeleton dynamics for protrusion of branches.

Disclosures: O.M. Lazo, None; **A. Couve**, None; **F.C.C. Bronfman**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.2

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH SBIR 5R44AG025641 - 05

Title: Efficacy of anti-Abeta13-28 antibodies in ameliorating congnitive deficits in a mouse model of Alzheimer's disease

Authors: *A. V. SAVONENKO[1], T. MELNIKOVA[1], H. KIM[1], D. GAINES[1], D. LEE[1], A. HIATT[2];
[1]Pathology, Johns Hopkins Univ., BALTIMORE, MD; [2]MAPP Biopharmaceutical, Inc, San Diego, CA

Abstract: Numerous therapeutic interventions are under investigation to prevent and treat Alzheimer's disease. Among the most advanced of these approaches are anti-amyloid-beta (Abeta) immunization strategies. When tested on AD transgenic models these strategies showed excellent results. However, results of clinical studies with active and passive immunotherapy did not meet original expectations of high efficacy in ameliorating functional deficits indicating that critical mechanisms required for the efficacy of these approaches are still poorly understood. In this study, we compared functional efficacy of three similar monoclonal anti-Abeta antibodies in amelioration of cognitive deficits in the APP/PS1 transgenic mouse model of Alzheimer's disease. The antibodies were raised against an internal domain of Abeta (Abeta13-28) and as detected by a surface plasmon resonance analysis had no significant differences in affinities to monomeric or oligomeric Abeta species. The passive immunization treatment (i.p. 250mkg of an antibody weekly) started when mice developed significant plague deposition and cognitive deficits. After one month of treatment, mice were tested in a series of water maze tasks that are conducted one after another to analyze reference, episodic-like and working memories in the same animals while the treatment continues. After the end of cognitive testing, the mice were sacrificed and brains processed for histological and biochemical analyses. As expected for antibodies against internal domain of Abeta, none of the antibodies resulted in any detectable hemorrhages in the brains of transgenic mice as compared to their aged-matched non-transgenic littermates. The analyses of cognitive performances indicated however that only one antibody produced significant amelioration of reference, episodic-like and working memory deficits in APP/PS1 mice. All beneficial effects of this antibody were cancelled when it was injected as an antibody-antigen complex with synthetic Abeta. The data on an antibody-driven sink effect, decrease in amyloid load, and activation of microglia will be presented to analyze requirements for functional efficacy of passive immunization.

Disclosures: **A.V. Savonenko:** Research Grant; SBIR NIH. **T. Melnikova:** SBIR NIH. **H. Kim:** None. **D. Gaines:** None. **D. Lee:** SBIR NIH. **A. Hiatt:** SBIR NIH. Employment; MAPP BIOPHARMACEUTICAL, INC.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing,

Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.3

Topic: C.02. Alzheimer's disease and other dementias

Support: France-Alzheimer association

Longevity program from the CNRS

National Foundation for Alzheimer's Disease and Related Disorders

National Institute on Aging (R01-AG020197)

Région Martinique

Title: In vivo follow-up of microhemorrhages during aging and anti-amyloid immunotherapy in the mouse lemur primate

Authors: *N. JOSEPH-MATHURIN[1], O. DORIEUX[1,2], A. KRASKA[1,3], M. SANTIN[1,4], P. HANTRAYE[1], J.-M. VERDIER[5], E. SIGURDSSON[6], N. MESTRE-FRANCÉS[5], M. DHENAIN[1];
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Abstract: Background:

Alzheimer's disease (AD) is a severe dementia characterized by intracerebral accumulation of amyloid (Aβ) proteins. Strategies to reduce Aβ1-42 β load as active anti-Aβ immunotherapy (AAAI) have been largely studied in AD transgenic mouse models and showed good results. For example K6Aβ1-30 in alum adjuvant decreases Aβ burden and prevents cognitive decline in transgenic mice [1]. However, clinical trials of AAAI have reported side effects like encephalitis and possibly microhemorrhages [2,3]. Mouse lemur primates can develop Aβ plaques with age and such a primate model may be more predictive than rodents of potential human side effects [4]. Here we studied, by magnetic resonance imaging (MRI), the effects of AAAI in these primates. Hypointense cerebral regions detected by MRI were evaluated in young animals and in older animals immunized with K6Aβ1-30 and Aβ1-42 in alum adjuvant.

Methods:

The study involved 9 young (1.9±0.2 years), 11 middle-aged (4.5±0.1 years) and 8 aged (5.9±0.1 years) animals. The older animals were treated with Aβ1-42 or K6Aβ1-30 vaccine and followed-up for 9 months by MRI (Pharmascan-Bruker 7T). All animals were scanned with a T2*-weighted sequence (TR/TE = 40/8, resolution = (234x234x234) µm3) while they were breathing carbogen. This procedure was used to improve microhemorrhage detection. The hypointense regions on these images were segmented. MR histology (TR/TE = 40/15, resolution = (25x25x132) µm3) and Perl's iron stained brain sections were performed to characterize the hypointense signals.

Results:

In vivo images of aged non-treated animals showed hypointense signal along ventricles. Analyses of all untreated animals highlighted a positive correlation between the volume of this phenomenon and age ($r=0.60$; $p<0.001$). Immunization with Aβ1-42 increased the size of the hypointense region ($F(2,5)=4.627$; $p<0.05$), compared to K6Aβ1-30 treatment. MR histology of immunized animals confirmed the presence of hypointense signal near ventricles and revealed other similar signals near vessels in basal temporal regions. Preliminary histological analyses of the brain tissue sections indicate that these two forms of hypointense signals correspond respectively to iron load in choroid plexus and microhemorrhages.

Conclusions:

Age associated increase of iron load was detected in choroid plexus in mouse lemur primates. Immunization with Aβ1-42 compared to K6Aβ1-30 worsened this phenomenon and appeared to lead to microhemorrhages.

References:

1-

Asuni et al, 2006

2-

Orgogozo et al, 2003

3-

Ferrer et al, 2004

4-

Mestre-Francès N et al, 2000

Disclosures: N. Joseph-Mathurin, None; **O. Dorieux**, None; **A. Kraska**, None; **M. Santin**, None; **P. Hantraye**, None; **J. Verdier**, None; **E. Sigurdsson**, None; **N. Mestre-Francés**, None; **M. Dhenain**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.4

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH grant AG18379

NIH grant AG18884

Alzheimer's Association (Zenith Award)

Title: Rivastigmine promotes alpha-secretase pathway of β-amyloid precursor protein (APP) processing by up-regulating ADAM complex and its implication in Alzheimer’s disease (AD)

Authors: *B. RAY[1], J. A. BAILEY[2], N. H. GREIG[3], D. K. LAHIRI[2];
[1]Indiana Univ., INDIANAPOLIS, IN; [2]Indiana Univ., Indianapolis, IN; [3]Natl. Inst. of Aging, Baltimore, MD

Abstract: Brain deposition of the amyloid β-peptide (Aβ), which originates from APP, is one of the major hallmarks of AD. APP is enzymatically processed by several secretases to produce secreted APP (sAPP), Aβ and p3 products. APP cleavage by α-secretase, an enzyme complex predominantly comprised 'a disintegrin and metalloproteinase' or ADAMs is considered to be non-amyloidogenic as this pathway precludes Aβ production. Hence, promoting this α-pathway could provide a novel strategy for AD treatment. Rivastigmine, a FDA-approved drug used for the treatment of mild AD patients, belongs to the cholinesterase inhibitor (ChEI) family. Previously, we have shown that apart from pro-cholinergic functions, some ChEIs have potential effects on the APP processing pathway (Lahiri et al., JPET, 2007).We hypothesized that rivastigmine has APP processing modulatory properties, and hence studied these effects in differentiated PC12 cells. NGF-differentiated PC12 cells were treated with three doses of rivastigmine (5-20μM) for 48 hr. Western immunoblotting of conditioned media (CM) samples showed a significant dose-dependent increase in levels of α-secretase-cleaved secretary product of APP (sAPPα) at 10μM and 20μM doses of rivastigmine vs. vehicle-treated samples. Further, both dimeric and oligomeric intracellular Aβ species were decreased by 10μM and 20μM rivastigmine treatment. Analyses of CM samples also revealed a significant dose-dependent decrease in β-secretase-cleaved product of APP (sAPPβ) with both doses of rivastigmine vs. vehicle-treated samples, suggesting a shift in APP processing towards the α-secreatse pathway. Further, rivastigmine treatment preserved secreted neuronal APP in degenerating primary cerebrocortical rat neuron cultures. There was no significant change in intracellular levels of β-site of APP cleavage enzyme (BACE-1) protein between the rivastigmine and vehicle-treated samples. We further studied the mechanism of rivastigmine's action and observed a significant dose-dependent increase in levels of intracellular ADAM9 and ADAM10 by both 10μM and 20μM rivastigmine treatment. Notably, this α-secretase-promoting effect of rivastigmine was not observed when differentiated PC12 cells were co-treated with the non-specific ADAM inhibitor, TAPI. Taken together, these results suggest that rivastigmine preserves neuronal sAPP and promotes the α-secretase pathway by up-regulating ADAM complex and, thereby, provides the first demonstration of an APP-modifying property by rivastigmine. Rivastigmine's dual function, pro-cholinergic and Aβ lowering properties warrant further investigation to optimize its beneficial effects.

Disclosures: B. Ray, None; **N.H. Greig**, None; **D.K. Lahiri**, None; **J.A. Bailey**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.5

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant AG18379

NIH Grant AG18884

Alzheimer's Association (Zenith Award)

Title: Novel effects of memantine on APP and synaptic proteins in different cell lines are mediated by intracellular targets

Authors: *J. A. BAILEY[1], B. RAY[2], P. K. BANERJEE[3], D. K. LAHIRI[2];
[1]Indiana Univ., INDIANAPOLIS, IN; [2]Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Forest Labs., Jersey City, NJ

Abstract: Memantine is a noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist approved for the treatment of moderate to severe Alzheimer's disease. This drug is thought principally to act through protection of neurons by inhibiting NMDA receptor-mediated toxicity, but other effects have not been excluded. For example, memantine has been proposed to act directly on the lysosome in cultured fibroblast cells. While the data on fibroblast cells are convincing, the uptake of memantine in neuronal cells is largely untested. We have observed that memantine can increase or preserve viability in a variety of cultured cell types, as well as increase synaptic markers in neuronal cells, and modulate APP cleavage. Interestingly, some of these effects can be observed in cell types with no apparent active NMDA receptors. To investigate the hypothesis that memantine produces these effects by uptake into neuronal and glial cells, we exposed primary embryonic (E16) rat cerebrocorical cultures, human SK-N-SH neuroblastoma, rat PC12 pheochromocytoma, and rat C6 glioma cells with a concentration range of memantine of 0.1 - 20 μM. After a 48-hour exposure, cells were lysed and analyzed by LC-MS to determine memantine concentrations. All cell types tested internalized memantine to some extent, and there was a range of uptake values (2-24%), which may correlate with cell division rate or cell type, though these associations are still under investigation. These data support the hypotheses that memantine has intracellular targets, and that intracellular memantine in neuronal and glial cultures are similar to effective concentrations measured in vivo. We propose that the effect of memantine on APP, synaptic proteins, and cell viability are mediated at least in part by intracellular targets in a broad range of cell types, and that some of these effects may be independent of the NMDA receptor.

Disclosures: **J.A. Bailey**, None; **D.K. Lahiri**, None; **P.K. Banerjee**, Forest Laboratories, Employment; **B. Ray**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.6

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH AG022103

NIH AG 023055

ZEN-00-2352

Title: Understanding the risk of homocysteine and cholesterol in AD: Therapeutic targets

Authors: *K. SAMBAMURTI[1], E. HOLLINGS[1], T. UTSUKI[1], N. H. GREIG[2], M. A. PAPPOLLA[1];
[1]Neurosciences, MUSC, CHARLESTON, SC; [2]Intramural Res. Program, Natl. Inst. on Aging, Baltimore, MD

Abstract: Homocysteine and cholesterol have both been identified as major risk factors for Alzheimer's disease (AD) and age-related macular degeneration (AMD). Interestingly, the amyloid beta protein of 42 aa (Aβ42) is deposited in extracellular lesions in both these diseases. Critical to the understanding of AD is the recognition that Aβ accumulation does not take place in most individuals although everyone expresses high levels of Aβ. We have determined that two major risk factors for AD and cardiovascular diseases, high homocysteine and hypercholesterolemia can lead to misprocessing of the Aβ42 precursor (APP) and therefore trigger the failure of Aβ homeostasis. We also studied the effects of known insults that lead to retinal degeneration in mouse models on APP processing. Our studies show that light damage resulted in an increase of membrane-bound C-terminal fragments of APP (CTFα/CTFβ) derived from α/β-secretase cleavage without detectable changes in the levels of full-length APP in mice undergoing light-induced photoreceptor degeneration. This accumulation can be explained by the failure of γ-secretase, which normally generates AB and efficiently eliminates the CTFs. Changes in CTFs of APP can be noted upon treatment of APP-expressing cells with either cholesterol, lovastatin, or isoprenoids, suggesting that multiple steps in the cholesterol biosynthesis pathway may regulate the turnover and homeostasis of APP. Further, we find that APP levels and processing may be regulated by the methionine-homocysteine cycle, suggesting that multiple metabolic pathways are responsible for maintaining homeostasis of APP and related proteins in the brain and eyes. Failure in maintaining any of these metabolic pathways may

trigger the failure of membrane protein turnover and homeostasis and lead to neurodegeneration, which may be prevented by management of these metabolic pathways.

Disclosures: K. Sambamurti, None; **E. Hollings**, None; **T. Utsuki**, None; **M.A. Pappolla**, None; **N.H. Greig**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.7

Topic: C.02. Alzheimer's disease and other dementias

Support: NIMH061412, Florida Biomedical Program

NINDS11323

Title: Functional analysis of methyl-substituted nicotines reveals different structural requirements for activation of α4β2 and α7 nAChRs

Authors: *H. XING[1], K. WILDEBOER[1], Y.-H. CHO[1], F. SOTI[1], J. LINDSTROM[2], W. KEM[1]; [1]Univ. Florida, Gainesville, FL; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: The manner in which nicotine's structure determines its nAChR agonist potency and efficacy is still incompletely understood. We have employed a "methyl substitution" scan approach to assess the importance of various portions of the N-methylated pyrrolidinyl ring of (S)-nicotine for interaction with α4β2 and α7 nAChRs, the two most abundant and widely distributed brain receptor subtypes. Selective agonists for these receptors are known to enhance cognitive function. We have synthesized and tested nicotine analogs with methyl substituents at each of the eight potential sites of methylation on the pyrrolidinium ring. Chiral HPLC was used to resolve racemic samples into their individual enantiomers; CD and NMR allowed assignment of methyl group configuration. We also determined the influence of replacing the nicotine 1'N-methyl with an ethyl group. Human nAChRs were expressed in *Xenopus* oocytes and studied using the two electrode voltage clamp method; peak currents were used to assess responses of both receptors and were normalized with respect to near maximal ACh responses. Most methyl substitutions led to a decrease in potency and/or efficacy; effects on the two nAChRs were often different. Quaternizing the N group by addition of a methyl substituent decreased potency ($1/EC_{50}$) >100-fold at both nAChRs. Replacement of the original 1'-N-methyl of nicotine with

an ethyl group almost abolished nicotine interaction at α4β2, but α7 efficacy was retained with only a ~2-fold decrease in potency. Adding a methyl at position 2' increased potency 3-fold at α7 without significantly affecting α4β2 potency and efficacy. Trans-3'methyl substitution enhanced α7 potency 2-fold but reduced α4β2 potency 2-fold. Although trans- and cis-4' methyl substitutions did not affect potencies at either receptor, efficacy was significantly reduced, particularly at α7. Cis-5' methyl substitution destroyed interaction with both α4β2 and α7, but trans-5' methylation only abolished interaction at α4β2; α7 potency and efficacy were reduced 2-fold. In conclusion, alkyl group enlargement at the 1'-N and methylation at the 5'-position is not well tolerated by α4β2, whereas α7 potency and efficacy is not significantly diminished. The differences in methylated-nicotine interaction with human α4β2 and α7 nAChRs suggest new ways in which nicotine and related compounds can be designed to more selectively and potently activate a particular receptor subtype.

Disclosures: H. Xing, None; **Y. Cho**, None; **J. Lindstrom**, None; **F. Soti**, None; **W. Kem**, None; **K. Wildeboer**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.8

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant RO1 AG20159

Title: Complement component C3 and its receptor complement receptor type 3 mediate the phagocytosis and clearance of Abeta by microglia

Authors: *H. FU[1], B. LIU[1], J. L. FROST[1], S. HONG[1], M. JIN[1], I. M. COSTANTINO[2], M. C. CARROLL[3], T. N. MAYADAS[4], D. J. SELKOE[1], C. A. LEMERE[1];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia in the elderly and is characterized by extracellular amyloid plaques and intracellular neurofibrillary tangles. The up-regulated production and activation of complement components and their receptors are found

within and around cerebral amyloid plaques in AD patients. Microglia, the principal immune effector cells in the CNS, can defend against pathogens through phagocytosis via complement component C3 and/or its receptor complement receptor type 3 (Mac-1, CD11b). We previously reported that the deficiency of C3 accelerated cerebral Aβ deposition and neuronal loss, possibly through inhibition of microglia-mediated uptake and clearance of Aβ. In the present study, we provide direct evidence that C3 and Mac-1 mediate the phagocytosis and clearance of fibrillar Aβ (fAβ) by microglia *in vitro* and *in vivo*. Using flow cytometry and confocal laser scanning microscope, we found that both primary mouse microglia and immortal murine microglial cell line were able to take up $fA\beta_{42}$ in a concentration- and time-dependent manner. The $fA\beta_{42}$ taken up by microglia was colocalized with lysosomal markers. Uptake of $fA\beta_{42}$ by primary microglia from mouse pups genetically deficient for C3 or Mac-1 was significantly decreased, compared with that of primary microglia from wild type C57BL/6 mouse pups. The knockdown of C3 or Mac-1 in primary microglia by transient transfection of siRNA against C3 or Mac-1 significantly inhibited $fA\beta_{42}$ uptake compared to cells transfected with control siRNA. *In vivo*, we found that Iba-1 and CD68 positive microglial cells took up cortically microinjected $fA\beta_{42}$, most of which was colocalized with the lysosomal marker, LAMP-1. Five days after the surgery, the fluorescent signal of fAβ left in the brain of C3 or Mac-1 knockout mice was significantly higher than that in wild-type mice. Together, these results demonstrate that C3 and its receptor Mac-1 are involved in the phagocytosis and clearance of Aβ by microglia, providing evidence consistent with a beneficial role for microglia in AD pathogenesis. Attempts to develop therapies aimed at removing Aβ by activating the beneficial role of microglia are warranted.

Disclosures: H. Fu, None; **B. Liu**, None; **J.L. Frost**, None; **S. Hong**, None; **M. Jin**, None; **I.M. Costantino**, None; **M.C. Carroll**, None; **T.N. Mayadas**, None; **D.J. Selkoe**, None; **C.A. Lemere**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS NS059005

Dana Foundation Neuroimmunology Program

Title: Microglial CX3CR1 deficiency restores β-amyloid clearance pathways in neurons and

slows progression of Alzheimer's disease

Authors: *S. E. HICKMAN[1], E. ALLISON[2], U. COLEMAN[2], N. ELPEK[2], T. R. MEMPLE[2], A. D. LUSTER[2], J. EL KHOURY[2];
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Abstract: CX3CR1 is a microglial chemokine receptor that binds fractalkine and regulates microglial recruitment to sites of inflammation and microglial-mediated neurotoxicity. To investigate the role of CX3CR1 in development and progression of Alzheimer's disease in the PS1-APP mouse model, we generated PS1-APP mice deficient in CX3CR1, and analyzed these mice for Alzheimer's-like pathology. We found that CX3CR1 deficiency was associated with a significant reduction in Aβ levels and in the number of senile-like plaques in the brain as compared with age-matched PS1-APP mice. Reduced Aβ level in the brain was associated with improved cognitive function. CX3CR1 deficiency was not associated with change in overall microglial numbers or in microglial association with senile plaques, and microglia maintained their ability to respond to acute injury *in vivo*. Analysis of gene expression in the brains of CX3CR1-deficient PS1-APP mice showed that levels of the Aβ degrading enzymes insulysin and neprilysin, highly expressed in neurons, were restored to levels observed in wild type mice, whereas PS1-APP mice have reduced levels of these enzymes. In contrast, level of the microglia specific enzyme MMP9, also reduced in PS1-APP mice, remained low in PS1-APP mice deficient in CX3CR1. Our data suggest a novel pathway where interaction of microglial CX3CR1 with neuronal fractalkine regulates gene expression in neurons and indicate that lowering CX3CR1 levels or inhibiting its activity in the brain may be a therapeutic strategy to reduce Aβ levels and stop or delay progression of Alzheimer's disease

Disclosures: S.E. Hickman, None; **E. Allison**, None; **U. Coleman**, None; **N. Elpek**, None; **T.R. Memple**, None; **A.D. Luster**, None; **J. El Khoury**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.10

Topic: C.02. Alzheimer's disease and other dementias

Support: BMBF Grant FK 0315089

BMBF Grant FK 0315223

BMBF Grant FK 3013185

Title: Inhibiton of glutaminyl cyclase(s) - New drug targets of Alzheimer's disease therapy

Authors: *H. U. DEMUTH[1,2], M. BUCHHOLZ[1], R. SOMMER[1], D. RAMSBECK[1], S. SCHILLING[1], U. HEISER[1];
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Abstract: We have validated that the metalloenzyme Glutaminyl Cyclase (QC) facilitates a pyroglutamate-formation (pGlu) not only from N-terminal glutamine but also from glutamate, promoting aggregation and toxicity of the pGlu beta-amyloid peptides (pGluAβ) in Alzheimer's disease (AD). Similarly, the amyloid peptides ADan and ABri are deposited in Familial British Dementia (FBD) and Familial Danish Dementia (FDD). These facts support the role of the pGlu-modification as a potential driving force for the amyloid formation process and represent an attractive target for therapeutic intervention in AD, FBD and FDD.
Moreover, the N-terminal pGlu modifications are also features of Monocyte-Chemoattractant Proteins, such as MCP-1. The pGlu-residue originates from cyclization of an N-terminal glutaminyl residue by QC *in vivo*. This posttranslational modification is essential for the stability of the chemokines against N-terminal degradation and for receptor agonist activity. Since compelling evidence suggests that neuroinflammation in general and the chemokine MCP-1 in particular plays a pivotal role early in AD we propose the inhibition of MCP-1 maturation as anti-inflammatory approach. Accordingly, the application of QC-inhibitors appears as double-egged sword fighting two hallmarks of AD: neurodegeneration and neuroinflammation.
Hence, we launched a drug discovery program which has approached the regulatory phase. Initial screening efforts identified imidazole containing thioureas as potent inhibitors of hQC. Starting from these ligands we conducted advanced combination of ligand- and structure based approaches, leading to new core structures and a modified metal binding groups, exhibiting activity in the nanomolar potency range. Starting from 1-(3-(1H-imidazol-1-yl) propyl)-3-(3,4-dimethoxyphenyl) thiourea the exchange of the thiourea by bio-isosteric replacements led to potent inhibitor classes whereas optimization of the metal-binding group was achieved utilizing QC homology and crystal structures revealing the binding modes of compounds within the active site.
We present here first insights into SAR of novel classes of QC inhibitors exhibiting a promising prediction of its blood-brain-penetration profile.
The efficacy of the QC inhibitors was assessed in a cell culture assay, directly monitoring the inhibition of pGluAβ3-42 formation. Conducting multiple pharmacological experiments we have demonstrated the dual function of QC-inhibitors in Alzheimer's disease, reducing the amount of highly toxic and amyloidogenic pGlu-peptides and ameliorating the neuroinflammation by suppression of biologically active MCP-1.

Disclosures: H.U. Demuth: Employment; Probiodrug AG. Research Grant; BMBF grants ## 0315089, 0315223, 3013185. **M. Buchholz:** Employment; Probiodrug AG. Research Grant; BMBF grants ## 0315089, 0315223, 3013185. **R. Sommer:** Employment; Probiodrug AG. Research Grant; BMBF grants ## 0315089, 0315223, 3013185. **D. Ramsbeck:** Employment;

Probiodrug AG. Research Grant; BMBF grants ## 0315089, 0315223, 3013185. **S. Schilling:** BMBF grants ## 0315089, 0315223, 3013185. Employment; Probiodrug AG. **U. Heiser:** Probiodrug AG. Research Grant; BMBF grants ## 0315089, 0315223, 3013185.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.11

Topic: C.02. Alzheimer's disease and other dementias

Title: Pyroglutamate-beta-amyloid induced neuropathology in two transgenic mouse models

Authors: *A. S. ALEXANDRU[1], W. JAGLA[1], S. GRAUBNER[1], R. SEDLMEIER[1], S. KOHLMANN[1], C. BÄUSCHER[1], A. P. OSMAND[2], S. SCHILLING[3], S. VON HÖRSTEN[4], H.-U. DEMUTH[3,5];
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Abstract: Over the past years, various mouse models have been generated in the context of Alzheimer’s syndrome helping to investigate various aspects of the disease. Addressing the recently described importance of the highly neurotoxic N-terminally truncated Aβ species, two different transgenic mouse lines were generated. The most toxic peptide pyroglutamated beta-amyloid 3-40/42 (pGlu-Aβ), formed *in vivo* by glutaminyl cyclase (QC), was introduced by expressing either truncated form AβGln3-42 (TBA2.1), or the mutated full-length APP (APP-NLQ-10). While in TBA2.1 the Aβ peptide expression is directed into the secretory pathway via fusion of the construct to pre-pro-thyroliberin, release of AβGln3-42 in APP-NLQ-10 relies on the β/γ-secretase-pathway after APP expression for subsequent generation of pGlu-Aβ. In order to investigate the influence of these different ways of introducing the pyroglutamated amyloid peptide into separate subcellular compartments, we analyzed mRNA levels and protein expression, and conducted front-to-back high sensitivity immunohistochemistry analysis across the brain. Both mouse lines exhibit significant levels of total Aβ and pGlu-Aβ, but differ in their Aβ/pGlu-Aβ-ratio. Additionally, albeit using the same promoter (Thy 1.2), known as enabling ubiquitous neuronal expression within the brain, Aβ immunoreactivity in TBA2.1 and APP-NLQ-10 is not confined to the same cell groups, but reveals a differential expression pattern. While in homozygous TBA2.1 mice by the age of 2-3 months a substantial neuropathological

phenotype of Aβ and pGlu-Aβ deposits, as well as neuroinflammation and hippocampal cell loss is apparent, homozygous APP-NLQ-10 mice of the same age show considerable intracellular Aβ and pGlu-Aβ immunoreactivity, but have no apparent neuroinflammatory or neurodegenerative phenotype. While behavioral analysis of TBA2.1 is very limited due to an early severe motor phenotype, APP-NLQ-10 shows no evidence of early motor impairment, but reveals altered behavioral responses in various paradigms including tests used for determination of affective behavior and cognition at a later age. These findings are important to further dissect the subcellular relevance of pGlu-Aβ production for unfolding initial cytotoxicity. Also, this later onset of neuropathology will enable a thorough analysis of behavior and might perhaps offer an extended window for treatability with QC inhibitors. Our findings, however, argue strongly for taking into account the importance of differences in cellular and subcellular distribution, as well as localization of amyloid species when investigating their mechanisms of neurotoxicity.

Disclosures: **A.S. Alexandru**, Ingenium Pharmaceuticals GmbH, Employment; **W. Jagla**, Ingenium Pharmaceuticals GmbH, Employment; **S. Graubner**, Ingenium Pharmaceuticals GmbH, Employment; **R. Sedlmeier**, Ingenium Pharmaceuticals GmbH, Employment; **S. Kohlmann**, Ingenium Pharmaceuticals GmbH, Employment; **C. Bäuscher**, Ingenium Pharmaceuticals GmbH, Employment; **A.P. Osmand**, None; **S. Schilling**, Stock Options, Ownership Interest; Probiodrug AG, Employment; **S. von Hörsten**, Consultant for Probiodrug AG, Consultant/Advisory Board; **H. Demuth**, Stock Options, Ownership Interest; CSO and Vice-CEO of Probiodrug AG, Employment.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.12

Topic: C.02. Alzheimer's disease and other dementias

Title: Transgenic mouse models with increased pyroglutamate-Abeta formation show early phenotypic changes

Authors: *S. SCHILLING[1], S. GRAUBNER[2], W. JAGLA[2], C. BÄUSCHER[2], S. KOHLMANN[2], M. MITROVIC[3], S. KURAT[3], B. HUTTER-PAIER[3], M. WINDISCH[3], H.-U. DEMUTH[1];
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Abstract: Pyroglutamate (pGlu)-modified Abeta peptides are found in amyloid deposits in sporadic and inherited Alzheimer's Disease. Formation of pGlu at the N-Terminus confers resistance against cleavage by proteases and peptidases, increases the cytotoxicity of the peptides and speeds up Abeta aggregate formation.
In order to further characterize the pathophysiologic potential of N-truncated and pGlu-modified Abeta peptides, we aimed at an increase of pGlu-Abeta formation in mouse models. In a first approach, APP-NLQ mice were generated, which express human APP carrying two familial AD mutations, a deletion of two amino acids and a point mutation at position three of Abeta resulting in a glutaminyl residue in neurons. Transgenic mice begin to accumulate Abeta peptides at 4-5 months of age. An ELISA analysis reveals a low overall Abeta load (below 100 ng/g brain wet weight), however, the deposited Abeta is completely N-terminally modified by a pGlu-residue (pGlu-Abeta3-x). The appearance of Abeta is accompanied by emotional changes, as assessed by evaluation of mice in a dark/light-box paradigm. A histochemical analysis of the Abeta deposition reveals an intracellular localization of Abeta in cortex and hippocampus.
In a second approach to increase the pGlu-Abeta formation, we crossbred APP_{SL} mice with transgenic mice, which express human Glutaminyl Cyclase (QC) neuron-specifically. Pyroglutamate-modified peptides were detected at an age of 7 months in the brain homogenates of double transgenic mice. At 9 months of age, the pGlu-Abeta load was 2-4 fold higher in double transgenics compared to APP_{SL}. Behavioral changes of APP_{SL}/hQC double transgenics started to develop at 6 months of age, as assessed in Morris water maze and contextual fear conditioning paradigms. Notably, the pGlu-Abeta(3-42) concentration was in the range of ng/g similar to APP-NLQ at the same age, whereas the total Abeta reached the range of µg/g at an age of 7-9 months in APP_{SL}/hQC mice.
Summarizing, to enforce the formation and deposition of pGlu-modified Abeta in mice, we developed and characterized two strategies, which either involved increased production of the direct pGlu-Abeta precursor Abeta3(Q)-42 (i.e. in APP-NLQ mice), or increased presence of the enzyme responsible for pGlu-Abeta production (QC in APP_{SL}/hQC mice). Although the pGlu-Abeta load in both models appear to be moderate, the formation of these species provoke early behavioral changes. The results support that N-terminal heterogeneity of Abeta influences the pathophysiologic potential of amyloid peptides and provide further evidence for a role of N-modified Abeta in human AD pathophysiology.

Disclosures: **S. Schilling**, Probiodrug AG, Employment; **S. Graubner**, Ingenium, Employment; **W. Jagla**, Ingenium, Employment; **C. Bäuscher**, Ingenium, Employment; **S. Kohlmann**, Ingenium, Employment; **M. Mitrovic**, JSW Life Science, Employment; **S. Kurat**, JSW Life Science, Employment; **B. Hutter-Paier**, JSW Life Sciences, Employment; **M. Windisch**, CEO JSW Life Science, Ownership Interest; **H. Demuth**, CSO Probiodrug AG, Ownership Interest; CEO Ingenium, Ownership Interest.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.13

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH grants

Alzheimer's Associaton

Title: Regulated secretory vesicles contain beta-amyloid peptide forms with neuropeptides and catecholamines that undergo co-secretion

Authors: *V. Y. HOOK[1], T. TONEFF[1], L. FUNKELSTEIN[1], M. ZIEGLER[1], H. CYNIS[2], H.-U. DEMUTH[2];
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Abstract: Accumulation of neurotoxic beta-amyloid peptides (Abeta) represent a major factor in the development of Alzheimer's disease that results in memory deficit. Abeta peptide forms include Abeta(1-40) and Abeta(1-42) forms (Abeta40 and Abeta42, respectively) and the N-terminal truncated and enzymatically modified pGlu-Abeta(3-42). Knowledge of the neurobiology of these Abeta peptides can enhance understanding of their cellular production, secretion, and functions. Because neurons undergo activity-dependent secretion of neurotransmitters and Abeta, this study sought to investigate the hypothesis of their common subcellular localization to regulated secretory vesicles. Analyses of dense core regulated secretory vesicles of neuronal-like chromaffin cells demonstrated the colocalization of Abeta peptide forms including pGlu-Abeta(3-42). These Abeta peptides are present with multiple neuropeptides of enkephalin, NPY, galanin, VIP, and somatostatin that undergo regulated secretion for cell-cell communication. Furthermore, these secretory vesicles contain the catecholamine neurotransmitters dopamine, norepipnephrine, and epinephrine that are co-secreted with Abeta and neuropeptides. These secretory vesicles contain the biosynthetic machinery for production of Abeta peptides, neuropeptides, and catecholamines. This organelle contains full-length APP, Abeta peptides, and components for beta- and gamma-secretases that produce Abeta. These APP processing components co-reside with proneuropeptide processing proteases as well as with catecholamine biosynthetic enzymes. The colocalization of Abeta with neuropeptides in secretory vesicles was further illustrated by cellular immunofluorscence confocal microscopy. These findings indicate the joint biosynthesis and secretion of Abeta peptides with diverse neuropeptides and catecholamine neurotransmitters, suggesting that Abeta peptide forms function with multiple neurotransmitter systems.

Disclosures: V.Y. Hook, None; **T. Toneff**, None; **L. Funkelstein**, None; **M. Ziegler**, None; **H. Cynis**, None; **H. Demuth**, None.

Nanosymposium

220. Alzheimer's Disease: Anti-Abeta Therapy, Pyroglutamate, APP Processing, Inflammation, Immunization

Location: Room 32B

Time: Sunday, November 14, 2010, 1:00 pm - 4:30 pm

Program Number: 220.14

Topic: C.02. Alzheimer's disease and other dementias

Title: Overexpression of glutaminyl cyclase, the enzyme responsible for pyroglutamate Abeta formation, induces behavioral deficits and glutaminyl cyclase knock-out rescues the behavioral phenotype in 5XFAD mice

Authors: *T. BAYER[1], S. JAWAHR[1], O. WIRTHS[1], S. SCHILLING[2], S. GRAUBNER[3], H.-U. DEMUTH[2];
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Abstract: Pyroglutamate-modified Abeta (AβpE3-42) peptides are gaining considerable attention as potential key players in the pathology of Alzheimer's disease (AD) due to their abundance in AD brain, high aggregation propensity, stability and cellular toxicity. Overexpressing AβpE3-42 induced a severe neuron loss and neurological phenotype in TBA2 mice. In vitro and in vivo experiments have recently proven that the enzyme glutaminyl cyclase (QC) catalyzes the formation of AβpE3-42. The aim of the present work was to analyze the role of QC in an AD mouse model with abundant AβpE3-42 formation. 5XFAD mice were crossed to transgenic mice expressing human QC (hQC) under the control of the Thy1 promoter. 5XFAD/hQC bigenic mice showed significant elevation of TBS, SDS and formic acid soluble AβN3pE peptides and aggregation in plaques. In 6-month-old 5XFAD/hQC mice, a significant motor and working memory impairment developed compared to 5XFAD. The contribution of endogenous QC was studied by also generating 5XFAD/QC-KO mice (mouse QC knockout). 5XFAD/QC-KO mice showed a significant rescue of the wild-type mice behavioral phenotype in the elevated plus maze test in comparison to both, 5XFAD and 5XFAD/hQC demonstrating the important contribution of endogenous mouse QC and transgenic overexpressed QC. These data clearly demonstrate that QC is the essential enzyme modulating AβpE3-42 levels in vivo and proves on a genetic base the concept, that therapeutic reduction of QC activity is an interesting new therapeutic approach for AD.

Disclosures: **T. Bayer**, Probiodrug, Consultant/Advisory Board; **S. Jawahr**, None; **O. Wirths**, None; **S. Schilling**, Probiodrug, Employment; **S. Graubner**, Ingenium, Employment; **H. Demuth**, CSO Probiodrug, Ownership Interest.

Nanosymposium

221. Autism: Genetic and Animal Models I

Location: Room 25A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 221.1

Topic: C.06. Developmental Disorders

Support: NIH DPI ODO388902

NIH 1R21 MH0878901

SFARI 137433

IBRO Outstanding Postdoctoral Fellowship

The Tashia and John Morgridge Endowed Postdoctoral Fellowship

CIRM TG201159

Title: Using iPS cells to identify the cellular basis of autism in patients with Timothy Syndrome

Authors: *S. P. PASCA[1], M. YAZAWA[1], T. PORTMANN[1], A. M. PASCA[1], O. SHCHEGLOVITOV[1], I. VOINEAGU[4], J. A. BERNSTEIN[2], D. H. GESCHWIND[4], J. HALLMAYER[3], R. E. DOLMETSCH[1];
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Abstract: Autism Spectrum Disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders characterized by language impairment, deficits in social interaction and stereotyped interests and behaviors. The underlying cellular and molecular defects that lead to ASDs are not understood. We have used induced pluripotent stem cells (iPSCs) to investigate the cellular basis of Timothy Syndrome (TS), a disorder that is associated with autism and is caused by a point mutation in the L-type calcium channel CaV1.2. We generated twenty iPSC lines from fibroblasts harvested from two patients with TS and from healthy controls. We generated neuronal precursors and neurons from these cells and have characterized them using immunocytochemistry, microarrays, calcium imaging and electrophysiology. From these studies we have identified a number of disease specific phenotypes in both the precursors and the neurons derived from TS patients. These results

suggest that iPSC derived neurons are a promising approach for studying the underlying cellular defects that lead to psychiatric disorders.

Disclosures: S.P. Pasca, None; **M. Yazawa**, None; **T. Portmann**, None; **A.M. Pasca**, None; **O. Shcheglovitov**, None; **J.A. Bernstein**, None; **J. Hallmayer**, None; **R.E. Dolmetsch**, None; **I. Voineagu**, None; **D.H. Geschwind**, None.

Nanosymposium

221. Autism: Genetic and Animal Models I

Location: Room 25A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 221.2

Topic: C.06. Developmental Disorders

Support: A.P. Giannini Foundation

NARSAD Young Investigator Award

NIH/NIMH 1K99MH090238-01

NIH/NIMH R01 MH081754-02R

Title: Modeling the functional genomics of autism using human neurons

Authors: *G. KONOPKA[1], E. WEXLER[2], E. ROSEN[3], L. CHEN[3], G. OSBORN[2], D. LU[2], F. GAO[3], G. COPPOLA[3], D. H. GESCHWIND[4];
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Abstract: Autism is one of the most common neurodevelopmental disorders, yet the etiology of the disease remains unknown. Genetic association studies and study of structural chromosomal variation have begun to provide insight into key molecules involved in both autism and the broader autism spectrum disorder (ASD). However, adjuncts to current animal models are necessary to provide higher throughput systems in which to try to mimic molecular and cellular aspects of ASD based on known human genetic risk factors. Furthermore, the disruption of highly evolved cognitive functions in patients with autism suggests that human or in some cases primate-specific molecular genetic interactions that underlie the development of these neural circuits, may be involved. From this perspective, human in vitro models to attain higher

throughput analysis of genes and pathways and to minimize evolutionary divergence would be quite useful. To directly address this need, we examined whether a human neuronal culture system based on primary neural progenitors and their progeny could be utilized in modeling some of the complex genetic features of autism. These normal human neuronal progenitors (NHNPs) were differentiated into a post-mitotic neuronal state through addition of specific growth factors. We examined whole genome gene expression throughout an 8 week time course of differentiation. After four weeks of differentiation, the cells displayed both morphological features and gene expression patterns indicative of a neuronal fate. Strikingly, a significant number of genes associated with ASD are either induced or repressed at this time point compared to undifferentiated cells. Moreover, we find many ASD genes highly connected to one another during the differentiation process using an unbiased assessment of underlying gene expression connectivity. Finally, the NHNP cells are genetically tractable, allowing for the manipulation of multiple candidate genes simultaneously or the administration of numerous environmental hazards. For example, by manipulating levels of FOXP2, a transcription factor know to directly regulate several ASD genes, we identify several direct targets of FOXP2 that are related either to disease or neuronal differentiation. Thus, NHNPs can be used to study signaling networks in ASD, the effects of mutation of several ASD candidate genes on neuronal differentiation gene expression, and the effects of extracellular molecules. These data should provide us with a better understanding of the signaling pathways disrupted in ASD.

Disclosures: G. Konopka, None; **E. Wexler**, None; **E. Rosen**, None; **L. Chen**, None; **G. Osborn**, None; **D. Lu**, None; **F. Gao**, None; **G. Coppola**, None; **D.H. Geschwind**, None.

Nanosymposium

221. Autism: Genetic and Animal Models I

Location: Room 25A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 221.3

Topic: C.06. Developmental Disorders

Title: In vitro studies of autism-related mutations of the contactin genes

Authors: *I. CLOËZ-TAYARANI[1], O. MERCATI[1,2], M. KONYUKH[1,3], C. LEBLOND[1,3], R. DELORME[4], M. LEBOYER[5], T. BOURGERON[1,3];
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Abstract: Autism spectrum disorders (ASD) are characterized by lifelong developmental

disabilities with impaired communication, restricted and repetitive behaviour. Individuals with ASD have difficulties in everyday social interactions. ASD have been shown to strongly depend on genetic factors. The genetics of ASD however is complex and these disorders may result from multigene interactions rather than rare mutations. The precise nature of the brain abnormalities still remains elusive. The results from our genetic studies in ASD patients have identified mutations in synaptic cell adhesion molecules including neuroligins and neurexins. These molecules play an important role in the maintenance of synapses. In the present study, we used the Illumina© BeadArray to genotype one million single nucleotide polymorphisms (SNP) covering the human genome in 250 affected individuals and 350 controls. We provide evidence for the existence of copy number variants as well as non synonymous heterozygous mutations in genes encoding several contactin members (contactins 3-6). The contactins belong to the cell adhesion molecules of the immunoglobulin superfamily and are closely related by their sequence, structure and neural properties. Our results indicate that identified mutations are distributed among the immunoglobulin and fibronectin domains of contactin molecules. We focused our analysis on mutations of contactin 4 and contactin 6 genes. We analyzed the cellular expression and membrane addressing of the mutated contactin 4 and 6 proteins in HEK 293 cells transfected with the corresponding mutated cDNAs. Our results indicate a clear cytoplasmic retention for some of the contactin 4 and contactin 6 mutations. Western blot analysis also revealed modifications in biochemical properties of these mutated proteins. Our current studies aim at identifying the consequences of these mutations on neurite outgrowth promoting activities of the contactin members using primary neurons in culture. We are also studying the effects of mutations on the binding of contactins to their known signalling partners. The identified mutations of contactin molecules in ASD patients may provide valuable insights into the role of individual immunoglobulin and fibronectin domains of contactin molecules. These approaches may also help in further understanding of the pathogenesis of ASD.

Disclosures: I. Cloëz-Tayarani, None; **O. Mercati**, None; **M. Konyukh**, None; **C. Leblond**, None; **R. Delorme**, None; **M. Leboyer**, None; **T. Bourgeron**, None.

Nanosymposium

221. Autism: Genetic and Animal Models I

Location: Room 25A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 221.4

Topic: C.06. Developmental Disorders

Support: CIHR Grant MOP14460

CIHR Grant MOP74564

Althea Foundation

Cure Autism Now

R37 MH049428

Nina Ireland

Genome Canada and Génome Québec

Title: A SNP associated with autism affects Dlx5/Dlx6 regulation in the forebrain

Authors: *L. POITRAS[1], M. YU[1], C. LESAGE-PELLETIER[1], R. B. MACDONALD[1], J.-P. GAGNÉ[2], G. HATCH[1], I. KELLY[2], S. P. HAMILTON[3], J. L. RUBENSTEIN[4], G. G. POIRIER[2], M. EKKER[1];
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Abstract: *Dlx* homeobox genes play a crucial role in the migration and differentiation of subpallial precursor cells that will give rise to various subtypes of gamma-amino-butyric-acid (GABA)-expressing neurons of the forebrain, including local-circuit cortical interneurons. Aberrant development of GABAergic interneurons has been linked to several neurodevelopmental disorders including epilepsy, schizophrenia, Rett syndrome and autism. Recent studies have associated *DLX* genes with human autism spectrum disorders (ASDs). Two *Dlx* bigene clusters, *DLX1/DLX2* and *DLX5/DLX6*, are found in autism susceptibility loci on chromosomes 2q31.1 and 7q21.3. In a search for autism genomic variants in the *DLX1/2* and *DLX5/6* loci, we identified thirty-one single nucleotide polymorphisms (SNPs) and two insertion/deletion polymorphisms. Interestingly, an adenine to guanine SNP was found in a *cis*-regulatory element (CRE) located in the intergenic region of the *DLX5/DLX6* bigene cluster, the I56i enhancer. The location of the SNP coincides with one of the two putative DLX binding sites within I56i. Furthermore, the I56i CRE was identified as one of 481 ultraconserved elements of the mouse genome (i.e., 100% identity with no insertion or deletions on DNA fragments longer than 200bp between orthologous regions of the human, rat and mouse genomes). Therefore, the identification of a SNP in the ultraconserved I56i enhancer is not only surprising but also suggests the SNP could have functional consequences for *Dlx5/Dlx6* regulation.
The I56i CRE is involved in *Dlx5/Dlx6* homeobox gene expression in the developing forebrain. Using transgenesis analysis, we have shown that the activity of this element was observed in a subset of the differentiating GABAergics interneurons that originated from the lateral, medial and caudal ganglionic eminences (LGE, MGE and CGE). The presence of the SNP results in lower I56i activity, predominantly in the MGE and CGE as well as in streams of neurons migrating to the cortex. Reduced activity is also observed in GABAergic interneurons of the adult somatosensory cortex. The SNP affects the affinity of Dlx proteins for their binding site in vitro and reduces the transcriptional activation of the enhancer by Dlx proteins. Affinity purification using I56i sequences led to the identification of a novel regulator of *Dlx* expression, the General Transcription Factor 2-I (GTF2I), that is among the genes most often deleted in

Williams-Beuren syndrome (WBS). This study illustrates the clear functional consequences of a single nucleotide variation in an ultraconserved non-coding sequence in the context of developmental abnormalities associated with disease.

Disclosures: L. Poitras, None; **M. Yu**, None; **C. Lesage-Pelletier**, None; **R.B. MacDonald**, None; **J. Gagné**, None; **G. Hatch**, None; **I. Kelly**, None; **S.P. Hamilton**, None; **J.L. Rubenstein**, None; **G.G. Poirier**, None; **M. Ekker**, None.

Nanosymposium

221. Autism: Genetic and Animal Models I

Location: Room 25A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 221.5

Topic: C.06. Developmental Disorders

Support: NIH R01MH080718 (Brodkin)

NIH ARRA Supplement 3R01MH080718-03S

Title: Elucidating neural circuitry underlying sociability, an autism relevant phenotype, in mouse models

Authors: *A. S. KREIBICH[1], M. TORRE[2], E. S. BRODKIN[2];
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Abstract: Reduced sociability (reduced tendency to seek social interaction) is among the most disabling and treatment-refractory symptoms of autism spectrum disorders (ASD). Therefore, there is a strong need for a better understanding of the fundamental neurobiology of sociability. Due to the experimental control they afford, mouse models will be very useful for this effort. Relative to C57BL/6J (B6) mice, BALB/cJ mice show low levels of sociability . In order to elucidate the neural circuitry underlying sociability, we mapped neural activation following social interaction in juvenile male B6 vs. BALB/cJ mice. We hypothesized that B6 and BALB/cJ mice would show differential levels of sociability and differential activation of amygdala nuclei following social interactions. We mapped neural activation with Fos immunohistochemistry, comparing the following 4 groups: 1) B6 mice “not exposed” (remaining in their home cages until perfusion); 2) B6 mice “exposed” to a stimulus mouse (gonadectomized A/J male mouse) in a social choice test; 3) BALB/cJ mice not exposed; 4) BALB/cJ mice exposed. The four groups were tested in a counterbalanced order, and all mice were perfused 1 hr after the beginning of the

exposure or nonexposure condition . The density of Fos immunoreactive cells was measured in multiple brain regions, and patterns of brain region activation were compared among the 4 groups. As in our previous studies, B6 mice were more sociable than BALB/cJ mice. Social exposure increased Fos staining in the basolateral amygdala (BLA) in B6 mice, but did not alter Fos levels in the BLA of Balb mice (one way ANOVA ($F_{3,21}=6.654$, $p=0.0032$). There were no strain differences in activation of medial or central amygdala nuclei. Furthermore, BALB/cJ, but not B6 mice, showed a significant decrease in Fos activation after social exposure in the CA3 region of hippocampus. These data further elucidate the brain regions that may underlie individual differences in sociability. Further elucidation and targeting of the primary neurotransmitters and cell types in the basolateral amygdala specifically may lead to novel approaches for rescuing sociability in ASD or other neurodevelopmental disorders. Future studies will extend this approach to mice with mutations in ASD-relevant genes.

Disclosures: A.S. Kreibich, None; **M. Torre**, None; **E.S. Brodkin**, None.

Nanosymposium

221. Autism: Genetic and Animal Models I

Location: Room 25A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 221.6

Topic: C.06. Developmental Disorders

Support: Ontario Mental Health Foundation - Postdoctoral Fellowship

Title: Using magnetic resonance imaging to probe structural changes in a mouse model of autism

Authors: *J. ELLEGOOD, J. P. LERCH, R. M. HENKELMAN;
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Abstract: Background - The Neuroligins and Neurexins are synaptic cell adhesion genes and disruptions in these genes have shown up in a wide array of Autism association studies (Jamain et al. 2003; Laumonnier et al. 2004). Mouse models with a Neuroligin3 knockout show reduced communication skills and a lack of social novelty preference (Radyushkin et al. 2009). A Neuroligin3 knockdown model, which displays an approximate 90% loss of Neuroligin3, has also displayed abnormal social interaction (Tabuchi et al., 2007).
Objectives - The purpose of this study was to assess differences in neuroanatomy and white matter microstructure between the Neuroligin3 mutant and wild-type mice.
Methods - 8 Neuroligin3 mutant and 8 male wild type fixed mouse brains, all 108 days old, were

examined.
MRI Acquisition - A 7.0 Tesla MRI scanner was used to acquire anatomical images of brains within skulls as well as Diffusion Tensor Images (DTI) to assess changes in the white matter. Total imaging time for a set of 3 brains imaged in parallel was ~12 h and 16 h for the two methods, respectively.
Data Analysis - We use image registration to align a neuroanatomical atlas defining 62 separate brain regions towards each scan. Volumes of individual structures for each mouse were then calculated. Changes in white matter were determined by registering fractional anisotropy (FA) maps to the same atlas and computing average FA values per structure. Group differences in volume or FA were calculated using t-tests, multiple comparisons controlled using the False Discovery Rate (FDR).
Results - Significant volume changes were found in 16 different regions, with FDRs of < 5%. Some of the notable regional changes were decreases in the corpus callosum (14%), fornix (14%), hippocampal formation (14%), internal capsule (15%), striatum (12%), and thalamus (15%). Despite the volume changes found in many white matter structures, such as the corpus callosum, internal capsule, and fornix, there were no significant FA differences detectable in the white matter of the Neuroligin3 mouse.
Conclusions - This study highlights volumetric changes in 16 different regions in the brain of the Neuroligin3 mouse. Furthermore, volume decreases are found in major white matter structures, such as the corpus callosum (p-value = 0.002 with an FDR value of 0.02), which may be related to those changes seen previously in human autism (Alexander et al. 2007), although in that study FA decreases were also found. A change in volume without a significant decrease in FA indicates that while there may be a loss in size of the white matter fiber bundles, the myelination and integrity of white matter tracts seem intact.

Disclosures: J. Ellegood, None; **J.P. Lerch**, None; **R.M. Henkelman**, None.

Nanosymposium

221. Autism: Genetic and Animal Models I

Location: Room 25A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 221.7

Topic: C.06. Developmental Disorders

Support: R01-MH081754-02R

R37 MH60233-06A1

Title: Identification of shared molecular pathways involved in autism by brain transcriptome

profiling

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Abstract: Autism is the most severe end of a spectrum of neurodevelopmental disorders characterized by repetitive behaviors, deficits in social behavior and language impairment. Autism spectrum disorders (ASD) are highly heritable, with concordance rates of 70-90% for monozygotic and 0-10% for dizygotic twins. Despite significant progress in understanding autism genetics, each of the genetic factors identified so far accounts for less than 1% of the cases, and no genetic cause responsible for the majority of ASD cases has yet been identified. A fundamental question regarding the pathogenesis of autism is whether the core behavioral and cognitive manifestations of autism result from the perturbation of specific neurobiological systems and molecular pathways, despite the wide variety of genetic causes. We aimed to identify molecular pathways involved in autism pathogenesis by combining genome-wide expression profiling of several brain regions from autistic cases and healthy controls, with network analyses. We profiled 3 brain regions: BA9, BA41/42 and cerebellum in 15 age matched ASD cases and controls from which high quality RNA could be reliably extracted. RNA integrity was tested by Agilent Bioanalyzer and array quality control metrics. We identified several hundred genes reliably differentially expressed (DE) at a stringent FDR of 1%. Genes differentially expressed between autistic and control samples were enriched for known autism susceptibility genes, and the clustering of individuals based on DE genes effectively differentiated between a large subset of cases and controls. The clustering indicates that more than half of the autistic subjects shared the major pattern of gene expression abnormalities. Weighted gene co-expression network analysis (WGCNA) showed that the autistic brain transcriptome preserves the general functional organization characteristic of normal brain tissue, supporting the robustness of our data. We identified two gene co-expression modules, showing strong correlation with the autism phenotype, one of which was enriched in genes involved in synaptic function while the other contained genes related to immune function. We further analyzed the enrichment of WGCNA modules for genes showing strong association signal in published GWAS studies.

Disclosures: I. Voineagu, None; **D. Geschwind**, None; **S. Horvath**, None; **F. Gao**, None.

Nanosymposium

221. Autism: Genetic and Animal Models I

Location: Room 25A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 221.8

Topic: C.06. Developmental Disorders

Support: Howard Hughes Medical Institute

NIMH Grant 1RC2MH089952

Title: Searching for recessive autism mutations in outbred populations using whole exome sequencing

Authors: *M. CHAHROUR[1], C. R. SCHUBERT[1], T. W. YU[1,2], C. R. STEVENS[3], R. HILL[1], S. B. GABRIEL[3], C. A. WALSH[1];
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[2]Massachusetts Gen. Hosp., Boston, MA; [3]The Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract: Autism is a neurodevelopmental disorder characterized by impaired communication skills, social behavior abnormalities, and stereotypies, with a heritability of 60-90%. The fact that no single genetic aberration accounts for more than 1% of cases suggests extreme heterogeneity, posing one of the major challenges in identifying genes. We hypothesized that at least a subset of autism is caused by recessive mutations, and used homozygosity analysis to dissect genetic heterogeneity. We analyzed the Autism Genetic Resource Exchange (AGRE) dataset to identify the most informative probands, and performed whole exome sequencing to find candidate pathogenic variants. Analysis was done using software that identifies runs of homozygosity (ROH) in affected individuals within a family. The ROH are more likely to carry recessive mutations and the analysis identified individuals enriched for ROH in their genome suggesting identity-by-descent. We identified several probands with large ROH and DNA samples for the top 18 informative individuals were subjected to whole exome sequencing with 92% coverage at 20X. The data generated was analyzed to map all SNPs, microinsertions, and microdeletions. On average we identified 34,581 variants per exome. Using an in-house analysis pipeline to annotate the mapped reads, we found that 15,145 variants per exome are potentially pathogenic. The common variants identified by the HapMap project, the 1000 Genomes project, and dbSNP130 were filtered out, and the remaining variants were considered candidates for pathogenic changes (an average of 1,210 variants per exome). Out of these, 53 were homozygous (35 in the coding sequence and 18 splice site changes) and 1,157 were heterozygous changes (614 in the coding sequence and 543 splice site changes). Initially we will restrict our attention to recessive mutations and variants that fall within identified ROH. Next, we will focus on variants inherited in a compound heterozygous state. We will resequence the identified variants in a collection of 500 control samples, and also compare sequences of affected individuals against those of their family members. Finally, additional autism patients will be screened for these variants. This will strengthen the hypothesis that disruption of a particular gene is causative of autism, and allow us to determine the frequency of a variant in affected populations. Uncovering the genes that contribute to autism is the first step towards therapeutic strategies.

Disclosures: M. Chahrour, None; **C.R. Schubert**, None; **T.W. Yu**, None; **C.R. Stevens**, None; **R. Hill**, None; **S.B. Gabriel**, None; **C.A. Walsh**, None.

Nanosymposium

222. Epilepsy: Mechanisms

Location: Room 24A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 222.1

Topic: C.07. Epilepsy

Support: NIH (NINDS & NCRR)

Title: Rapamycin suppresses mossy fiber and somatostatin interneuron axon sprouting but not epileptogenesis in a mouse model of temporal lobe epilepsy

Authors: *P. BUCKMASTER[1], X. WEN[2], F. LEW[3];
[1]Dept Comparative Med., [2]Stanford Univ., Stanford, CA; [3]Washington State Univ., Pullman, WA

Abstract: Patients with temporal lobe epilepsy display many pathological changes in the dentate gyrus, including hilar neuron loss, granule cell axon (mossy fiber) sprouting, GABAergic axon sprouting, hilar ectopic granule cells, and others. It is unclear which of these circuit anomalies, if any, contribute to epileptogenesis. Ideally, one would like to selectively block specific changes to identify those that affect the development of spontaneous seizures. Recent evidence suggests the mTOR inhibitor, rapamycin, might be useful in this regard. GIN mice, which express GFP in a subset of somatostatin interneurons, were treated with pilocarpine to induce status epilepticus. Beginning 1 d later, mice were treated daily with rapamycin (3 mg/kg, ip). After 2 months, mice were perfused and stereological methods used to measure GFP-positive axon length and aberrant Timm staining in the granule cell layer + molecular layer. Epileptic rapamycin-treated mice displayed significantly less mossy fiber and GFP-positive axon sprouting in the granule cell layer + molecular layer than epileptic vehicle-treated mice. The optical fractionator method was used to measure numbers of granule cells, Nissl-stained hilar neurons, and Prox1-immunoreactive hilar ectopic granule cells. Hilar neuron loss, number of granule cells, and number of Prox1-immunoreactive hilar ectopic granule cells were similar in epileptic rapamycin- and vehicle-treated mice. To evaluate epileptogenesis, mice were video-monitored daily 9 h/d during the second month after pilocarpine-induced status epilepticus. The frequency and severity of seizures was similar in both groups. To more rigorously evaluate epileptogenesis, mice were video-EEG monitored with an electrode implanted in the hippocampus. Recordings were obtained daily 9 h/d during the third month after status epilepticus. Again, frequency and severity of seizures was similar in rapamycin- and vehicle-treated mice. One possible interpretation of these data is that axon sprouting in the dentate gyrus is not epileptogenic but loss of hilar neurons and generation of ectopic granule cells might be. However, mossy fiber and somatostatin axon sprouting might have opposing effects, and rapamycin might affect epileptogenesis through other mechanisms

that were not evaluated in the present study. Nevertheless, these findings suggest that targeting signal transduction mechanisms is a useful strategy to more selectively test the epileptogenicity of circuit changes in temporal lobe epilepsy.

Disclosures: P. Buckmaster, None; **X. Wen**, None; **F. Lew**, None.

Nanosymposium

222. Epilepsy: Mechanisms

Location: Room 24A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 222.2

Topic: C.07. Epilepsy

Support: Epilepsy Research UK Grants A0702 and A0937

Wellcome Trust Grant 074771

Title: High frequency network activity preceding epileptic seizures in vitro

Authors: *J. G. JEFFERYS[1], P. JIRUSKA[2], W.-C. CHANG[2], A. F. BUJAN[3], J. CSICSVARI[4], R. W. DEARDEN[3];
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Abstract: Pre-ictal changes in EEG can occur in both humans and animal models. Using rat hippocampal slices in vitro we found low-amplitude high-frequency activity (HFA; >100Hz) building up over periods of tens of seconds preceding seizures in CA1. Similar HFA appeared in both the low calcium (<0.2mM) and high potassium (8.5 mM) models. The increase in HFA amplitude leading up to each seizure was associated with increases in multiunit activity, the global synchrony index and with decreases in the signal complexity. In this study we examined the cellular mechanism of HFA and its pre-ictal dynamics.
At the cellular level, HFA cycles were associated with significant increases in the probability of action potential firing of all pyramidal cells, but only of a subset of interneurons. Cellular firing properties, together with the size and shape of the cycles of HFA, suggested that each cycle was produced by the action potential co-firing of 5-10 pyramidal cells within a ~5ms time window. Spike train analysis demonstrated relatively low rates of coincident firing. However, testing the significance of coincident firing revealed greater than chance coincident firing (within 5 ms) of 4% of the 1354 pairs of pyramidal cells ($p<0.001$ in all cases) suggesting involvement of fast synchronizing mechanism in generation of HFA. The presence of pre-ictal HFA in the low

calcium model implicates non-synaptic mechanisms of synchronization, and the failure of the gap junction blockers octanol and carbenoxolone to suppress this HFA suggests that ephaptic interactions play the key role. The firing rates of both pyramidal cells and interneurons accelerated as the next seizure approached, but remained substantially slower than the frequency of HFA. While neuronal firing accelerated as the seizure approached, HFA frequency decreased from ~230 Hz to ~160 Hz, a difference that may be due to increases in neuronal synchronization. Epileptic seizures usually appear abruptly on the macroscopic scale, as they do in the two models reported here. However transition to seizure can be preceded by more subtle changes in neuronal dynamics, as proved to be the case in the two distinctive models used here. The progressive increases in neuronal firing and synchronization over the tens of seconds preceding seizures demonstrates a pre-ictal state that may prove useful in early seizure detection and in attempts to block transition to seizure.

Disclosures: P. Jiruska, None; **W. Chang**, None; **A.F. Bujan**, None; **J. Csicsvari**, None; **R.W. Dearden**, None; **J.G. Jefferys**, None.

Nanosymposium

222. Epilepsy: Mechanisms

Location: Room 24A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 222.3

Topic: C.07. Epilepsy

Support: Dedicated Health Research Funds of the University of New Mexico School of Medicine

Title: Inhibition of temporal lobe epilepsy development in rat by NKCC1-block

Authors: *W. S. MUELLER[1], D. E. BRAGIN[2], S. PETERSON[4], J. A. CONNOR[3];
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Abstract: Development of temporal lobe epilepsy (TLE) following status epilepticus (SE) is not well understood. Tissue obtained from TLE patients permits the study of pathological changes in human brain after years of established epilepsy, but study of plasticity in the early, latent period (that leads to recurrent spontaneous seizures in the chronic period), requires animal model studies. Most published SE rat models of TLE eventually reproduce most clinical and neuropathological features of human TLE, including ultimate preferential loss of entorhinal cortex (EC) layer3 (L3) pyramidal neurons (PNs). However, these SE models show so many

early changes that it is difficult to identify the alterations crucial for occurrence of seizures. We therefore employed a more mild lithium-pilocarpine SE protocol (benzodiazepine 1 hour after onset of SE, instead of 2 or more hours) in order to enhance the opportunity to observe specific changes. This model also better reproduces the latent period of several months in humans. Studying L5 neurons of the deep EC during the latent period we have demonstrated a depolarizing shift of GABAAergic PSPs, caused by progressive re-expression of the Cl- inward transporter NKCC1, as well as a progressive downregulation of the Cl- outward transporter, KCC2. These changes are highly specific for the deep EC, suggesting that antiepileptic drugs designed to enhance GABAergic transmission might actually increase epileptiform activity in the EC while inhibiting motor cortex and output.
Here we tested 1) if occurrence of seizures predicts early L3 PN loss, and 2) in how far bumetanide, an inhibitor of NKCC1 that largely restores IPSP reversal potential, may suppress EC seizures during and beyond treatment. Seizure occurrence was determined with video recordings of SE control rats and SE rats that had been chronically treated with bumetanide from 1 week post SE for 2-3 months, and sacrificed 6 weeks later. Neurotrace staining showed absence of any significant loss of L3 PNs 3 weeks post SE. Bumetanide treated rats showed greatly lower seizure occurrence during (-80%) and beyond bumetanide treatment (-85%).
In conclusion, our results demonstrate that early EC L3 PN loss is not necessary for occurrence of seizures, but may result from later downstream effects. Further, bumetanide is effective in suppressing seizures in the early chronic period during as well as weeks beyond the treatment period. Apparently, EC epileptiform activity or seizures which occur in absence of bumetanide have induced additional chronic changes that facilitate epileptic activity.

Disclosures: W.S. Mueller, None; **D.E. Bragin**, None; **S. Peterson**, None; **J.A. Connor**, None.

Nanosymposium

222. Epilepsy: Mechanisms

Location: Room 24A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 222.4

Topic: C.07. Epilepsy

Support: NIH/NINDS

Title: Hilar and CA3 contributions to recurrent excitatory connectivity of dentate granule cells in a model of temporal lobe epilepsy--A laser scanning glutamate uncaging study

Authors: *W. ZHANG[1], J. R. HUGUENARD[2], P. S. BUCKMASTER[1];
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CA

Abstract: Enhanced recurrent excitation in dentate granule cells has been intensively investigated as a potentially pro-epileptic change in temporal lobe epilepsy (TLE). Previous studies suggest that neurons in the hilus contribute little, if any, to recurrent excitation of granule cells in epileptic animals, most likely because of the loss of mossy cells and amputation of axons in slice preparations. However, other studies suggest that mossy cells and CA3 pyramidal cells might sprout axons and synapse with granule cells after deafferentation lesions and in epilepsy models. Furthermore, hilar ectopic granule cells are generated in models of temporal lobe epilepsy, and they have been shown to project axon collaterals to the molecular layer where the dendrites of normally positioned granule cells are located. In this study, we sought to test the hypothesis that neurons in regions other than the granule cell layer contribute to enhanced recurrent excitation of granule cells in epileptic pilocarpine-treated rats. Laser scanning photostimuli randomly, systematically, and focally activated neurons in the granule cell layer, hilus, and proximal CA3 pyramidal cell layer in a grid pattern with 60 μm spacing. By uncaging glutamate on neurons in scanning areas while recording evoked EPSCs of granule cells (V_{hold} = -70 mV), we obtained maps of excitatory connectivity in 400 μm-thick slices (control = 6, epileptic = 11 rats) and measured the proportion of stimulation sites in each region that evoked EPSCs. For all regions combined, more excitatory synaptic responses were evoked in epileptic rats (7 ± 1% of stimulation sites) than in controls (3 ± 1%, $P<0.05$). Consistent with previous reports, a higher proportion of sites in the granule cell layer evoked EPSCs in epileptic rats compared to controls (7 ± 2% versus 3 ± 2%). However, in addition to the granule cell layer, stimulation sites in the hilus (4 ± 2% in control, 7 ± 2% in epileptic) and proximal CA3 pyramidal cell layer (2 ± 1% in control, 7 ± 3% in epileptic) also demonstrated increased probabilities of evoking EPSCs in granule cells in epileptic animals. These findings suggest that neurons in the hilus and proximal CA3 region contribute to enhanced recurrent excitatory connectivity of dentate granule cells in TLE.

Disclosures: W. Zhang, None; **J.R. Huguenard**, None; **P.S. Buckmaster**, None.

Nanosymposium

222. Epilepsy: Mechanisms

Location: Room 24A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 222.5

Topic: C.07. Epilepsy

Support: R21NS061069

1F31NS064694

Title: Persistent and resurgent sodium currents are increased in EC layer II neurons in a rat model of temporal lobe epilepsy

Authors: *M. K. PATEL[1], E. H. BERTRAM[3], N. J. HARGUS[2];
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Abstract: Temporal lobe epilepsy (TLE) is a common form of adult epilepsy involving the limbic structures of the temporal lobe. Layer II neurons of the entorhinal cortex (EC) form the major excitatory input into the hippocampus via the perforant path and consist of non-stellate and stellate neurons. These neurons are spared and hyper-excitable in TLE. Since sodium (Na) channels play a critical role in action potential (AP) generation and conduction we sought to determine if Na channel gating parameters and expression levels were altered in TLE. Specifically we focused on persistent (I_{NaP}) and resurgent (I_{NaR}) Na currents since these two currents arise mainly from activation of the $Na_v1.6$ Na channel isoform and are major contributors to the generation of AP bursts.
I_{NaP} currents were recorded in EC layer II non-stellate and stellate neurons using voltage ramps. Both TLE stellate and non-stellate neurons had larger I_{NaP} current amplitudes when compared to control neurons. In non-stellate neurons control I_{NaP} currents had an amplitude of -121.3 ± 26.1 pA (n = 9) and were significantly ($P<0.01$) increased in TLE to -358 ± 46.2 pA (n = 7). In a similar manner, TLE stellate neurons also had increased I_{NaP} current amplitudes. Amplitudes were increased from -153.5 ±18.9 pA (n = 9) under control conditions to -356.8 ± 31.7 pA (n = 7: $P < 0.01$) in TLE.
I_{NaR} current amplitudes were also increased in TLE. I_{NaR} currents in non-stellate neurons were profoundly increased from -514.8 ± 72.4 pA (n = 6) in control to -1394.6 ± 82.1 pA (n = 7: $P < 0.001$) in TLE. I_{NaR} currents in stellate neurons were also significantly larger in TLE compared to controls. Control amplitudes were increased from -568.9 ± 58.9 pA (n = 7) to -1477.8 ± 75.2 pA (n = 7: $P < 0.001$) in TLE. Families of I_{NaR} currents were evoked to construct current voltage plots. TLE non-stellate neurons had significantly ($P<0.05$) hyperpolarized I_{NaR} $V_{1/2}$ values (-61.3 ± 1.9; n=7 in control compared with -68.0 ± 2.3; n=8 in TLE). Slopes were also slowed in TLE (-5.0 ± 0.6 in control compared with -6.2 ± 0.5 in TLE). In contrast to non-stellate neurons, I_{NaR} $V_{1/2}$ values in stellate neurons were unchanged (-70.1 ± 2.8; n=10: in control compared with -65.4 ± 2.9; n=6 in TLE). Slope values were slowed in TLE (-4.0 ± 0.4 in control compared with -7.0 ± 0.4 in TLE; $P<0.05$ Holm-Sidak method). Immunohistochemistry experiments revealed increased staining intensity of $Na_v1.6$ along the axon initial segment (AIS) in TLE brain slices when compared to control. We propose that increases in I_{NaR} and I_{NaP} in TLE may contribute to the generation of AP bursts previously reported in EC layer II neurons in TLE, leading to seizure generation and spread within the limbic system.

Disclosures: M.K. Patel, None; **N.J. Hargus**, None; **E.H. Bertram**, None.

Nanosymposium

222. Epilepsy: Mechanisms

Location: Room 24A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 222.6

Topic: C.07. Epilepsy

Support: DFG grant SP108/14-1

DFG grant SP108/14-2

Helmholtz programme "Function and Dysfunction of the Nervous System

Title: Multi-receptor analyses of human epileptic neocortex

Authors: *N. PALOMERO-GALLAGHER[1], A. SCHLEICHER[2], H. J. BIDMON[2], H. W. PANNEK[3], E. J. SPECKMANN[4,5], K. ZILLES[1,2];
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Abstract: A disturbed balance between excitatory and inhibitory neurotransmitter receptors is thought to underlie the pathogenesis of focal temporal epilepsy. The factors implicated in the initiation, maintenance and propagation of epileptic seizures remain largely unknown. Although glutamate and GABA are considered the key players in temporal lobe epilepsy, reports concerning alterations of these two neurotransmitter systems remain controversial, and other neurotransmitters have been implicated in the pathophysiology of the disease. We applied intracellular recording techniques and in vitro receptor autoradiography to characterize epileptiform discharges and quantify densities of glutamate (AMPA, kainate, NMDA), GABA ($GABA_A$, $GABA_B$), acetylcholine (M_1, M_2, nicotinic), noradrenaline (α_1, α_2), serotonin ($5\text{-}HT_{1A}$, $5\text{-}HT_2$) and adenosine (A_1) receptors as well as of central and peripheral benzodiazepine (BZ) binding sites in biopsies from the middle temporal gyrus of patients with pharmaco-resistant focal temporal lobe epilepsy (n=36) and autopsy controls (n=10).
Epileptic tissue presented spontaneous (ss, 75% of cases) or non-spontaneous (n-ss, 25% of cases) spiking activity. Receptor laminar distribution patterns were comparable between epileptic and control tissue, though we found a considerable inter-individual variability in epilepsy-related density alterations. AMPA, kainate, M_1, M_2, nicotinic and $5\text{-}HT_{1A}$ receptor densities were increased in ss and n-ss cases. NMDA and A_1 receptor densities were decreased and those of α_{2h} and peripheral BZ receptors were increased in ss cases. Central BZ binding site and α_1 receptor densities were increased in n-ss cases. Central BZ binding site and $5\text{-}HT_{1A}$ receptor alterations correlated negatively with seizure frequency in n-ss cases. Kainate and nicotinic alterations correlated negatively with illness duration in ss cases. Thus, neocortical temporal lobe epilepsy is

associated with a generalized receptor imbalance resulting in a net potentiation of excitatory neurotransmission. Alterations of the peripheral BZ receptor in particular highlight the fact that not only neurons, but also astrocytes are impaired by seizure activity. The involvement of neuromodulatory neurotransmitters in epilepsy expands the horizon of molecular targets for future antiepileptic drugs and could represent the concept needed to solve the problem of drug resistance.

Disclosures: N. Palomero-Gallagher, None; **A. Schleicher**, None; **H.J. Bidmon**, None; **H.W. Pannek**, None; **E.J. Speckmann**, None; **K. Zilles**, None.

Nanosymposium

222. Epilepsy: Mechanisms

Location: Room 24A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 222.7

Topic: C.07. Epilepsy

Support: Telethon Italy Grant GGP07278

Title: Cortical interictal activity in vivo: Electrophysiology and 2-photon imaging

Authors: M. BRONDI[1,2], S. SULIS SATO[1,2], G. DE VITO[2], L. DE VIVO[2], S. LANDI[2], *G. RATTO[3,2];
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Abstract: Interictal activity (IA) is an important feature of epilepsy, and it is characterized by brief (<0.3 s) quasi-periodic depolarizations of the EEGs observed in epileptic patients and in experimental models of epileptiform activity both *in vitro* and *in vivo*. IA is produced by the hypersynchronous firing of large neuronal ensemble but little is known about the neuronal mechanisms involved at the onset of synchronisation and at the end of firing.
To shed light on the dynamics of the neural population and on the involvement of astrocytes during the interictal phase we have developed a focal model of IA consisting in the local administration of the GABAaR inhibitor Bicuculline in the mouse visual cortex. The Ca activity of neurons and astrocytes was imaged by *in vivo* 2-photon microscopy after loading with the Ca indicator OG-BAPTA1 together with the astrocyte-specific dye SR-101. The field potential was recorded in the injected volume by an extracellular electrode, thus allowing the simultaneous acquisition of both electrical activity and Ca changes.
We found that during interictal activity neurons, but not astrocytes, responded with a very fast

Ca change to most if not all events, as shown comparing the field recording with the OG fluorescence changes. Time based cross-correlation analysis between electrophysiology and Ca changes highlights a strong synchronization between the field recording and the activity of single neurons. However, the degree of correlation differed between cells and varied during the recording, suggesting that neurons contribute variably to the overall activity. Furthermore, the visual stimulation of the eye controlateral to the recorded cortex showed that during the treatment with Bicuculine a single flash of light was sufficient to trigger a large interictal event. By studying the timing statistics of evoched and spontaneous interictal events, we have determined the existence of a well defined refractory period that follows each interictal burst. Finally, in a transgenic mice expressing the Green Fluorescent Protein under the GAD67 promoter (**Chattopadhyaya *et al.* 2004)** we have recorded Ca changes in pyramidal and putative fast spiking interneurons. Here we observed that the peak of the field recordings correlated with the peak of the Ca changes in GFP-positive neurons, while pyramid showed a slower change and a more prolonged plateau, suggesting the presence of a strong and fast recruitment of inhibitory interneurons at the onset of the hypersynchronous activity.

Disclosures: M. Brondi, None; **S. Sulis Sato**, None; **L. De Vivo**, None; **S. Landi**, None; **G. Ratto**, None; **G. De Vito**, None.

Nanosymposium

222. Epilepsy: Mechanisms

Location: Room 24A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 222.8

Topic: C.07. Epilepsy

Support: EPOCH

Title: Regulation of the innate immune system in human TLE: Simultaneous measurement of multiple proteins by multiplex-immunoassay

Authors: *A. A. KAN[1], W. DE JAGER[2], M. DE WIT[1], P. GOSSELAAR[3], P. C. VAN RIJEN[3], O. VAN NIEUWENHUIZEN[4], P. N. E. DE GRAAN[1];
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Abstract: Temporal lobe epilepsy (TLE) is the most common type of focal epilepsy in adults. Therapy resistance develops in approximately 75 % of the patient population. A subset of these

patients is treated by surgically removing the epileptic focus. The etiopathology of TLE is unknown and drug therapy so far is only directed at seizure suppression.
Several lines of evidence implicate the immune system in the etiology of TLE in both animal models of this disease and in human patients (Vezzani et al. 2008. Epilepsia 49-S2: 24-32). Recently, we have performed an unbiased transcriptome analysis in hippocampal biopsies from mesial TLE (mTLE) patients with and without hippocampal sclerosis (HS, non-HS) versus autopsy controls. Several key members of the innate immune system were found to be differentially regulated (van Gassen et al. 2008. Epilepsia; 49:1055-1065) including members of the chemo and cytokine family.
In this study we aim to simultaneously measure protein levels of multiple components of the innate immune system to generate an immune profile for TLE.
We performed a quantitative multiplex bead-based immunoassay for 25 components of the innate immune system. We analyzed hippocampal and cortical homogenates from patients operated for mTLE with and without HS, and autopsy controls. We also analyzed CSF samples from some of these patients.
Our data shows that hippocampal biopsy material from HS, non-HS and autopsy controls each show a distinct immunological profile. This profile reveals, beside several immune components previously associated with TLE (eg. IL1-beta, IL-6), also other regulated components of the immune system. These proteins we are presently investigating in human TLE tissue using immunocytochemistry. Animal studies will be required to investigate whether differential regulation of these immunological proteins is a cause or consequence of seizures. The identification of distinct immune profiles in TLE helps to gain more insight in the role of the immune system in epileptogenesis and might provide new handles toward therapeutic and diagnostic tools.

Disclosures: A.A. Kan, None; **W. de Jager**, None; **M. de Wit**, None; **P. Gosselaar**, None; **P.C. van Rijen**, None; **O. van Nieuwenhuizen**, None; **P.N.E. de Graan**, None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.1

Topic: C.09. Demyelinating Disorders

Support: MDA440467

Title: Myelin thickness in the heterozygous R98C knock-in mouse model of CMT1B does not respond to neuregulin I type III overexpression

Authors: M. SHY, *Y. BAI, X. WU, L. M. DILLON, J. KAMHOLZ, A. PATZKO;
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Abstract: The R98C mutation in myelin protein zero (*MPZ*) causes a severe early-onset form of CMT1B and a similar neuropathy in knockin mice. Heterozygous animals (R98C/+) are characterized by abnormally thin myelin which rarely surpasses 0.5 μm even in large diameter axons. Neuregulin I type III (Nrg1*) signaling regulates myelin thickness through signal transduction pathways initiated by ErbB2/3 signaling and transgenic overexpression of Nrg1* in neurons causes Schwann cell hypermyelination. We hypothesized that thin myelin in the R98C knockin animals may result from impaired or partial responses to Nrg1* mediated signals. We therefore wished to determine whether crossing R98C/+ mice with mice that over-express Nrg1* would increase myelin thickness beyond 0.5 μm and rescue the phenotype of the knockin animals. Nrg1* overexpressor transgenic (Nrg1* tg) mice performed significantly ($p<0.05$) worse both on the rotarod (124 sec) and the four limbs grip test (190 g) than wild type (wt) littermates (198 sec, 219 g). They also show a tendency to weakness on the hind limb grip test (Nrg1* tg 72 g, wt 92 g). However, motor nerve conduction velocities (MNCV: 36 m/s) and compound muscle action potentials (CMAP: 7,1 mV) were the same in Nrg1* tg and wild type animals. Crossbred animals (Nrg1* tg, R98C/+) performed identically on the rotarod (85 sec) and grip test (four limbs: 160 g, hind limb: 72 g) to R98C/+ mice. There was no improvement of MNCV (16 m/s, CMAP: 3.3 mV) compared to R98C/+ mice. Electron microscopy revealed unchanged G-ratio (0.8) despite a very limited number of hypermyelinated axons. RT-PCR showed 4 to 14 fold Nrg1* overexpression in the spinal cord and Western-blot analyses confirmed increased Nrg1* protein expression in sciatic nerves. Further experiments are ongoing to analyse Nrg1* driven signal transduction pathways. Our preliminary data suggest that Nrg1* mediated signaling is not able to regulate myelin thickness in R98C neuropathy and to clinically or morphologically improve it. Determining why R98C knockin mice are resistant to over-expression of Nrg1* signaling should provide insight into the molecular basis for hypomyelination in the animals.

Disclosures: M. Shy: Speakers Bureau/Honoraria; Dr. Shy is an invited speaker of Athena Diagnostics.. **Y. Bai:** None. **X. Wu:** None. **L.M. Dillon:** None. **J. Kamholz:** None. **A. Patzko:** None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.2

Topic: C.09. Demyelinating Disorders

Support: NIH Grant 5RO1HD055461

Hunter's Hope foundation

Title: Bone marrow transplantation worsens the altered olivocerebellar dynamics in murine model of globoid cell leukodystrophy

Authors: *M. SANDS[1], A. S. REDDY[2], D. F. WOZNIAK[3], S. C. FOWLER[4];
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Abstract: Tremor (twitching) is the defining characteristic of the twitcher mice, an authentic model for Globoid Cell Leukodystrophy (GLD; Krabbe's disease). The physiological basis of tremor in the twitcher mice is poorly understood. Two types of tremor can be phenotypically defined-resting and intentional tremor. Resting tremor, present only at rest, is caused by basal ganglia lesions and has a frequency of 6-8 Hz. Intentional tremor, present only during movement, is caused by lesions in Olivocerebellar circuits and has a frequency of about 12 Hz. The tremor in the twitcher mice was characterized by force plate actometer. Our studies show that the tremor seen in the twitcher mice resembles that of lesions in the olivocerebellar circuits, with a characteristic peak in the power spectrum at about 12 Hz. Further, this characteristic frequency was absent in the power spectra of low mobility bouts, proving that it is intentional tremor. This was further confirmed by harmaline, a drug known to induce tremor by altering olivocerebellar properties. In the wildtype, harmaline induces a characteristic tremor with a frequency of about 12 Hz. In the twitcher mice, harmaline does not alter the tremor phenotype, indicating that the olivocerebellar properties are altered even before harmaline injection. Previous studies have shown that severe inflammation is present in the cerebellum, as shown by the presence of CD11b+ cells (macrophages) in the white matter. Thus, cerebellum is a major site of pathology in the twitcher mice, and should be a targeted for therapy.
In the second part of the study, we evaluated the efficacy of various therapies using the force plate actometer. Currently, gene therapy, bone marrow transplantation(BMT) and substrate reduction therapies are used for treating the disease in pre-clinical studies. Previously, it was shown that combining CNS directed gene therapy (using Adeno-associated virus 2/5; AAV2/5) and BMT, a synergistic improvement in lifespan could be obtained. Interestingly, animals treated with AAV2/5 and BMT showed a worsening of tremor phenotype, whereas animals treated with gene therapy alone fared better. When animals treated with BMT only were tested, they showed a worsening of the tremor phenotype. Thus, BMT worsens cerebellar damage that already exists in the twitcher mice. Detailed histological studies of the cerebellum and brainstem are underway. Our study highlights the pathology of cerebellum and brainstem in the twitcher mice and emphasizes the need for therapies that do not worsen the pathology. Additionally, the force plate actometer could be a valuable tool for evaluating the efficacy of the various therapies in the twitcher mice.

Disclosures: M. Sands, None; **A.S. Reddy**, None; **D.F. Wozniak**, None; **S.C. Fowler**, None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.3

Topic: C.09. Demyelinating Disorders

Title: Demyelinating effect of autoantibodies in a multiple sclerosis disease model is prevented by administration of phage displaying the immunodominant epitope of myelin

Authors: *B. SOLOMON;
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Abstract: Introduction
Experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS), can be elicited in mice by inoculating various putative MS antigens or their corresponding encephalitogenic peptides with appropriate adjuvants. The presence of myelin oligodendrocyte glycoprotein (MOG)−specific antibodies in actively demyelinating lesions of MS indicates that, as in MOG−induced EAE, these autoantibodies may play an integral role in the formation of vesiculated, disrupted myelin sheaths. Here we present a novel strategy of antigen-specific therapy to treat EAE mice based on nasally administered filamentous phage displaying the immunodominant epitope (MOG_{36-44}). Bacteriophages are the most numerous life forms on earth; their natural occurrence and contact with humans is therefore constant and intensive. Moreover, their lack of tropism for mammalian cells advocates their potential use as therapeutic agents.
Experimental
We induced EAE in C57BL/6 mice immunized with fragments of MOG and the disease developed approximately two weeks later. The proposed antigen is based on engineered filamentous phage (using f88 phage display system) displaying over 150 copies of myelin oligodendrocyte glycoprotein fragment (MOG 36-44) on the major coat protein. The phage-MOG was nasally administrated to naïve and EAE diseased mice using different protocols.
Results
The results presented here indicate that nasal administration of phages presenting MOG_{36-44} to EAE C57BL/6 mice reduced anti-MOG antibodies in the brain and periphery and improved the clinical score of treated animals. Moreover, the treatment prevented demyelination and significantly reduced monocyte chemoattractant protein 1(MCP-1) and other proinflammatory cytokines.
Brain biochemistry markers for neuronal damage, astrocytosis and detection of phagocytic cells

indicated lower inflammation in the CNS. The positive effect of this treatment was achieved even after single nasal administration at disease onset, pointing to a possible repair mechanism other than nasal tolerance.

Conclusions

Brain delivery of MOG via filamentous phages suggests that the improved clinical effects obtained in EAE mice may be due to depletion of MOG autoantibodies *in situ* and/or stimulation of immune mechanisms
towards induced tolerance in the periphery, indicating that the humoral immune system in MS would be a reasonable therapeutic option.

Disclosures:

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.4

Topic: C.09. Demyelinating Disorders

Support: NIH

Title: Unfolded protein response (UPR) activation in a mouse model of early-onset CMT 1B

Authors: M. A. SAPORTA[1], Y. BAI[1], B. SHY[1], A. PATZKO[1], M. PENNUTO[4], M. CROWTHER[5], M. FELTRI[4], *J. A. BENJAMINS[2], D. KIRSCHNER[5], C. SOUTHWOOD[3], A. GOW[3], L. WRABETZ[4], M. SHY[1];
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Abstract: R98C mice are an authentic model of early onset CMT 1B, an inherited demyelinating neuropathy associated with mutations in the Myelin Protein Zero (MPZ) gene. Our previous work has demonstrated that both heterozygous and homozygous mice have a clinical, neurophysiologic and morphological phenotype that recapitulates the human disease. We have also showed that mutant P0 R98C protein is retained in the endoplasmic reticulum of Schwann cells (SCs) raising the possibility that UPR activation plays a role in the toxic gain of function associated with this condition. In this study, we have further demonstrated that all three main UPR pathways are active in our model. Interestingly, apoptosis was not demonstrated in our colony, as it would be expected in the context of UPR activation. Instead, a paradoxical increase in the number of SCs and increased SCIP/Oct6 and c-jun expression in sciatic nerves were

observed, suggesting a change in SC phenotype towards a less differentiated profile, especially in homozygous R98C mice. We have also identified SC expressing c-jun and myelin basic protein concomitantly, suggesting that these are not physiologic immature SCs. We are now working in determining the exact developmental time point when UPR activation occurs and whether it is directly related to the change in SC phenotype. To study the influence of the PERK pathway of the UPR in the phenotype of the R98C mice, we cross bred this colony with Chop-null mice. Chop is a downstream mediator of the PERK pathway. The phenotype of the resulting strain was characterized by nerve conduction studies and morphometric and ultrastructural analysis. Although no significant improvement was detected after Chop ablation in nerve conduction velocity, sciatic nerve pathology or X-ray diffraction analysis, a significant rescue of compound muscle action potential amplitude was observed in heterozygous, chop null mice. We are currently working in characterizing the mechanisms behind this effect. In summary, our study demonstrates that UPR activation does not lead to apoptosis in Schwann cells. Instead, increased proliferation and a change towards a less differentiated phenotype are seen. Whether these are direct consequences of UPR activation remains to be determined.

Disclosures: M.A. Saporta, None; **Y. Bai**, None; **B. Shy**, None; **A. Patzko**, None; **M. Pennuto**, None; **M. Crowther**, None; **M. Feltri**, None; **J.A. Benjamins**, None; **D. Kirschner**, None; **C. Southwood**, None; **A. Gow**, None; **L. Wrabetz**, None; **M. Shy**, None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.5

Topic: C.09. Demyelinating Disorders

Support: NIH NS050705

NIH NS50345

Title: Connexins in central myelinating glia: Heteromeric interactions as a potential pathogenic mechanism for Cx32 and Cx47 related diseases

Authors: *C. K. ABRAMS[1], J. ORTHMANN MURPHY[2], M. M. FREIDIN[1], S. S. SCHERER[2], K. A. KLEOPAS[3];
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Abstract: Oligodendrocytes couple to astrocytes and oligodendrocytes via gap junctions. Astrocytes express connexin43 (Cx43) and Cx30, whereas oligodendrocytes express Cx32 and Cx47. We have shown previously that the channels that form between oligodendrocytes are likely restricted to Cx47/Cx47 and Cx32/Cx32 homotypic channels, and that heterotypic channels between astrocytes and oligodendrocytes are comprised of Cx43/Cx47 and Cx30/Cx32 (Orthmann-Murphy et al., (2007) J Neurosci 27:13949-13957). Here we assess whether the connexins expressed by the same cell type can form heteromeric hemichannels and whether heteromeric interactions play a role in central nervous system manifestations of Cx32 associated X-linked Charcot Marie Tooth Disease ($CMTX_{CNS}$) or Cx47 associated Pelizaeus-Merzbacher-like Disease (PMLD) and Hereditary Spastic Papaparesis (HSP). Using dual whole-cell patch clamp assays, wild-type Cx32 and Cx47 do not appear to form heteromeric hemichannels when expressed together in cells and paired with cells expressing one of the oligodendrocyte or astrocyte connexins. In addition, co-immunoprecipitation experiments failed to reveal a biochemical interaction between Cx32 and Cx47. Cell pairs where one cell expresses a $CMTX_{CNS}$ mutant and wild type Cx47 and the other expresses Cx43 show a significant reduction in junctional current compared to cell pairs with Cx32 wild-type and Cx47 wild-type in one cell and Cx43 in the other. Furthermore, co-immunoprecipitation experiments show a biochemical interaction between mutant forms of Cx32 and Cx47 wild-type. These findings suggest that loss of Cx47 function may contribute to the $CMTX_{CNS}$ phenotype. We are currently investigating whether the phenotypes of Cx47 associated PMLD and/or HSP can be explained by heteromeric interactions with wild-type Cx32.

Disclosures: C.K. Abrams, None; **M.M. Freidin**, None; **S.S. Scherer**, None; **J. Orthmann Murphy**, None; **K.A. Kleopas**, None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.6

Topic: C.09. Demyelinating Disorders

Support: ANR Grant ANR-08-BIOT-016-01

Title: Novel compounds to promote remyelination in multiple sclerosis

Authors: *T. BORDET[1], K. MAGALON[2], C. ZIMMER[2], G. TARDIF[1], J. KHALDI[2], C. BOURBON[3], C. CHAIMBAULT[1], M. MICHAUD[1], G. DUHAMEL[3], S. CONFORT[3], M. CAIRE[2], R. M. PRUSS[1], A. VIOLA[3], P. DURBEC[2];

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Abstract: Multiple sclerosis (MS) is a chronic degenerative and debilitating disease of the central nervous system (CNS) characterized by inflammation and demyelination. Despite the improved relapse management provided by immune modulating treatments, progression of the handicap in MS patients remains because of progressive and irreversible axonal degeneration. Based on these observations, early neuroprotection and myelin repair strategies should be considered as additional therapeutic requirements for MS patients. Importantly, experimental evidence indicate that new myelin sheaths in MS lesions are formed by oligodendrocyte progenitor cells (OPC) present throughout the adult CNS or newly generated from adult stem cells present in the subventricular zone (SVZ). However this process may be limited because of insufficient OPC recruitment or impaired maturation process. Several growth factors have been shown to affect OPCs survival and proliferation such as platelet-derived growth factor (PDGF), neurotrophin-3 and glial growth factor-2 while insulin-like growth factor (IGF1) and T3 hormone promote their maturation. Mimicking action of theses growth factors with a drug is one of the challenges we addressed.
We previously identified a new class of cholesterol-oxime compounds for their neuroprotective activities. Among these compounds, olesoxime (TRO19622) has been shown to accelerate axon regeneration and remyelination in the peripheral nervous system after sciatic nerve crush. This regeneration / remyelination process was associated with functional recovery of conduction velocities in motor axons (Bordet et al., JPET 322:709-720, 2007). Here, we demonstrate that in vitro treatment with olesoxime dose-dependently accelerates OPC differentiation from neural progenitors derived from neonatal murine SVZ by promoting their maturation. Importantly treatment with olesoxime strongly enhanced myelination in co-cultures of dorsal root ganglion neurons and OPC and in neonatal rat brain slices. Since this compound was selected for its motor neuron survival promoting activity, we identified new optimized small molecules originating from the same cholesterol-oxime family of compounds to promote OPC maturation making these drugs ideal candidates for MS. Compounds are currently under evaluation in widely-recognized preclinical models of MS. Positive preclinical evidence of their efficacy in these models will provide the necessary proof of concept to motivate further development of this class of compounds for treatment of MS.
Support: The Agence National pour la Recherche, BiotecS grant [ANR-08-BIOT-016-01]

Disclosures: **T. Bordet**, Trophos SA, Employment; **G. Tardif**, Trophos SA, Employment; **M. Michaud**, Trophos SA, Employment; **C. Chaimbault**, Trophos SA, Employment; **R.M. Pruss**, Trophos SA, Employment; **K. Magalon**, None; **C. Zimmer**, None; **J. Khaldi**, None; **M. Caire**, None; **P. Durbec**, None; **A. viola**, None; **C. Bourbon**, None; **G. Duhamel**, None; **S. Confort**, None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.7

Topic: C.09. Demyelinating Disorders

Support: NIH Grant 5RO1HD055461

Hunter's hope foundation

Title: Forebrain, cerebellar and spinal cord directed AAV2/5 gene therapy augments therapeutic effect of bone marrow transplantation in murine model of globoid cell leukodystrophy

Authors: *A. S. REDDY[1], J. H. KIM[2], S.-K. SONG[2], R. KLEIN[3], M. S. SANDS[4]; [1]Neurosci., Washington Univ. in St.Louis, SAINT LOUIS, MO; [2]Radiology, [3]Intrnl. Med., Washington Univ. in St.Louis, Saint louis, MO; [4]Intrnl. Med., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Globoid-cell Leukodystrophy (GLD; Krabbe's disease) is an inherited demyelinating disease caused by the deficiency of lysosomal enzyme Galactosylceramidase (GALC). Bone Marrow Transplantation (BMT) is currently the only available therapeutic option in humans. It was previously shown that there is dramatic synergy between CNS-directed AAV2/5 gene therapy and myeloreductive BMT in the murine model of Krabbe's disease (Twitcher). However, the CNS-directed gene therapy was limited to forebrain and thalamus. Cerebellum and spinal cord are also known to be major sites of the disease. This is clinically evidenced by tremor and hindlimb paralysis, and histologically by demyelination and infiltration of CD11b+ cells. In the current study, an improvement in the CNS gene delivery was made by the addition of intrathecal and intracerebellar injections. This improved CNS targeting of AAV2/5 further improved the efficacy. In the current study, AAV2/5 alone improves the lifespan from a median of 42 days in the untreated twitcher to 72 days, compared to a lifespan increase to about 49 days in the previous study. When AAV2/5 is combined with BMT, the median lifespan is increased to 123 days (range: 92-228 days) compared to 105 days in the previous study. This increase is associated with a corresponding improvement in the motor performance on constant and accelerated rotarod. There is also an improvement in the axial and radial diffusivity by diffusion tensor imaging of the spinal cord of treated animals. These clinical improvements are accompanied by histological improvement in myelination and reduction of CD11b+ cells in the CNS and PNS in combination treated animals. A quantitative decrease in T cells and activated microglia/macrophages (CD45+CD11b-hi)is observed by flow cytometry of the brains of treated animals. Similarly, animals receiving therapy showed normalization of some chemokines (notably CXCL1) that are elevated in the brains and spinal cords of twitcher mice. All the above mentioned improvements were greater in combination treated animals compared to either therapy alone. The current study maximizes gene delivery to the CNS using AAV2/5. It also highlights the validity of combination therapy when the therapeutic intervention occurs at a presymptomatic stage.

Disclosures: A.S. Reddy, None; **J.H. Kim**, None; **S. Song**, None; **R. Klein**, None; **M.S. Sands**, None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.8

Topic: C.09. Demyelinating Disorders

Support: MDA440467

Title: Oral curcumin treatment of the R98C knock-in mouse model of CMT1B

Authors: *A. PATZKO, I. KATONA, M. A. SAPORTA, Y. BAI, A. JANI-ACSADI, L. M. DILLON, M. E. SHY;
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Abstract: We investigated whether orally administered curcumin would improve the phenotype of the R98C knock-in mouse model of severe early-onset Charcot Marie Tooth disease type 1B (CMT1B). CMT1B is the second most frequent form of autosomal dominant CMT1 and results from mutations in myelin protein zero (*MPZ*). Misfolded MPZ is retained in the cytoplasm of myelinating Schwann cells. We hypothesized that curcumin might be an effective treatment, as it has been shown to reduce ER retention of mutant MPZ and PMP22 protein aggregates. Mice were treated with daily gastric lavage of curcumin (100mg/kg/day) dissolved in alimentum (CA) or in oil (CO) beginning at 4 days of age as well as with two curcumin derivatives dissolved in oil; phosphatidylcholine curcumin (PCC), and a fluorinated curcumin analogue (CDF). PCC is engineered to increase GI absorption and CDF to increase tissue retention time of curcumin. CA and CDF treated heterozygous animals (R98C/+) showed a modest tendency to perform better than their non-treated littermates on the rotarod (29% improvement) but not four limb or hind limb grip tests. However, following treatment with PCC or CO the R98C/+ mice improved significantly ($P<0.05$) and performed identically to wild type animals (+/+) on rotarod and grip tests. Compound muscle action potentials improved significantly ($P<0.05$) in PCC or CO treated R98C/+ animals (untreated R98C/+ 37 mV; PCC treated R98C/+ 58mV), but did not improve in CA or CDF treated animals. Nerve conduction velocities did not improve with any therapy. Electron microscopy revealed no changes in the G-ratio following any treatment (+/+ 0.67; R98C/+ 0.77; R98C/R98C very thin, non compacted myelin). Mass spectrometry confirmed the presence of curcumin in sciatic nerve samples (696 ng/g). CDF treated mice showed no

significant differences compared to curcumin treated animals. Further investigations with immunohistochemistry, Western blot and RT-PCR are ongoing. We suppose that the vehicle used to dissolve curcumin might influence its effect. Our data suggest significant clinical improvement in R98C/+ mice treated with PCC or CO accompanied by neurophysiological changes. CA or CDF treated R98C/+ mice show mild clinical improvement, however, without neurophysiological and morphological changes.

Disclosures: A. Patzko, None; **I. Katona**, None; **M.A. Saporta**, None; **Y. Bai**, None; **A. Jani-Acsadi**, None; **L.M. Dillon**, None; **M.E. Shy**, Dr. Shy is an invited speaker of Athena Diagnostics., Speakers Bureau/Honoraria.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.9

Topic: C.09. Demyelinating Disorders

Support: NIH grant 5R01NS048952-05 to M.T. Philipp

Title: Human oligodendrocytes MO3.13 conditioned in a three-dimensional culture system as a model to study oligodendrocyte injury induced by the Lyme disease spirochete Borrelia burgdorferi

Authors: *G. RAMESH[1,2], K. H. BENTRUP[4], B. PAHAR[3], M. T. PHILIPP[2,4];
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Abstract: Lyme neuroborreliosis, a disease of both the central and peripheral nervous systems is caused by the spirochete *Borrelia burgdorferi (Bb)*. Recently we reported that the interaction of *Bb* with brain parenchyma elicits inflammatory mediators from glial cells, and glial (oligodendrocyte) and neuronal apoptosis. Oligodendrocytes, the myelin-producing cells in the CNS, are the major targets of injury in multiple sclerosis, a CNS disease that may show clinical similarities with Lyme neuroborreliosis. In order to evaluate the role of inflammation in oligodendrocyte death as induced by *Bb*, we evaluated the suitability of a human cell line of primary oligodendrocytes, MO3.13 grown in both the conventional two-dimensional (2D) *in vitro* tissue cultures, and three dimensional (3D) rotatory wall vessel (RWV) cultures (Synthecon Inc.). 3D culture has been reported to enhance the phenotype of cells to one that is closer to that

seen *in vivo*. The expression of the oligodendrocyte phenotypic markers myelin basic protein (MBP), myelin proteolipid protein (mPLP), oligodendrocyte marker (O4), galactoceramide (GALC) and 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) was evaluated by immunofluorescence staining and confocal microscopy. MBP expression was also quantified using flow cytometry. Enhanced expression of MBP and mPLP was observed in cultures that were conditioned in a 3D environment followed by chemical differentiation in 2D (grown in medium containing phorbol myristate acetate and devoid of serum) as compared to those grown and chemically differentiated only in 2D, while the expression of O4, GALC and CNPase was unaffected. Importantly, the morphology of cells conditioned in 3D, followed by chemical differentiation, showed more pronounced processes, which indicates enhanced differentiation, as compared to 2D cultures. In addition, we observed that live *Bb* induces 6-27-fold higher levels of oligodendrocyte apoptosis as compared to background levels in 48 hours *in vitro*, as measured by active caspase-3 activity, which supports our previous *in vivo* findings indicating that *Bb* could contribute to oligodendrocyte apoptosis. Three-fold higher levels of oligodendrocyte apoptosis were observed in 3D conditioned cultures compared to that of 2D cultures, in cells from early passages, but this difference in susceptibility to apoptosis was not evident in cultures from later (older) passages. In conclusion, the human oligodendrocyte cell line MO3.13 grown in 3D represents a novel and more appropriate *in vitro* approach to study oligodendrocyte injury, a phenomenon possibly contributing to the pathogenesis of Lyme neuroborreliosis.

Disclosures: G. Ramesh: Research Grant; NIH grant 5R01NS048952-05 to M.T. Philipp. **K.H. Bentrup:** None. **B. Pahar:** None. **M.T. Philipp:** NIH grant 5R01NS048952-05 to M.T. Philipp.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.10

Topic: C.09. Demyelinating Disorders

Title: Cerebrospinal fluid derived from progressive multiple sclerosis patients promotes neuroectodermal differentiation of human neural precursor cells in vitro

Authors: *M. CRISTOFANILLI, S. A. SADIQ;
Multiple Sclerosis Res. Ctr. of New York, NEW YORK, NY

Abstract: Adult multipotent neural precursor cells (NPCs) have the capacity for self-renewal and to differentiate into functional cells (e.g. neurons, astrocytes or oligodendrocytes) within discrete tissue-specific germinal niches, such as the subventricular zone of the lateral ventricles

and the subgranular zone of the dentate gyrus of the hippocampus. Due to their intrinsic plasticity NPCs can be considered an essential part of the cellular mechanism(s) by which the central nervous system (CNS) tries to repair itself after an injury. Although developing evidence indicate that endogenous neurogenesis and gliogenesis occur as part of an 'intrinsic' self-repair process during the course of inflammatory CNS disorders, such as multiple sclerosis (MS), there are no convincing explanations about the overall incapacity of the endogenous stem cells to promote full and long-lasting CNS repair in progressive forms of MS.
There are evidence suggesting that endogenous NPCs, while contributing to CNS repair in MS, may also become the target of the disease itself. Recent data suggest that inflammatory components, such as CNS-infiltrating blood-borne inflammatory mononuclear cells, reactive CNS-resident cells (for example astrocytes, brain endothelial cells and microglia) and humoral mediators (for example cytokines and chemokines), can be responsible for such failure. This is because they can affect proliferation and differentiation of NPCs, either directly or indirectly through the uncoordinated re-expression of developmental genetic programs that regulate stem cells.
In this project, we investigated the effect of applications of cerebrospinal fluid (CSF) derived from progressive MS patients on the survival, proliferation, and differentiation properties proprieties of human neural stem cells in vitro. We found that CSF derived from primary progressive (PPMS) and secondary progressive (SPMS) patients increased the differentiation of embryonic and fetal derived neural stem cells toward mature brain cells (astrocytes, oligodendrocytes, and neurons) compare to control CSF. Our findings suggest that the failure of repair mechanisms in progressive forms of MS is not the result of inhibition of differentiation pathways. .

Disclosures: M. Cristofanilli, None; **S.A. Sadiq**, None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.11

Topic: C.09. Demyelinating Disorders

Support: NHMRC Project Grant

Title: Modulating Bone Morphogenic Protein signalling during cuprizone-induced demyelination alters oligodendrocyte numbers

Authors: *T. J. KILPATRICK, J. SABO, H. CATE;

Ctr. for Neurosci, The Univ. Melbourne, Carlton Sth, Vic, Australia

Abstract: Enhancement of oligodendrocyte regeneration is a promising strategy to repair demyelinating diseases of the central nervous system. Myelin injury induces several growth factors, including those that could modulate oligodendrogliogenesis. We previously showed that bone morphogenic protein (BMP) signalling is increased in myelin lesions during cuprizone-induced demyelination and that BMP4 inhibits oligodendrogliogenesis by decreasing adult neural precursor cell proliferation and oligodendrocyte differentiation *in vitro*. Here, we report the effects of modulating BMP signalling upon adult oligodendrocyte progenitor cells (OPCs) during demyelination. We used osmotic mini-pumps to infuse BMP4, its endogenous antagonist Noggin or vehicle into the lateral ventricle during cuprizone-induced demyelination. BrdU was added to drinking water to mark proliferation and BrdU and lineage specific proteins were detected in sections by immunohistochemistry. p-SMAD 1/5/8 immunoreactivity in the corpus callosum was significantly increased by infusion of BMP-4 ($p<0.01$; n=4,5) and decreased by Noggin ($p<0.05$; n=4,6). When the tissue was assessed after 6 weeks of cuprizone challenge, BMP4 infused mice had increases in proliferation, Olig2+, and Olig2-BrdU double positive cells in the midline corpus callosum compared to vehicle infused mice ($p<0.01$; n=6,5), while there was no significant difference in Noggin infused mice. However, when an additional cohort was assessed after 1 week of recovery following 6 weeks of cuprizone, Noggin infused mice had increases in the number of Olig2+, BrDU and Olig2-BrdU double positive cells that co-labelled with CC1, a mature oligodendrocyte marker, in the midline corpus callosum compared with vehicle ($p<0.05$; n=4,4), while there was no significant difference in BMP4 infused mice. Thus, although BMP infusion resulted in increased OPC proliferation during demyelination, our results suggest that Noggin infusion may ultimately have a more beneficial effect on repair given that Noggin, and not BMP4, infusion increased oligodendrocyte numbers during recovery. We are currently examining how modulation of BMP signalling also affects other neural cell populations within the myelin lesion and how these activities, in combination, influence remyelination.

Disclosures: T.J. Kilpatrick, None; **H. Cate**, None; **J. Sabo**, None.

Nanosymposium

223. Demyelinating Disorders: Cellular Mechanisms

Location: Room 31C

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 223.12

Topic: C.09. Demyelinating Disorders

Title: Intrathecal methotrexate reduces demyelination and astrogliosis in a non-inflammatory demyelination model

Authors: *S. A. SADIQ[1], A. W. NASSERY[2], A. M. MUELLER[2];
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Abstract: Objective:
We hypothesize that the benefits of intrathecal methotrexate (ITMTX) on progressive MS is only partially related to its anti-inflammatory properties. Consequently, we investigated its influence on a non-inflammatory CNS demyelination model.
Background:
MS patients with a progressive disease respond poorly to currently available anti-inflammatory disease modifying agents. Intrathecal administration of the antifolate drug methotrexate has a beneficial impact on the disease progression of severe primary and secondary progressive MS cases.
Astrocytic activation and subsequent astrogliosis occur in progressive MS as well as in the cuprizone-induced model of corpus callosum demyelination and are considered to be hallmarks of scar formation in the CNS.
Methods:
To induce corpus callosum demyelination, male C57Bl/6 mice were fed with cuprizone mixed into ground chow. Methotrexate was administered intracerebroventricularly (icv) by osmotic pumps. Brain tissue sections were stained for astrocytic markers, myelin and microglial cells. Random sections were analysed per animal by a blinded investigator.
Results:
After being fed with cuprizone for four weeks, mice were characterized by strong demyelination and an increase in GFAP+ astrocytes and MAC3+ microglial cells within the corpus callosum as compared to naive mice. ITMTX significantly decreased the demyelination and number of astrocytes in the corpus callosum. ITMTX starting after two weeks of cuprizone administration also decreased astrogliosis and microglial activation in the corpus callosum at the end of six weeks of cuprizone administration.
Contrastingly, an icv administration of methotrexate after a cuprizone feeding period of 6 weeks neither delayed remyelination nor influenced the number of astrocytes or microglial cells in the corpus callosum.
Discussion:
The pathophysiological basis of the cuprizone-induced demyelination model are not primarily related to inflammatory mechanisms. Similarly, the progressive forms of MS are less dependent on inflammatory events than its relapsing forms.
We were able to establish an inhibition of demyelination and astrogliosis by ITMTX in the corpus callosum of cuprizone fed mice. This corroborates that the beneficial impact of ITMTX on disease progression is not solely mediated by its anti-inflammatory properties. Moreover, the inhibition of astroglial activation suggests that ITMTX may influence the generation of astrocytic scars in MS lesions.

Disclosures: S.A. Sadiq, None; **A.M. Mueller**, None; **A.W. Nassery**, None.

Nanosymposium

224. Neurotoxicity and Neurodegeneration II

Location: Room 23A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 224.1

Topic: C.02. Alzheimer's disease and other dementias

Support: University of Newcastle grant G0187902

NHMRC grant #572601

Title: Gene expression studies in three different mouse models support the case for neurologic sequelae in iron overload disorders and provide new insights into mechanism

Authors: *D. JOHNSTONE[1], B. ACIKYOL[1], R. M. GRAHAM[2], D. TRINDER[2], J. K. OLYNYK[2], R. J. SCOTT[1], P. MOSCATO[1], E. A. MILWARD[1];
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Abstract: Severe disruption of brain iron homeostasis can cause fatal neurodegenerative disease. However there is disagreement over the neurologic effects of milder, more common iron disorders such as hereditary hemochromatosis, which is usually caused by mutations in the *HFE* gene or occasionally the transferrin receptor 2 (*TFR2*) gene. Genome-wide brain gene expression was assessed in two mouse models of hemochromatosis ($Hfe^{-/-}$, $Tfr2^{Y245X}$), short-term dietary iron-supplemented mice and wildtype controls (age 10 wks, n=4/group). All models and in particular the $Hfe^{-/-}$ brain showed numerous significant gene expression changes ($p<0.05$), relating to many important brain systems. Notably $Hfe^{-/-}$ brain showed changes of at least 2-fold for prominent genes relating to neurotransmission (e.g. glutamate NMDA receptor *Grin1*, GABA receptor *Gabbr1*) and synaptic plasticity (e.g. FBJ osteosarcoma oncogene *Fos*, calcium/calmodulin-dependent protein kinase II α *Camk2a*). There were also changes for disease-related genes. Several genes causatively linked to neuronal ceroid lipofuscinosis showed expression changes - labile iron accelerates lipofuscin generation. Few key genes in other neurodegenerative diseases showed changes except genes relating to Alzheimer's disease (AD), including the genes encoding amyloid precursor protein, *tau*, apolipoprotein E, presenilin 1 and other γ-secretase components. Unexpectedly these were all down-regulated which may, if anything, partially protect against AD. γ-Secretase activity mediates Notch signaling and there was also down-regulation of genes for Notch signaling intermediaries. 'Hairy and enhancer of split' *Hes1* and *Hes5*, downstream targets of the Notch canonical pathway, were also down-regulated, confirming that changes for γ-secretase complex and Notch signaling genes have functional consequences. Furthermore, heterozygosity for loss-of-function mutation in *NOTCH3* is associated with headaches and fatigue and there was a 2-fold decrease in *Notch3* expression, which could partly account for these symptoms in hemochromatosis patients. There were also changes for the calcium channel gene *Cacna1a*, associated with familial hemiplegic migraine,

and circadian rhythm genes that may affect fatigue e.g. neuronal PAS domain protein 2. Many of these effects were also observed in *Tfr2*^{Y245X} mice or dietary iron-supplemented mice and were further validated by real-time reverse transcription PCR. The findings provide strong evidence that iron overload can considerably disrupt brain gene expression and give new insights into mechanisms which underlie neurologic signs and symptoms in patients with iron disorders.

Disclosures: D. Johnstone, None; **B. Acikyol**, None; **R.M. Graham**, None; **D. Trinder**, None; **J.K. Olynyk**, None; **R.J. Scott**, None; **P. Moscato**, None; **E.A. Milward**, None.

Nanosymposium

224. Neurotoxicity and Neurodegeneration II

Location: Room 23A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 224.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NS036147

NS025372

Title: Geldanamycin protects against ischemia/reperfusion-related cerebrovascular injury

Authors: *C. M. MAIER[1], C. GOEDERS[2], N. EISERT[2], L. XU[2], P. NARASIMHAN[2], P. CHAN[2], R. GIFFARD[2];
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Abstract: Abstract
Effective stroke therapies require recanalization of occluded cerebral blood vessels; however early reperfusion can cause blood-brain barrier (BBB) injury, leading to cerebral edema and/or devastating brain hemorrhage. These complications of early reperfusion, significantly limit the benefits of stroke therapies. Thus, there is an urgent need to develop strategies aimed at reducing BBB damage and increasing the therapeutic window of currently approved stroke treatments. In the present study we used an animal model that facilitates identification of specific free radical-associated components of the reperfusion injury process In this model, KO (knockout) mice containing 50% activity of the mitochondrial antioxidant manganese-SOD (superoxide dismutase) (SOD2-KO) undergo transient focal ischaemia (30 min) followed by reperfusion (72 h). These animals have delayed (>24 h) BBB breakdown associated with activation of matrix metalloproteinase-9 (MMP-9), inflammation and a high brain haemorrhage rate. These adverse consequences are absent from wild-type littermates and SOD2 overexpressors. Using this model,

we have assessed the effects of Geldanamycin (GA), a benzoquinone ansamycin that binds HSP90, releases heat shock factor 1 and induces heat shock proteins (HSPs). Western blots showed the GA administered at 1ug/gram, i.p., 2 days prior to stroke onset, increased brain HSP70 expression by 44% compared with saline injections (n=4 or 5 per group, p=0.0004 by Student t-test) . Following ischemia/reperfusion, there was trend toward an increase in HSP 70 expression in the ischemic hemisphere of GA-treated vs. Saline-treated animals (p=0.0892). Our data also showed reduced MMP-9 expression in the cerebrovasculature and brain parenchyma. TTC measurements and immunohistochemistry showed a concomitant significant reduction in hemorrhage rates, infarct and edema.

Disclosures: C.M. Maier, None; **N. Eisert**, None; **L. Xu**, None; **P. Narasimhan**, None; **P. Chan**, None; **R. Giffard**, None; **C. Goeders**, None.

Nanosymposium

224. Neurotoxicity and Neurodegeneration II

Location: Room 23A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 224.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NS 432851

S10 RR15685-01

Title: Tetrahydrobiopterin is critical to the survival of thalamus neurons in vitro

Authors: *L. YU[1], J. WHITSETT[2], J. VASQUEZ-VIVAR[2], S. TAN[1];
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Abstract: Introduction: Deficiency of tetrahydrobiopterin (BH_4), a cofactor of nitric oxide synthase (NOS) can cause motor deficits in early childhood. We have shown that BH_4 is a developmental factor determining vulnerability of the immature fetal rabbit brain to hypoxic-ischemic injury and subsequent motor deficits in newborns. BH_4 concentration rapidly increases in the perinatal period with the highest concentrations in the thalamus compared to basal ganglia (BG) and cortex. We have shown that in the developing rabbit the nNOS mRNA level was also highest in the thalamus and in the near term rabbit exceeded adult brain levels.
Objective: We hypothesized that the neurons in the thalamus were more dependent on BH_4 for their survival than neurons in the basal ganglia and cortex.
Methods: Tissue from thalamus, BG and cortex were extirpated from E22 New Zealand white

rabbit fetal brains that were subjected to 40 minutes global hypoxia and compared to that from control rabbits. Brain tissue was dissociated into single cell suspension and then cultured in either medium containing BH_4 (10μM) or sepiapterin, an analog of BH_4, or vehicle. At 24 hr, the supernatant in the wells was replaced by fresh media and the supernatant analyzed. At 48 hours, the supernatant and the attached cells were analyzed by flow cytometry by a Live/Dead assay with proper precautions.
Results: The live and live/dead ratio for attached cells at 48 hr was significantly the lowest for thalamus compared to that for BG and cortex. Supplementation of BH_4 or sepiapterin resulted in significant increase in survival in the thalamus but did not increase survival in BG or cortex. The beneficial effect of sepiapterin was less in hypoxic group compared to the control group.
Conclusion and speculation: BH_4 is critical to the survival of neurons in the thalamus as neuronal culture in the absence of BH_4 in the milieu decreases cell survival. This requirement is more pronounced for neurons in the thalamus than BG or cortex. Supplementation of BH_4 partially protects the thalamic neurons but does not protect as much neurons from other regions. It is speculated that the greater dependence of BH_4 in the thalamus may be related to the higher nNOS levels.

Disclosures: L. Yu, None; **J. Whitsett**, None; **J. Vasquez-Vivar**, None; **S. Tan**, None.

Nanosymposium

224. Neurotoxicity and Neurodegeneration II

Location: Room 23A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 224.4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effects of nigella sativa oil on haloperidol induced deficit in rat model

Authors: *T. MALIK[1,2,3], D. HALEEM[2], S. H. HASAN[3], S. PERVEZ[3], T. FATIMA[4];
[1]Itasca, IL; [2]Neurochemistry and Biochem. Neuropharm. unit, Biochem. Dept., The Univ. of Karachi, Karachi, Pakistan; [3]Dept. Pathology and Microbiology, Histopathology unit,, [4]Dept. of Biol. and Biomed. Sci., The Aga Khan Univ. Hosp., Karachi, Pakistan

Abstract: The neuropathological status of Haloperidol (HP) induced Extrapyramidal symptoms (EPS) remains unclear, but several lines of evidence suggest that persistent neuronal alterations in the basal ganglia cause EPS produced by HP provoked oxidative stress. The goal of this study was to evaluate the possible protective effects of the antioxidative agent “Nigella sativa (NS)” oil on HP induced neuropathological alterations and related motor symptoms in the rodent striatum (Str). To achieve these objectives HP was administered alone and with NS oil. EPS was

monitored in HP treated groups and the animals treated with NS alone and placebo. In the HP treated group displayed a high degree of motor impairment ($p < 0.00$)shown on rota rod experiment, vacuous chewing movement ($p < 0.00$) shown grossly disturbed the large fraction of the cytoarchitectonic pattern ($p<0.05$), histopathology with nerve cell depletion concomitant shrunken cytoplasm, nuclear membrane breakdown and chromatin disorganization. Scarring was also a prominent feature owing profusion of astrogliosis in the dorso and ventro lateral regions of the caudate putamen and in the core of nucleus accumbens. Moderate levels of halo and pyknotic neurons were also observed in HP treated rodents. The morphological HP induced neuronal changes were almost absent in the HP plus NS treated groups ($p < 0.00$). However minor astrogliosis was observed with no obvious indication of cell loss and 82% normal neuronal densities were observed using quantitative, analytic approach in the NS plus HP treated Str. We conclude that NS therapy has preventive effects on HP induced neuronal degeneration in the Str. We believe that further preclinical research into the utility of NS may indicate its usefulness as a protective agent from irreversible EPS during neuroleptic treatment

SCALE	HISTOPATHOLOGICAL SCREENING CRITERIA				TREATMENT AND EFFECTS	
Level	**Neuronal Chromaticity**	**Cytoarchitectonic Pattern**	**Neuronal Density**	**Astrogliosis Status**	**Treatment Groups**	**Results**
0	Normal histological features	Normal histological features	Normal histological features	Normal histological features	Controls and NS	*($p<0.05$)
1	Slight changes with some signs of hyperchromaticity	Disruption of cytoarchitectonic patterns	No obvious Atrophy	No obvious astrogliosis.	-	-
2	Moderate level Hyperchromaticity	Disrupted cytoarchitectonic patterns	Presentation of Some small / Atrophic neurons	Slight indications of astrogliosis. No obvious indications of cell loss	NS+HP	*($p<0.00$)
3	Hyperchromatic and small neurons		Clear signs of cell loss	Increased astrogliosis	HP	*($p<0.05$)
4	Large fraction of hyperchromatic neurons	Cytoarchitectonic pattern was grossly disrupted the large fraction of the neurons seen pyknotic	Prominent areas of cell loss	Marked astrogliosis.	HP	*($p<0.00$)

Histopathological screening of striatum in Nigella sativa (NS) and Haloperidol (HP) treated group of animals

Disclosures: T. Malik: None. **D. Haleem:** None. **S.H. Hasan:** None. **S. Pervez:** None. **T. Fatima:** None.

Nanosymposium

224. Neurotoxicity and Neurodegeneration II

Location: Room 23A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 224.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS045103

NIH Grant P01 NS058484

NIH Grant P20RR20171

Title: Small GTPase Rit promotes neuronal survival via a p38 MAPK cascade

Authors: W. CAI[1], J. L. RUDOLPH[1], J. BRELSFOARD[2], K. E. SAATMAN[2], *D. A. ANDRES[1];
[1]Mol. & Cell. Biochem., [2]Spinal Cord and Brain Injury Center, Dept. Physiol., Univ. of Kentucky Col. of Med., LEXINGTON, KY

Abstract: The mammalian Rit and Rin proteins, along with the Drosophila homologue Ric, comprise an evolutionarily conserved subfamily of the Ras-related GTPases. Here, we identify an unexpected role of Rit GTPase in neuronal survival in response to reactive oxygen species (ROS) stress. Primary hippocampal neurons derived from Rit knockout mice display increased apoptosis and selective disruption of p38 MAP kinase signaling in response to ROS exposure, but not following trophic factor withdrawal, when compared with those derived from wild-type littermates. In stark contrast, neurons expressing constitutively active Rit (Rit Q79L) display significantly higher resistance to ROS and inhibition of the p38 MAPK pathway (SB203580) disrupted Rit-mediated neural protection. Importantly, following moderate traumatic brain injury, a commonly used brain injury model known to induce the generation of ROS, higher levels of degenerating neurons were observed in the hippocampus of Rit knock-out mice than wild-type brain injured mice, particularly within the dentate gyrus. Taken together, these data suggest that the Rit-38 MAPK signaling cascade is a key molecular mechanism controlling whether oxidative damage results in neuronal death or recovery.

Disclosures: W. Cai, None; **J.L. Rudolph**, None; **J. Brelsfoard**, None; **K.E. Saatman**, None; **D.A. Andres**, None.

Nanosymposium

224. Neurotoxicity and Neurodegeneration II

Location: Room 23A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 224.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Fellowship LE1846/2-1 from the Deutsche Forschungsgemeinschaft

Alzheimer's Association IRG-07-58874

Title: Characterisation of a new ATF4 isoform that is expressed in neuronal cells selected for resistance against oxidative stress and mediates xCT expression

Authors: *J. LEWERENZ[1,2], H. SATO[3], P. MAHER[4];
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[3]Yamagata Univ., Tsuruoka, Japan; [4]Salk Insitute for Biol. Studies, La Jolla, CA

Abstract: Oxidative glutamate toxicity is a form a neuronal cell death in which high extracellular glutamate inhibits cystine uptake via the cystine/glutamate antiporter system xc-. Cystine is the rate limiting substrate for the synthesis of the important antioxidant glutathione. As a consequence, inhibition of system xc- leads to glutathione depletion and subsequent cell death in response to oxidative stress.
Oxidative stress is pathophysiologically important in many neurodegenerative diseases. To understand how nerve cells defend themselves against oxidative stress, we selected murine hippocampal HT22 cells resistant to oxidative glutamate toxicity. Glutamate resistance in these cells is associated with a strong upregulation of system xc-.
We then asked, how system xc- is upregulated in these cells. Using different luciferase xCT promoter constructs, we show that the very proximal region of of the xCT gene promoter is responsible for the upregulation of xCT transcription in glutamate-resistant HT22 cells. The amino acid response element located in this region is known to bind the nuclear factor ATF4. In line with this, ATF4 protein is up-regulated in glutamate-resistant HT22 cells compared to wild-type HT22 cells. Knock-down of ATF4 in glutamate-resistant HT22 cells decreases system xc- activity and glutamate resistance, indicating that ATF4 is at least in part responsible for the resistant phenotype.
Interestingly, the ATF4 protein in glutamate-resistant HT22 cells exhibits an approximately 5 kD higher apparent molecular weight compared to wild-type HT22 cells. Our results suggest that this is not due to posttranslational modifications because recombinant ATF4 expressed in glutamate-resistant cells shows no size shift. Moreover, ATF4 in glutamate-resistant HT22 cells is not upregulated due to higher protein stability. Upon eIF2alpha phosphorylation, a strong inducer of ATF4 protein translation, glutamate-resistant HT22 cells express classical ATF4, whereas high molecular weight ATF4 is down-regulated. Induction of classical, but not

expression of high molecular weight ATF4, is associated with the expression of the ATF4 downstream target, CHOP. These findings indicate that the new ATF4 is a functionally distinct protein. In addition, PC12 cells selected for resistance against the amyloid-beta peptide express a high molecular weight ATF4 protein with similar properties.
In summary, our findings suggest that ATF4 is involved in the protective response against oxidative stress in nerve cells. Moreover, we describe a new ATF4 variant with properties distinct from classical ATF4.

Disclosures: J. Lewerenz, None; **H. Sato**, None; **P. Maher**, None.

Nanosymposium

224. Neurotoxicity and Neurodegeneration II

Location: Room 23A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 224.7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: T32MH067631

Title: The HIV glycoprotein gp120 dysregulates fast axonal transport through an axon-autonomous mechanism

Authors: *S. H. BERTH, G. MORFINI, T. SARMA, S. T. BRADY;
Anat. and Cell Biol., Univ. of Illinois-Chicago, CHICAGO, IL

Abstract: Sensory neuropathies are the most prevalent neurological complication of HIV infection: close to 30% of all HIV patients develop sensory neuropathies during the course of their disease. The most common form of neuropathy is distal sensory polyneuropathy (DSP), a neuropathy directly attributable to HIV infection. DSP is characterized by a progressive distal to proximal dying-back degeneration of long sensory axons from dorsal root ganglion (DRG) neurons, resulting in debilitating pain. A significant body of research has documented the neurotoxicity of gp120, an HIV glycoprotein that is overproduced and shed by macrophages, which surround DRG neurons and their axons. However, mechanisms linking gp120 to dying back degeneration remain uncertain. We found that F11 cells, a hybrid cell line made from rat dorsal root ganglion and mouse neuroblastoma cells, internalized gp120 with a distinct time course in a process independent of CXCR4 activation. Gp120 internalization was further characterized through immunolocalization studies. To examine the effects and distribution of gp120 in the axonal compartment of neurons, microfluidic chambers that isolate axons and cell bodies have begun to be used for analysis of gp120 effects on axons. Further, functional studies

in isolated squid axoplasm revealed that gp120 reduced both retrograde and anterograde fast axonal transport (FAT) through abnormal activation of selected kinases and phosphatases. Biochemical studies in cultured cells confirmed and extended these observations by examining the role of CXCR4 in mediating activation of these kinases. Given the unique reliance of neurons on FAT, our studies suggest that gp120-induced activation of phosphotransferases in the axonal compartment might represent a critical pathogenic event in DSP.

Disclosures: S.H. Berth, None; **G. Morfini**, None; **T. Sarma**, None; **S.T. Brady**, None.

Nanosymposium

224. Neurotoxicity and Neurodegeneration II

Location: Room 23A

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 224.8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NH&MRC Grant 572550

NH&MRC Grant 254670

Neutrauma Research Program, Western Australia

Title: Early increases in oxidative stress in nerve vulnerable to secondary degeneration: Near infra-red light treatment

Authors: *L. FITZGERALD, C. A. BARTLETT, J. WELLS, J. RODGER, A. R. HARVEY, S. A. DUNLOP;
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Abstract: Following brain and spinal cord injury, tissue outside the direct trauma site succumbs to delayed damage known as secondary degeneration, resulting in an area of tissue damage greater than can be accounted for by the initial insult and further loss of function. We have comprehensively characterised a model of secondary degeneration in the rat central nervous system, in which the optic nerve (ON) is partially transected, resulting in clear spatial separation of tissue undergoing secondary degeneration from the initial injury. Using this model, we have recently demonstrated that within five minutes of injury, MnSOD (oxidative stress marker) co-localizes within the astrocytic network of nerve vulnerable to secondary degeneration, spreading to RGC somata vulnerable to secondary degeneration by 24 hours. Secondary astrocyte hypertrophy is evident in the nerve from 3 hours. We have extended these findings to

demonstrate that immunointensity of the advanced glycation end product N(epsilon)-(carboxymethyl) lysine and Aquaporin4 also significantly increase in astrocytes of ON vulnerable to secondary degeneration by 24 hours ($p \leq 0.05$). These changes are accompanied by decreases in catalase activity ($p \leq 0.05$). Post operative treatment with near infra-red light (670nm, WARP10 LED array) significantly reduces early increases in MnSOD immunoreactivity and prevents the later increases in numbers of MnSOD immunoreactive aggregates in areas of the ON vulnerable to secondary degeneration ($p \leq 0.05$). Importantly, visual function is restored to normal with near infra-red light treatment, as assessed by optokinetic nystagmus and Y-maze pattern discrimination tests ($p \leq 0.05$). Oxidative stress spreading via the astrocytic network is an early event during secondary degeneration and its containment, perhaps with near infra-red light, may help to prevent further damage to the nerve.
We thank Professor J.T Eells for the kind gift of the WARP10 LED array.

Disclosures: **L. Fitzgerald**, National Health & Medical Research Council, Australia Grant ID: 572550. Neurotrauma Research Program, Western Australia, Research Grant; **C.A. Bartlett**, None; **J. Wells**, None; **J. Rodger**, None; **A.R. Harvey**, None; **S.A. Dunlop**, National Health and Medical Research Council Grant ID: 254670, Neurotrauma Research Program, Western Australia, Research Grant.

Nanosymposium

225. New Molecular Targets in Psychotic Diagnoses

Location: Room 4

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 225.1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: DFG GRK 1033

DFG Ko1679/3-1

SMRI 02R-186

Title: DISC1 aggregates recruit DTNBP1: Interaction of two top schizophrenia susceptibility proteins

Authors: P. OTTIS, R. LELIVELD, V. BADER, S. TROSSBACH, *C. KORTH;
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Abstract: Background: DISC1 and DTNBP1 (dystrobrevin-binding protein 1, or dysbindin) both

have been genetically linked to schizophrenia. Extensive genetic studies and reverse genetics through animal modeling have established DISC1 and DTNBP1 as two of the best investigated schizophrenia susceptibility genes. Significant decreases in dysbindin protein levels have been reported to be associated to schizophrenia in post mortem schizophrenic brains and we previously demonstrated insoluble DISC1 in post mortem brains of a subpopulation of sporadic CMD patients.
Methods: Insoluble proteins were purified from post mortem brains (SMRI Consortium Collection) and probed for the presence for dysbindin and DISC1 by Western blotting. Co-localization studies were performed on Dysbindin and DISC1, both over-expressed in co-transfected cell-models using both fluorescent fusion-tags and immunofluorescence. Determination of the interacting domains was achieved applying various truncation constructs of both proteins. Biochemical characterization of DISC1-dysbindin complexes along with characterization of other associated or bridging proteins was performed.
Results: Dysbindin immunoreactivity in the insoluble fraction purified from post mortem brains of CMD brains but not normals was present in 80% of those post mortem cases of the SMRI Consortium Collection that showed also imunoreactivity to DISC1. Brains with a variety of neurodegenerative diseases did not show dysbindin or DISC1 immunoreactivity in insoluble fractions. When transiently overexpressed in human neuroblastoma cells, DISC1 assembled in to several aggresomes per cell whereas dysbindin showed a monodispersed cytosolic distribution. Co-expression of dysbindin and DISC1, however, demonstrated that dysbindin was recruited into the DISC1 aggresomes that where large and occurred only once per cell. Interaction assays and further biochemical characterization of the aggregates including proteomic analyses will be reported.
Conclusions: Our results suggest that aggregated, insoluble DISC1 can recruit cellular soluble dysbindin and thus potentially influence its functionality. A subgroup of patients with chronic mental disease was identified to display both insoluble DISC1 and dysbindin as evidence that 1. insoluble human DISC1 interacts with human dysbindin and 2. recruitment of dysbindin by insoluble DISC1 could be an important disease mechanism for mental disease in vivo.

Disclosures: P. Ottis, None; **C. Korth**, None; **S. Trossbach**, None; **V. Bader**, None; **R. Leliveld**, None.

Nanosymposium

225. New Molecular Targets in Psychotic Diagnoses

Location: Room 4

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 225.2

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Rotary Health Research Fund

Rebecca L. Cooper Medical Research Foundation

NHMRC Fellowship 400016

Title: Decreased mu opioid receptor availability in subjects with schizophrenia who died by suicide

Authors: *E. SCARR[1,2], T. T. MONEY[1,2], B. DEAN[1,2];
[1]Mental Hlth. Res. Inst., Parkville, Australia; [2]Psychiatry, Univ. of Melbourne, Melbourne, Australia

Abstract: Previous studies have shown the mu-opioid receptor to be altered in the brains of subjects who died by suicide [(1-3)]. Therefore, we wished to determine whether this system has a role in suicide by subjects with schizophrenia.
As the opioidergic system, in particular the mu receptor, has previously been shown to be altered in the brains of people who died as the result of suicide, we wished to determine whether this system has a role in suicide by subjects with schizophrenia.
Mu receptor levels were determined using *in situ* radioligand binding and autoradiography with [^{3}H]DAMGO as the ligand and Western blots using tissue from the dorsolateral prefrontal and anterior cingulate cortices and caudate putamen from 12 subjects with schizophrenia who died by suicide, 26 subjects with schizophrenia who died from other causes and 20 control subjects. [^{3}H]DAMGO binding density ($p = 0.36$) and mu receptor protein levels ($p = 0.37$) did not vary with diagnoses in any of the brain regions studied. However [^{3}H]DAMGO binding density, but not mu protein levels, was significantly decreased in all regions from subjects who died as a result of suicide (BA 9 $p<0.01$; BA 24 $p<0.001$; CPu $p<0.05$).
Our data shows that [^{3}H]DAMGO binding, but not mu protein levels, are decreased in tissue from subjects with schizophrenia who died by suicide, indicating that mu opioid receptor availability is decreased in the brains of these people. This in turn suggests that people with schizophrenia who die as a result of suicide have higher central levels of endogenous ligands for the mu opioid receptor than those people with schizophrenia who die from other causes. A better understanding of the factors that may contribute to the high suicide rate associated with schizophrenia may help us develop more effective strategies to prevent this devastating event.
References:
1. Gabilondo,AM, Meana,JJ, Garcia-Sevilla,JA (1995): Increased density of mu-opioid receptors in the postmortem brain of suicide victims. *Brain Res* 682: 245-250.
2. Gross-Isseroff,R, Dillon,KA, Israeli,M, Biegon,A (1990): Regionally selective increases in mu opioid receptor density in the brains of suicide victims. *Brain Res.* 530: 312-316.
3. Zalsman,G, Molcho,A, Huang,Y, Dwork,A, Li,S, Mann,JJ (2005): Postmortem m-opioid receptor binding in suicide victims and controls. *Journal of Neural Transmission* 112: 949-954.

Disclosures: **E. Scarr**, AstraZeneca, Speakers Bureau/Honoraria; **T.T. Money**, None; **B. Dean**, Janssen-Cilag, Speakers Bureau/Honoraria; Eli Lilly, Speakers Bureau/Honoraria; Bristol-Myers Squibb, Consultant/Advisory Board.

Nanosymposium

225. New Molecular Targets in Psychotic Diagnoses

Location: Room 4

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 225.3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant 5R01MH086601-02

Title: VU0152100, a selective positive allosteric modulator of M4 muscarinic acetylcholine receptors, produces antipsychotic-like activity and enhancement of cognition in rats

Authors: *C. K. JONES[1,4,5], N. BYUN[2], J. D. ROSANELLI[4], M. BUBSER[4], T. M. BRIDGES[4], C. W. LINDSLEY[4,3], P. J. CONN[4];
[2]Inst. of Imaging Sci., [3]Dept. of Chem., [1]Vanderbilt Univ. Med. Ctr., Nashville, TN; [4]Dept. of Pharmacol., Vanderbilt Program in Drug Discovery, Vanderbilt University Medical Center, TN; [5]Tennessee Valley Healthcare Syst., U.S. Dept. of Veterans Affairs, Nashville, TN

Abstract: Recent studies indicate that selective activators of specific subtypes of muscarinic acetylcholine receptors (mAChRs) may provide a novel approach for the treatment of psychotic symptoms and behavioral disturbances associated with schizophrenia and Alzheimer's disease (AD). For example, the M1/M4-preferring mAChR agonist xanomeline produces robust decreases in psychotic symptoms, behavioral disturbances, and in some of the cognitive impairments in schizophrenic and AD patients. At present, the relative contributions of M1 and M4 mAChRs to the clinical effects of xanomeline or its effects in associated animal models remain unknown. Recent findings using postmortem brain tissue from schizophrenia patients and M4 knockout (KO) mice suggest that selective activation of M4 does contribute to the effects of xanomeline and that selective activators of M4 may have exciting potential as novel antipsychotic and cognitive enhancing agents. Unfortunately, previous attempts to develop highly selective agonists of M4 have failed due to the high sequence conservation of the orthosteric acetylcholine (ACh) binding site of the mAChRs. Over the last several years, we and others have developed a novel approach to selectively activating individual mAChR subtypes, especially M4, using highly selective positive allosteric modulators (PAMs). These compounds do not activate M4 directly, but dramatically potentiate the response of the receptor to ACh. Recently, we reported that pharmacologic characterization of VU152100, a potent and systemically active M4 PAM. In the present studies, we describe the effects of selective activation of M4 by VU0152100 in several preclinical models predictive of antipsychotic-like activity in rats, specifically amphetamine and phencyclidine (PCP)-induced hyperlocomotion and disruption of prepulse inhibition (PPI) of the acoustic startle reflex, and enhancement of

hippocampal-mediated learning and memory tasks. Interestingly, VU0152100 produced antipsychotic-like activity and enhancement of cognition at doses that did not produce adverse effects associated with activation of peripheral mAChRs in vivo. VU0152100 also produced efficacy across these preclinical models in a dose range that did induce catalepsy or impair general locomotor output. These results represent an important breakthrough in this field and provide further support for the concept that highly selective M4 activators, such as M4 PAMs, have potential for development as novel antipsychotic and cognitive enhancing agents.

Disclosures: C.K. Jones, None; **N. Byun**, None; **J.D. Rosanelli**, None; **M. Bubser**, None; **T.M. Bridges**, None; **C.W. Lindsley**, None; **P.J. Conn**, None.

Nanosymposium

225. New Molecular Targets in Psychotic Diagnoses

Location: Room 4

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 225.5

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant K99 MH085004

NIA Grant ROI AG06173

Title: DISC1 acts downstream of APP and DAB1 in cortical development

Authors: *T. L. YOUNG-PEARSE[1], S. SUTH[2], E. LUTH[2], A. SAWA[3], D. J. SELKOE[1]; [1]Dept Neurol, BWH/Harvard Med. Sch., BOSTON, MA; [2]BWH/Harvard Med. Sch., Boston, MA; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Although clinically distinct, schizophrenia and Alzheimer's disease are common and devastating disorders that profoundly impair cognitive function. For Alzheimer's disease, key mechanistic insights have emerged from genetic studies that identified causative mutations in Amyloid Precursor Protein (APP) and Presenilin. Several genes have been associated with schizophrenia and other major psychoses, and understanding their normal functions will help elucidate the underlying causes of these disorders. One such gene is Disrupted in Schizophrenia-1 (DISC1). DISC1 and APP have been implicated separately in cortical development, with each having roles in both neuronal migration and neurite outgrowth. Here, we report a biochemical and functional interaction between DISC1 and APP. Using in utero electroporation in the living rat brain, we show that DISC1 acts downstream of APP and Disabled-1 to regulate cortical precursor cell migration. Specifically, overexpression of DISC1 rescues the migration defect

caused by a loss of APP or DAB1 expression. Moreover, knock-down of APP in cultured embryonic neurons results in altered subcellular localization of DISC1. This effect is rescued by APP expression, but deletion of the DISC1 binding domain prevents this rescue. Using transfected cells and normal brain tissue, we show that APP and DISC1 co-immunoprecipitate and that the intracellular domain of APP interacts with the N-terminal domain of DISC1. Based on these findings, we hypothesize that the APP cytoplasmic region transiently interacts with DISC1 to help regulate the translocation of DISC1 to the centrosome, where it plays a key role in controlling neuronal migration during cortical development.

Disclosures: T.L. Young-Pearse, None; **D.J. Selkoe**, None; **S. Suth**, None; **E. Luth**, None; **A. Sawa**, None.

Nanosymposium

225. New Molecular Targets in Psychotic Diagnoses

Location: Room 4

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 225.6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NARSAD

NINDS

NIMH

NIDA

Title: Glutathione un-GLU-ed: A role for glutathione in neuronal glutamate metabolism

Authors: *M. KOGA[1], M. MESSMER[1], A. SAWA[1,2], S. H. SNYDERR[1,2,3], T. W. SEDLAK[1]; [1]Dept. of Psychiatry and Behavioral Sci., [2]Dept. of Neurosci., [3]Dept. of Pharmacol., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Glutamate is the principal excitatory neurotransmitter of the brain and participates in pleiotropic physiologic and pathologic processes. Glutamate neurotransmission is crucial to learning and memory, while glutamate excitotoxicity is a mediator of stroke damage. Multiple lines of evidence have suggested deficient glutamate neurotransmission in schizophrenia, particularly via NMDA receptors. While release of glutamate from neurotransmitter vesicles has been intensively studied, substantially less is known of how neuronal glutamate levels are

regulated prior to synaptic release.
Glutathione is a tripeptide composed of the amino acids glutamate, cysteine, and glycine, and is abundantly present at millimolar concentrations in neurons. Glutathione, participates in a variety of cellular processes, including antioxidant and drug detoxification pathways, and diminished glutathione levels have repeatedly been found to be associated with schizophrenia.
We hypothesized that glutathione may be a potentially significant reservoir for glutamate in neurons. To test this we employed inhibitor drugs (2I4C, acivicin, and BSO) that target enyzmes of the glutathione metabolic cycle and measured glutamate in PC12 and HT22 neurons via the glutamate oxidase method. We found that acivicin and 2I4C, drugs that block liberation of glutamate from glutathione, resulted in up to a 60% decrease in glutamate levels. BSO, which prevents synthesis of glutathione from glutamate, had the reverse effect, significantly increasing glutamate levels and decreasing glutathione. Similar trends were observed in cultured primary neuronal cells. Reduced glutamate levels were not the result of cytotoxicity as neuronal viability was not substantially altered via drug treatment.
These findings suggest that glutathione may be an important neuronal reservoir of glutamate that contributes to baseline glutamate levels. Our model could also bridge two independent lines of research in schizophrenia, that of reduced glutathione levels and glutamatergic dysfunction in schizophrenia.

Disclosures: M. Koga, None; **M. Messmer**, None; **A. Sawa**, None; **S.H. Snyderr**, None; **T.W. Sedlak**, None.

Nanosymposium

225. New Molecular Targets in Psychotic Diagnoses

Location: Room 4

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 225.7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH

NARSAD

Title: Specific regulation of NRG1 isoform expression by neuronal activity

Authors: *X. LIU, W.-C. XIONG, L. MEI;
Inst. of Mol. Med. and Genet. and Dept. of Neurol., Med. Col. of Georgia, AUGUSTA, GA

Abstract: Neuregulin 1 (NRG1) is a large family of EGF domain-containing trophic factors.

Generation of these isoforms is complex, and thought to be contributed by alternative splicing and usage of different promoters (Mei and Xiong, Nature Rev Neurosci, 9:437-452, 2008). It would be important to understand mechanisms of NRG1 isoform expression because genetic studies have identified NRG1 as a susceptibility gene of schizophrenia and in particular, some NRG1 appeared up-regulated in brain regions of schizophrenic patients. Moreover, although six types of NRG1 have been identified in human, whether they all exist in rodents remain unclear. To these ends, we have systematically characterized the spatial and temporal expression of six different isoforms and investigated regulatory mechanisms. Specific primers were designed for each of the six types and used for RT-PCR with rat brain cDNA as template and resulting products were sequenced. We found that five of the six types (I, II, III, IV, and V) were expressed in rat brains; however, expression of type VI was undetectable. Sequence analysis revealed that rodent type IV NRG1 transcript may use ATG in the immunoglobin (Ig) domain as translation initial site. Real time PCR analysis indicates that most abundant isoforms were type I, II and III, while expression levels of type IV and V were lower. Different types have unique expression patterns during development. For example, type IV was barely detectable after birth. Intriguingly, expression of some, but not all, NRG1 isoforms is regulated by neuronal activity. mRNA levels of type I and IV were elevated in the brain of rat injected with kainic acid. Accordingly, treatment of cultured neurons with KCl or bicuculline increased their mRNA levels. Results indicate that a CREB binding site in the type IV promoter region is critical for regulation by neuronal activity. Our studies indicate that expression of NRG1 isoforms is regulated by distinct mechanisms, which contributes to versatile functions of NRG1 and pathologic mechanisms of brain disorders including schizophrenia.

Disclosures: X. Liu, None; **W. Xiong**, None; **L. Mei**, None.

Nanosymposium

225. New Molecular Targets in Psychotic Diagnoses

Location: Room 4

Time: Sunday, November 14, 2010, 1:00 pm - 3:00 pm

Program Number: 225.8

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH grant MH085208

NIH grant MH086385

NIH grant MH64168

NIH grant MH60877

Title: Microarray analysis of subtypes of pyramidal and nonpyramidal neurons from auditory cerebral cortex in schizophrenia

Authors: J. F. SMILEY[1,2], H. M. CHAO[1,3], A. J. DWORK[4,5], M. J. ALLDRED[1,3], *I. ELAROVA[1], D. C. JAVITT[1,3], S. D. GINSBERG[1,3];
[1]Nathan Kline Inst., ORANGEBURG, NY; [2]City Col., New York, NY; [3]NYU Med. Sch., New York, NY; [4]New York Psychiatric Inst., New York, NY; [5]Columbia Univ., New York, NY

Abstract: Schizophrenia is associated with altered neurotransmission by both glutamatergic and GABAergic neurons in the cerebral cortex, and there is evidence that specific cortical pathways and neuronal subtypes are selectively vulnerable in this disease. To further characterize the specificity of schizophrenia pathology, we used microarray analysis to compare mRNA expression levels in isolated neuron subtypes of auditory association cortex, in schizophrenia (n=15) and age-matched nonpsychiatric (n=15) brains. Laser capture microdissection (LCM) was used to isolate calbindin (CB) and parvalbumin (PV) subtypes of GABAergic interneurons, as well as glutamatergic pyramidal neurons of lower layer III. These cell types represent distinct components of cortical circuitry; parvalbumin neurons are mainly involved in feed-forward inhibition, calbindin cells are more involved in feedback or modulatory inhibition, and layer III pyramidal cells are mainly feed-forward principal neurons. RNA was harvested from homogeneous cell populations acquired via LCM, amplified via terminal continuation (TC) RNA amplification, and hybridized to custom-designed microarrays containing 576 transcripts relevant to neuroscience and neuropsychiatric disorders. Preliminary analysis showed that pyramidal neurons have about twice as many significantly altered genes in schizophrenia compared to either CB or PV neurons. CB neurons were distinguished from the other cell types by their general tendency of reduced expression for most differentially regulated genes. An analysis of the types of genes changed in schizophrenia was obtained using Chi-squared analysis to look for clusters of changes within 21 predefined gene ontology groups on the microarray. Among the most significant group changes were the monoamine-related transcripts, including dopamine and norepinephrine receptors, which were especially up-regulated in CB and PV neurons. Overall, the findings indicate that the profile of gene expression changes in the cerebral cortex in schizophrenia is strikingly different in distinct neuronal subpopulations, and some changes will be overlooked by measuring expression changes in the whole cerebral cortex.

Disclosures: J.F. Smiley, None; **H.M. Chao**, None; **A.J. Dwork**, None; **M.J. Alldred**, None; **I. Elarova**, None; **D.C. Javitt**, None; **S.D. Ginsberg**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.1

Topic: D.04. Vision

Support: NIH R01 EY015219

Canadian Foundation for Innovation LOF Grant

Title: Retinotopically organized resting-state functional connectivity of human visual cortex in amblyopia

Authors: *L. B. LEWIS[1], F. CARBONELL[2,3], A. SHMUEL[2,3], J. D. MENDOLA[1];
[1]Dept. of Ophthalmology, [2]Departments of Neurol. and Neurosurg. and Biomed. Engin., McGill Univ., Montréal, QC, Canada; [3]Departments of Neurol. and Neurosurg. and Biomed. Engin., Montreal Neurolog. Inst., Montréal, QC, Canada

Abstract: Introduction
Amblyopia is a developmental visual disorder associated with loss of monocular acuity and sensitivity as well as alterations in binocular integration. Abnormal connections in occipital cortex are known to underlie this loss, but the extent to which these abnormalities are regionally or retinotopically specific has not been fully determined. Furthermore it is unknown whether abnormalities exist in functional connectivity in amblyopia only during active vision, or whether they exist more generally during states of rest.
Methods
Amblyopic subjects (n=4, mean age=32.0 yrs, std=10.6 yrs) and normally sighted subjects (n=8, mean age=30.6 yrs, std=8.3 yrs) were scanned at 3 Tesla. Resting state functional MRI was obtained (TR=2s, TE=30ms, matrix=64x64, 40 slices, voxel size=2x2x2mm) in near darkness with alternating blocks of eyes open vs. eyes closed. Visual areas V1, V2, V3, V3A/B, V4, MT, LO1 and LO2 of each subject were defined according to conventional phase encoding retinotopy. Each area was divided into subarea ROIs according to eccentricity. Pre-processing of the resting-state time series was applied, including motion correction and modeled physiological noise removal. The first principal component, that accounted for most of the variance in the global average of the pre-processed time-series, was removed. Visual-area and eccentricity-specific time-courses were integrated over each of the retinotopically defined ROIs. Resting state functional-connectivity measures between all pairs of ROIs were obtained by computing the correlation coefficients between the corresponding time-courses.
Results
The global comparison of amblyopic and normal groups (with eye condition and eccentricity combined), indicates reduced connectivity between areas V2, V3, V3A/B, V4 and MT in the amblyopic subjects. Consistently, this extrastriate network has previously been associated with binocular integration and stereopsis. In addition, preliminary analysis shows that in amblyopic adults, when eyes were open vs. closed, there was an eccentricity dependent effect; regions of occipital cortex that represent central areas of visual space were more correlated than in normally sighted subjects, whereas regions of occipital cortex that represent peripheral areas of visual space were less correlated than in normally sighted subjects. This might reflect a signature of

compensation for the central vision loss. These findings suggest that amblyopia results in abnormalities in functional connectivity not only during active vision, but also during states of rest. We conclude that abnormal anatomical connections may underlie these effects.

Disclosures: L.B. Lewis, None; **F. Carbonell**, None; **A. Shmuel**, None; **J.D. Mendola**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.2

Topic: D.04. Vision

Support: NIH R01-EY02966

NIH R01-EY16281

NIH T32-EY07143-13

Title: Contour binding increases coherence between local field potentials at distant sites in cortex

Authors: *A. MARTIN[1], R. VON DER HEYDT[1,2];
[1]Neurosci., The Johns Hopkins Univ., BALTIMORE, MD; [2]Krieger Mind Brain Inst., The Johns Hopkins Univ., Baltimore, MD

Abstract: Information about an object can be represented across long distances in visual cortex, within a cortical processing area as well as between areas. Synchrony of neuronal firing has been proposed as a way of binding the distant elements of an object together, but synchrony might also result from a specific connectivity that enables feature grouping and object-based attention (Craft et al., J Neurophysiol 2007). In a previous study of spike activity we did not find significant synchronization between neurons coding for features of the same object compared to features of different objects (Dong et al., J Vision 2007).
We have now studied coherence in the local field potential (LFP) with a similar same-object/different-objects paradigm, but using a task that controlled the monkey's attention. LFPs and single-neuron activity were recorded from 2 electrodes separated by 3-10mm cortical distance in macaque areas V1 and V2. The monkeys maintained fixation while attending one of several figures presented parafoveally or in the near periphery. Edges of the figures were presented in the preferred receptive fields of neurons recorded simultaneously from each

electrode. The edges could either be part of the same or different figures (thus the edges were bound together or not) and the animal could attend the figure with edges in the receptive fields or another figure elsewhere in the visual field. We found a robust increase of coherence between LFPs with binding. The spectral range of the increased coherence was in the beta and low gamma bands. The effects of attention on coherence were subtle.
We conclude that binding of contour elements increases coherence of LFP between the sites representing these elements, even when they are widely separated in cortex. Since the LFP is considered to be the sum of synaptic inputs to the local area, this increased coherence with binding suggests that the neurons responding to bound elements have common reentrant inputs that act to group the elements together.

Disclosures: A. Martin, None; **R. von der Heydt**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.3

Topic: D.04. Vision

Support: NIH grant R01 EY017077

Title: Correlated firing between primate prefrontal neurons before and after learning to perform a cognitive task

Authors: *C. CONSTANTINIDIS[1], X.-L. QI[1], T. MEYER[1,2];
[1]Wake Forest Univ. Sch. of Med., Winston-Salem, NC; [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The primate prefrontal cortex is essential for learning and performing cognitive operations, however little is known about how learning affects prefrontal neuronal activity and functional connectivity. To address this issue we recorded activity from prefrontal neurons of monkeys before and after they were trained to perform visual working memory tasks. Prior to training, the animals fixated while the stimuli that we eventually used in a cognitive task were presented passively. A total of 1365 neurons were recorded during this phase. After training, the animals were required to remember the stimulus spatial locations and features and indicate if two stimuli presented in sequence were the same or different. Another 1343 neurons were then recorded from the same animals. After learning to perform the task we observed a greater number of neurons activated by the stimuli, and higher average firing rate. In order to understand the nature of changes in effective connectivity between prefrontal neurons we examined trial-to-

trial covariations in firing rate (noise correlation) as well as cross-correlation of spike trains. This analysis was performed on 823 pairs of neurons recorded simultaneously prior to training, from separate electrodes 0.5 to 1 mm apart and 556 pairs of neurons recorded after training in the same fashion. Noise correlation decreased after learning to perform the task. The effect was evident in all task periods but was most pronounced during the stimulus presentation period. Similarly, the strength of cross-correlation peaks, estimated based on the number of spikes in the 5 ms around the center of the cross-correlation histogram decreased after training. The results indicate that training is characterized by a decorrelation of the discharges of prefrontal neurons, which is evident both at long (noise correlation) and short (cross-correlation) time scales of neuronal synchronization. The finding reveals that prefrontal neuronal circuits undergo a functional reorganization after training, reflecting more distributed functional inputs. This decrease in correlation may improve the sensitivity of information representation in neuronal populations after training.

Disclosures: C. Constantinidis, None; **X. Qi**, None; **T. Meyer**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.4

Topic: D.04. Vision

Support: NIH Grant MH68004

Title: Temporal frequency tuning in non-retinotopic cortex

Authors: *J. O. GARCIA, E. A. HECKER, D. A. BRIDWELL, R. SRINIVASAN;
UC Irvine, IRVINE, CA

Abstract: fMRI studies exploring the topographic organization of visual cortex have previously limited visual field map delineation to the robust response of neurons to visual stimulation within a particular region of space. In these studies, an orderly mosaic of visual field maps emerges, with distinct functional significance and response properties (e.g., Wandell et al., 2005), but this method is biased to neurons with small receptive field sizes and has only recently been extended beyond occipital cortex. EEG studies, limited by the spatial resolution of their method, cannot define analogous visual field maps; instead, these studies exploit the superior temporal resolution of EEG by using a periodic stimulus to impose synchrony on populations of neurons. This frequency-tagging (SSVEP) method uses different frequencies of visual flicker and the

corresponding EEG response at these frequencies to target specific brain networks, often extending beyond retinotopic cortex. These frequency-tagged networks exhibit dynamic properties that depend on frequency and have been used to isolate brain networks with different functional properties (e.g., sensitivity to attention). Few studies have investigated the corresponding dependence of the fMRI signal on the temporal frequency of visual input, but Srinivasan et al. (2007), using whole field stimulation with a reversing checkerboard, observed fMRI responses in non-retinotopic frontal cortex increases responsivity to specific temporal frequencies (3-5 Hz). In the present study, we have measured fMRI responses to two stationary flickering checkerboards, presented in the lower half of each visual field at different reversal frequencies (2-15Hz). We have also used a non-periodic random broadband flicker stimulus (RBBF) to confirm the response is specific to the periodicity of the stimulus. Overall, the RBBF condition elicited the most activity in early visual areas (V1-V3) in the contralateral hemisphere, but showed a complex pattern of frequency tuning in the ipsilateral hemisphere. We observed a bias to the 7.5Hz flicker in many regions of cortex, including DLFPC, MFC, and IPS, but these non-retinotopic cortical regions also show a more variable response to flicker at the different frequencies. We propose temporal frequency tuning to be another topographic organizing principle of the human brain that may be used to identify brain regions within a functional network extending beyond retinotopic cortex. These results have broad implications to studies of visual field mapping and concurrent imaging of fMRI and EEG.

Disclosures: J.O. Garcia, None; **E.A. Hecker**, None; **R. Srinivasan**, None; **D.A. Bridwell**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.5

Topic: D.04. Vision

Title: Visual information represented at different levels of functional hierarchy in inferior temporal cortex of monkeys revealed by machine learning

Authors: *M. TANIFUJI[1], G. UCHIDA[1], T. SATO[1], J. KITAZONO[2], M. OKADA[2];
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Abstract: Inferior temporal (IT) cortex is considered to be essential for visual perception and recognition of objects. In the previous study (Sato et al., Cereb. Cortex, 19, 1870-, (2009)), we

showed that neurons with similar object selectivity are locally clustered and form functional columns in IT cortex of macaque monkeys. Furthermore, we recently found a larger functional structure (functional domains) that covers multiple columns with similar object selectivity. These results suggest IT cortex is hierarchically organized in function from single cells, columns, then to functional domains.
What is the essential information represented at different levels of the hierarchy and how does the information differ among different levels? Particularly, there was a functional domain specific to faces. Thus, more specifically, we can ask whether the face domain represents the face category and local clusters within the face domain represent facial features. If these local clusters represent facial features, combinations of these clusters in the face domain can be used to identify individual faces. To address the question, we investigated performance of a regularized linear classifier for categorization and identification of faces with different level of functional hierarchy. We first trained the classifier so as to categorize images into face and non-face object. We also trained the classifier to identify a particular face from other faces. Although local clusters essential for the face categorization appeared across different functional domains, the ones with positive weight parameters appeared only in the face domain. This result suggests that the differential activation of domains to faces is essential for face categorization. On the other hand, spatial distributions of the local clusters essential for the face identification had no relation to the domain structures. Thus, it is not the case that the local clusters in the face domain are essential and critical for face identification, but the local clusters outside of the face domains are also and actually even more useful for face identification. We also trained the classifier to identify a particular non-face object from other non-face objects, and found no specific role of functional domains for identification of non-face objects. Altogether, these results suggest that the functional domain represents information about object category such as faces, whereas local clusters (presumably columns) represent generic visual features useful for identifying objects in general.

Disclosures: M. Tanifuji, None; **T. Sato**, None; **J. Kitazono**, None; **M. Okada**, None; **G. Uchida**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.6

Topic: D.04. Vision

Support: DARPA Young Faculty Award

Title: A place area in the macaque occipitotemporal sulcus localized by functional magnetic resonance imaging

Authors: *S. KORNBLITH[1,2,3], D. Y. TSAO[3];
[1]Los Angeles, CA; [2]Brain and Cognitive Sci., MIT, Cambridge, MA; [3]Biol., Caltech, Pasadena, CA

Abstract: In humans, functional magnetic resonance imaging has identified an area in posterior parahippocampal cortex that exhibits significantly stronger activation to images of scenes than to images of faces or objects. In three macaques, we localized a potentially homologous area bilaterally in the fundus of the occipitotemporal sulcus that responded significantly more strongly to images of both empty and furnished familiar and novel indoor scenes than to images of scrambled scenes, familiar and unfamiliar objects, textures, and scenes in which the background was blurred but the objects were left intact. In line with previous human findings (Epstein & Kanwisher, 1998), the region exhibited a reduced response to images of scenes in which the walls and floor were rearranged such that they no longer formed a coherent geometry. This region potentially serves a role in visual guidance of spatial navigation.

Disclosures: S. Kornblith, None; **D.Y. Tsao**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.7

Topic: D.04. Vision

Support: PHS Grant DA023427

Title: Predicting face-selective fusiform voxels from diffusion-based connectivity alone

Authors: *Z. M. SAYGIN, D. E. OSHER, R. R. SAXE, J. D. E. GABRIELI;
MIT, CAMBRIDGE, MA

Abstract: Structural connectivity is the substance of neural communication and as such, defines the capabilities of a network. Accordingly, functional regions should be characterizable by their unique pattern of connectivity. We present the use of structural connectivity to define a region that can currently only be defined by functional activity, the fusiform face area (FFA). Diffusion-weighted data were acquired from 13 healthy subjects (mean age=21, 5M:8 F) using echo planar

imaging (64 slices, voxel size 2x2x2mm, 128x128 base resolution, diffusion weighting isotropically distributed along 60 directions, b-value 1000s/mm2). Automated cortical and subcortical parcellation was performed to define specific cortical and subcortical regions in each individual's T1 scan which were then registered to each individual's diffusion images, and used as the seed (fusiform gyrus) and target regions for fiber tracking. Probabilistic diffusion tractography was carried out using FSL-FDT with 25000 streamline samples. Each fusiform voxel was assigned a probability of connectivity to each target region. We also acquired event-related fMRI data while the same subjects viewed face and scene stimuli. Each subject's functional image for the contrast of face>scene was registered to his/her diffusion-weighted image. We built a regression model using leave-one-out cross-validation (LOOCV): the model was trained to predict the functional T-value (face>scene) for each fusiform voxel based on connectivity data in 12/13 subjects, and tested using the remaining subject's data. Our results indicate that, by using only connectivity probability, we were able to successfully identify face-selective voxels and dismiss scene-selective and non-selective voxels in 11 out of 13 subjects' fusiform gyri. We also compared our model's predictions against the functional activation predicted from a LOOCV group analysis of faces>scenes generated in normalized space (SPM-8 EPI template, random-effects test). The predictions from the group analysis were accurate in only 5 out of the 13 subjects. The predictions based on connectivity were closer to the actual T-values than those predicted by a group analysis in all but 1 subject. We demonstrate here the ability to predict functional activation from structural connectivity. This method will not only inform researchers of the structure-function relationship of functional brain regions, but will also be useful for studying populations who are ill-suited for long anatomical scanning or fMRI, such as infants, lower functioning individuals with autism, or other individuals who are unable to undergo brain imaging without anesthesia.

Disclosures: Z.M. Saygin, None; **D.E. Osher**, None; **R.R. Saxe**, None; **J.D.E. Gabrieli**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.8

Topic: D.04. Vision

Support: NIH EY016464

Title: Early vs. late components of category selectivity in the parahippocampal place area: A rapid acquisition fMRI study

Authors: *S. BOUVIER, R. EPSTEIN;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Complex patterns of category selectivity have been demonstrated in several visually-responsive cortical regions. For example, the parahippocampal place area (PPA) responds more strongly to scenes than to other visual stimuli, and it also shows preferences among non-scene stimuli, responding more to buildings than to other objects. We hypothesized that these different aspects of category selectivity might be attributable to early (feedforward) vs. late (recurrent) processing, and thus might impact PPA response at different points in time. To test this, we scanned subjects using a rapid acquisition fMRI protocol (TR = 250 ms) while they viewed four types of images (duration 500 ms; ISI 3 s) in a 2 x 2 design. Images had a large central foreground item that was either a building or a non-building object; independently, these objects were either presented on a scenic background or in isolation with no background. To compute the influence of scenic components, we subtracted responses to stimuli without backgrounds from those with scenic backgrounds. To compute the influences of foreground item category, we subtracted responses to non-building object stimuli from responses to building stimuli. Response differences related to foreground item category (building vs. non-building) were significantly delayed (550 ms) compared to response differences related to the presence or absence of scenic backgrounds. We hypothesize that the delayed enhancement of activity for buildings relative to non-building objects may reflect feedback from other cortical regions that identify foreground object category; in this case, enhancing PPA response when the foreground object has navigational relevance. In contrast, scene vs. non-scene discrimination may occur more quickly because it operates on feed-forward calculations intrinsic to the PPA.

Disclosures: S. Bouvier, None; **R. Epstein**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.9

Topic: D.04. Vision

Support: NIH Grant EY15000

Stanford Bio-X Graduate Student Fellowship

Title: Abstracting visual information: Reading dynamic word forms

Authors: *A. M. RAUSCHECKER, R. F. BOWEN, L. M. PERRY, A. M. KEVAN, R. F. DOUGHERTY, B. A. WANDELL;
Med. Scien Training Program, Stanford Univ., STANFORD, CA

Abstract: Intro: Humans are highly practiced at reading words defined by contours. Yet, we can also read words defined by less typical stimulus cues, such as motion contrast. How are words represented by these different features routed through cortex? We measured responses in multiple visual areas, including the visual word form area (VWFA), to words defined by three types of stimulus cues: contours, luminance, and motion. We measured responses in the VWFA and distinct visual areas to understand the change in the pattern of activation as we varied the basic features of these letter strings.
Methods: In an event-related fMRI experiment, we asked subjects to make a lexical decision (word/pseudoword) on letter strings defined by contours, luminance (black dots inside the word, white dots outside the word), or motion (dots move right inside the word, dots move left outside the word). We manipulated the visibility of words by changing the amount of noise in the stimulus cue. For contour-defined words, this meant scrambling the phase of the image; for words defined by dots (luminance or motion), we varied the coherence of the dot signals. In each subject, we defined early visual areas (V1-V4) by retinotopic mapping, and we defined areas hMT+ and VWFA by independent functional localizers. During the main experiment, we measured the response in each of these areas to increasing levels of visibility for letter strings defined by different stimulus cues.
Results: Using contour-defined words, the VWFA response increased with word visibility, as shown previously (Ben-Shachar et al., 2007). Using motion contrast or luminance cues, the VWFA response increased with word visibility in a similar fashion, suggesting that the VWFA response is sensitive to word stimuli regardless of the basic visual cues used to define them. Human MT+ responses increased with word visibility, but only for motion defined stimuli. The responses in V1 did not increase with word visibility for any stimulus cue.
Conclusions: The VWFA responds to visual word stimuli defined by contour, luminance, and motion. Such cue invariance suggests that letter string in many forms, not only in the overlearned contour-defined form, engage the VWFA. These results support the hypothesis that the VWFA receives signals about visual word forms from early visual areas irrespective of the stimulus defining features. Just as the lateral occipital complex as a whole may have a special role in processing visual objects (Grill-Spector et al., 1998), the VWFA may be at the interface between perception and language. It extracts stimulus information of any kind that is related to letter strings by receiving relevant information from multiple visual areas.

Disclosures: A.M. Rauschecker, None; **R.F. Bowen**, None; **L.M. Perry**, None; **A.M. Kevan**, None; **R.F. Dougherty**, None; **B.A. Wandell**, None.

Nanosymposium

226. Extrastriate Cortex: Functional Organization I

Location: Room 33C

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 226.10

Topic: D.04. Vision

Support: Human Frontier Science Program, RGY0080/2008

NSERC, 375457-09

Title: Retinotopically organized resting-state functional connectivity within and between areas of the human visual cortex

Authors: *K. CHA[1,2], L. B. LEWIS[3], F. CARBONELL[1], J. D. MENDOLA[3], A. SHMUEL[1,2,4]; [1]Montreal Neurolog. Institute, Dept. of Neurol. and Neurosurg., [2]Program in Neurosci., [3]McGill Vision Research, Dept. of Ophthalmology, [4]Dept. of Biomed. Engin., McGill Univ., Montreal, QC, Canada

Abstract: Recent fMRI studies demonstrated resting-state functional connectivity (RS-FC) which has been used to group cortical areas to functional networks. It has been hypothesized that RS-FC is mediated by thalamo-cortical and cortico-cortical connections. However, the functional specificity of inter-areal RS-FC remains unknown. Here we aimed at testing whether **RS-FC within and between areas of the human visual cortex are retinotopically organized.**
Eight subjects were scanned at 3 Tesla. Visual areas V1, V2, V3, V3A, V3B, V4, LO1, LO2 and V5/MT were defined according to retinotopy. The resting-state time series were band-pass filtered (0.01-0.1 Hz cut-off). The first principal component, which accounted for most of the variance in the global average, was removed. Visual-area and eccentricity specific time-courses were integrated over sub-area eccentricity-based ROIs. RS-FC measures between all pairs of ROIs were obtained by computing the correlation coefficients between the corresponding time-courses.
Prior to removing the global average signal, all visual areas showed highly correlated activity (r=0.40±0.07). Following the removal of the global average signal, most pairs of visual areas remained positively correlated (r=0.16±0.08). Pairs of spatially adjacent areas, such as V1-V2, V2-V3, V3-hV4, V3B-LO1 and LO1-LO2 showed high correlations. Although inter-areal correlations within hemispheres were stronger, the patterns of the RS-FC between areas prevailed across hemispheres. ROIs in lower areas, such as V1 and V2, showed high correlations only with ROIs in proximal areas of the hierarchy, such as V3 and V4. In contrast, ROIs from higher visual areas correlated broadly with other areas. Within area RS-FC decreased with increasing eccentricity difference between the ROIs: ROIs of central and intermediate eccentricity within an area and one hemisphere showed higher correlations compared to the corresponding ROIs of central and peripheral eccentricities. Correlating sub-regions of a visual area with sub-regions from other areas revealed that RS-FC is graded according to retinotopic layout: the closer in visual field the representations of cortical regions are, the stronger the RS-FC between them. V5/MT was more correlated with peripheral regions from other areas, while LO1 and LO2 were more correlated with central regions from other areas.

These findings demonstrate within-area and inter-areal, inter-hemispheric, retinotopically-specific patterns of RS-FC. We expect that, to a first approximation, these RS-FC measures reflect network specificity and functional organization of the anatomical connections between visual areas.

Disclosures: K. Cha, None; **L.B. Lewis**, None; **F. Carbonell**, None; **J.D. Mendola**, None; **A. Shmuel**, None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.1

Topic: D.18. Brain-Machine Interface

Support: EU Grant Presenccia

EU Grant Renachip

EU Grant Synthetic Forager

Title: A brain-computer interface system for the real-time analysis of position

Authors: *C. GUGER[1], T. GENER[2], G. EDLINGER[1], S. BERMUDEZ I BADIA[3], P. VERSCHURE[3], M. SÁNCHEZ VIVES[2];
[1]G.Tec Guger Technologies OEG, Schiedlberg, Austria; [2]IDIBAPS, Barcelona, Spain; [3]SPECS, UPF, Barcelona, Spain

Abstract: Brain-computer interfaces are using the EEG, the ECoG and activity of action potentials as inputs to analyze brain activity for communication purposes and/or the control of external devices. The major difference of the three concepts is their spatial resolution. Surface EEG provides a spatial resolution of several cm, ECoG of several mm and neural spikes of several μm. EEG and ECoG based BCI systems use mostly motor imagery and evoked potentials. BCI systems based on spikes use action potentials from the primary motor area that allow e.g. rats or monkeys to control robotic devices. Thus far it is not know whether a BCI system can be developed that utilizes the states of more central structures. In order to address this question we used the activity of hippocampal place cells to predict the position of an animal in real-time.
Spike activity from the hippocampus was recorded and analyzed in real-time. The rat was

running in a box of 80x80 cm and the movement was captured with a video tracking system. First data was acquired to calculate the rat's trajectories and to identify place fields. Then a Bayesian classifier was trained to predict the position of the rat given its neural activity. This information was used in a subsequent trial to predict the rat's position in real-time.
The real-time experiments were successfully performed with 2 rats and yielded an error between 13 and 27 % using 4-5 neurons. The experiment showed successfully that the position reconstruction can be done in real-time and that the place cells were stable enough to generalize from the training run to the real-time reconstruction experiment. The performance depends mainly on the number of cells, the firing rate and the data window length.
For a BCI application based on place cells it has to be assumed that a rat could be trained in a closed loop experiment to modulate its own place cell activity. In a first run the rat learns its environment and has particular patterns of place cell activity at various places in the arena. We ask whether if the animal would only receive a rewarding stimulus when it visits a particular position in space where a specific place cell fires it would enhance the activity of this place cell. It has to be tested if the rat is able to modulate voluntarily this special place cell firing.
The closed loop experimentation and analysis system proposed here allows to evaluate the quality of place cell recordings in real-time and helps to reduce the recording time. Furthermore, it can be used to change e.g. arena properties during the recording to investigate changes of the place fields. As such it introduces a new range of closed loop systems for the study of cognition and behavior and its neuronal substrate.

Disclosures: **C. Guger:** Employment; g.tec Guger Technologies OEG. **T. Gener:** None. **G. Edlinger:** g.tec Guger Technologies OEG. **S. Bermudez i Badia:** None. **P. Verschure:** None. **M. Sánchez Vives:** None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.2

Topic: D.18. Brain-Machine Interface

Support: Land-Sachsen-Anhalt Grant MK48-2009/003

NINDS NS21135

Title: Comparing the information content of EEG, MEG and ECoG signals in a finger movement task

Authors: *F. QUANDT[1,2], C. REICHERT[1], H. HINRICHS[1], S. DÜRSCHMID[1], E. F. CHANG[3], R. T. KNIGHT[2], J. W. RIEGER[2,1];
[1]Dept. of Neurol., Otto-von-Guericke-University, Magdeburg, Germany; [2]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA; [3]Univ. of California, San Francisco, CA

Abstract: It is a widely debated issue in Brain Machine Interface (BMI) research which recording technique is most suitable to decode different brain states for control of external devices e.g. a neuroprosthesis. To address this question, we designed a finger-tapping task and compared the information content of the brain signal, related to individual finger movements, between non-invasive 32-channel EEG, 248-sensor MEG and in invasive ECoG recordings. The objective was to accurately decode on a single trial basis which finger was moved by the subject. Subjects were instructed to briefly press a button using the thumb, the index, the middle, or the little finger in response to a visual cue. A one-vs-rest linear Support Vector Machine (SVM) was trained to separate the four different finger classes. We used either the down-sampled and low-pass filtered time series or different frequency bands as input for the SVM. Depending on the recording modality we found a significant difference in performance across the two types of features.
The results show that the classification of single movements in each of the four fingers was well above the chance level of 25% (95% confidence interval from permutation test: 28%), for both invasive and non-invasive recordings. The classifiers trained on the non-invasively recorded signals performed best on the raw time series. On the data of our 13 MEG subjects we reached a mean decoding accuracy of 57%. The results of the scalp EEG data were less robust (average accuracy across blocks: 43%). As opposed to the non-invasive approaches, the ECoG signal showed reliable high gamma activation in single trials over motor related brain areas. The information content of those activations was sufficient to separate the brain signal of the four fingers and yielded the highest prediction rates of 80%, and 60% in our two subjects.
In conclusion, we show that it is possible to use non-invasively and invasively measured brain activity to reliably decode in single trials which finger was moved. Furthermore, the high gamma band, which appears to be more somatotopically organized than the lower frequency bands, is highly informative for decoding in the ECoG signal, but not in the single trials of the MEG data. Even though, in general the non-invasive recordings are thought to hold less information than ECoG recordings for decoding different finger movements, the MEG signal proved to be quite characteristic for the four different fingers. This demonstrates that the optimal recording method and its information content for a Brain Machine Interface can be specific for the executed task.

Disclosures: F. Quandt, None; **C. Reichert**, None; **H. Hinrichs**, None; **S. Dürschmid**, None; **E.F. Chang**, None; **R.T. Knight**, None; **J.W. Rieger**, None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.3

Topic: D.18. Brain-Machine Interface

Support: EU project BrainAble ICT-2010-247447

Title: A hybrid brain-computer interface based on motor imagery and steady-state visual evoked potentials

Authors: *C. BRUNNER, B. Z. ALLISON, C. ALTSTÄTTER, C. NEUPER;
Inst. for Knowledge Discovery, Graz Univ. of Technol., Graz, Austria

Abstract: A brain-computer interface (BCI) is a communication channel that does not rely on activity from peripheral nerves or muscles. Instead, different brain signals are measured and used directly, such as event-related desynchronization (ERD), steady-state visual evoked potentials (SSVEPs), P300 or slow cortical potentials. Unfortunately, none of these approaches work for all users. This study compares two BCI approaches (ERD and SSVEP) within subjects, and also evaluates a novel hybrid BCI based on a combination of these signals.
We recorded data across three conditions in a randomized order. In the first condition, subjects imagined moving their hands or feet (ERD). In the second condition, subjects focused on one of two flickering lights (SSVEP). In the third condition, subjects simultaneously performed both tasks. We analyzed the data online with logarithmic band power features at frequencies consistent with ERD and SSVEP activity, and subjects received online feedback based on their performance. Subjects also completed questionnaires.
All subjects could simultaneously perform the imagined movement and visual tasks even though they had no training. All subjects showed both SSVEP and ERD activity during the hybrid BCI condition. Subjects' performance in the hybrid condition was not significantly different from the SSVEP condition, but the ERD condition was significantly worse than either the SSVEP or the hybrid condition. Subjects generally considered the hybrid condition moderately more difficult, but all of them were able to successfully complete the hybrid task.
Results support the hypothesis that subjects who do not have strong ERD activity might be more effective with an SSVEP BCI, and suggest that SSVEP BCIs are less prone to illiteracy. A simultaneous hybrid BCI is feasible, although the current hybrid approach, which involves combining ERD and SSVEP in a two-choice task to improve accuracy, is not significantly better than a comparable SSVEP BCI.
Switching to an SSVEP BCI could increase reliability in subjects who have trouble producing the EEG activity necessary to use an ERD BCI. On the other hand, subjects who are able to produce strong ERD patterns could switch to an ERD BCI, because the flickering lights can be annoying. Subjects who are proficient with both BCI approaches might be able to combine these approaches in different ways and for different goals.

Disclosures: C. Brunner, None; **B.Z. Allison**, None; **C. Altstätter**, None; **C. Neuper**, None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.4

Topic: D.18. Brain-Machine Interface

Support: EU FP7 Project BrainAble, Project Number 247447

EU FP7 Project Future BNCI, Project Number 248320

Title: Toward a multidimensional "hybrid" BCI based on simultaneous SSVEP and ERD activity

Authors: *B. Z. ALLISON[1], C. BRUNNER[1], S. GRISSMANN[2], C. NEUPER[2];
[1]Tech. Univ. of Graz, Graz, Austria; [2]Dept. of Psychology, Karl Franzens Univ., Graz, Austria

Abstract: Hybrid Brain-Computer Interface (BCI) research is a principal interest in our group at the Laboratory of Brain-Computer Interfaces. A hybrid BCI is a BCI combined with another interface, and we focus on "pure" hybrid BCIs that combine two BCIs. These two BCIs can operate sequentially and/or simultaneously. While TUG has developed sequential hybrid BCIs, we focus here on hybrid BCIs that combine two BCI approaches simultaneously.
We first conducted an offline study with three conditions. All three conditions involved trials in which the user was cued with a left or right arrow. In the "ERD only" condition, these cues instructed the subject to imagine moving the left or right hand. In the "SSVEP only" condition, these cues instead instructed the subject to focus on a left or right flickering LED. In the "hybrid" condition, these cues instructed the subject to imagine left/right hand movement and focus on the left/right LED. Results from 14 subjects showed that some subjects had poor ERD or SSVEP data, indicating they would be unable to attain effective control with either approach. However, data from the hybrid condition suggested that all subjects would attain accurate control in the hybrid condition. Hence, the hybrid approach can improve accuracy relative to a conventional "simple" BCI, and this improvement could make the difference between a frustrating failure and an effective communication system. Later work further confirmed that subjects did indeed generate both SSVEP and ERD activity during the hybrid condition, and identified other opportunities for improving classification accuracy.
We then tested an online version of this hybrid BCI approach with realtime feedback. Results showed that subjects could simultaneously control SSVEP and ERD activity in a BCI. This was also the first online study to compare SSVEP vs. ERD BCIs within subjects. SSVEP BCIs were more accurate, but questionnaires showed that some subjects considered the flickering lights in our SSVEP approach annoying. These results could help identify the best BCI for each user.

In current work, subjects trained to use SSVEP activity to control a cursor in the horizontal dimension while simultaneously using ERD activity to move the cursor up and down. Performance differed considerably across subjects. We report on performance with different 2 dimensional tasks, improvement during training, and subjective report based on questionnaires.

Disclosures: B.Z. Allison: Research Grant; EU FP7 Project BrainAble, Project Number 247447, EU FP7 Project Future BNCI, Project Number 248320. **C. Brunner:** None. **S. Grissmann:** None. **C. Neuper:** None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.5

Topic: D.18. Brain-Machine Interface

Title: Connectivity-based parcellation of human thalamus and its relation to electrode position in deep brain stimulation for essential tremor

Authors: *M. BECKMANN, L. LÄER, A. GHARABAGHI;
Universitätsklinik für Neurochirurgie, Tübingen, Tübingen, Germany

Abstract: Thalamic ventral intermediate nucleus (VIM) has successfully been used as a target for deep brain stimulation (DBS) in patients suffering from Essential Tremor (Zhang et al., J Neurosurg, 2009). Pre-surgical targeting has, however, proved difficult due to the homogeneity of human thalamus on conventional MR images. Standard indirect target estimation, on the other hand, cannot account for individual anatomical or connectional differences. DTI studies of human thalamus have used attempts requiring the definition of target regions prior to analysis (Behrens, Nat Neurosci, 2003; Yamada, AJNR, 2010). Here, we evaluate the possibility of identifying thalamic subregions of distinct anatomical connectivity using a user-independent a-priori diffusion tractography-based parcellation approach. Secondly, we reconstruct electrode position from post-surgical MR images.
Methods: T1 and diffusion-weighted images were acquired in five patients undergoing DBS surgery for Essential Tremor (3T Siemens TimTrio, 64 diffusion directions, b=1500 s/mm2, 2.5mm isotropic resolution). Image analysis and electrode reconstruction used FSL and Amira, respectively. Multi-slice 3D thalamus masks were drawn on each patient's T1 brain scan and served as seeds for multi-fibre probabilistic tractography. Probability of connection between each thalamic seed voxel and the rest of the brain was stored in a connectivity matrix. Subsequently, k-means clustering was used to group seed voxels of similar connectivity profiles (Klein et al.,

Neuroimage, 2006). Finally, electrode position was reconstructed from the artifact observed on post-surgical T1 MR images.
Results and Discussion: We reliably identified seven subregions of distinct connectivity profiles within each individual's thalamus. With no prior knowledge of possible connection targets several lateral clusters in anterior-posterior sequence were discriminated, resembling known histological layout (Morel, 2007). This fast and user-independent connectivity-based approach might prove promising for future use in DBS targeting.

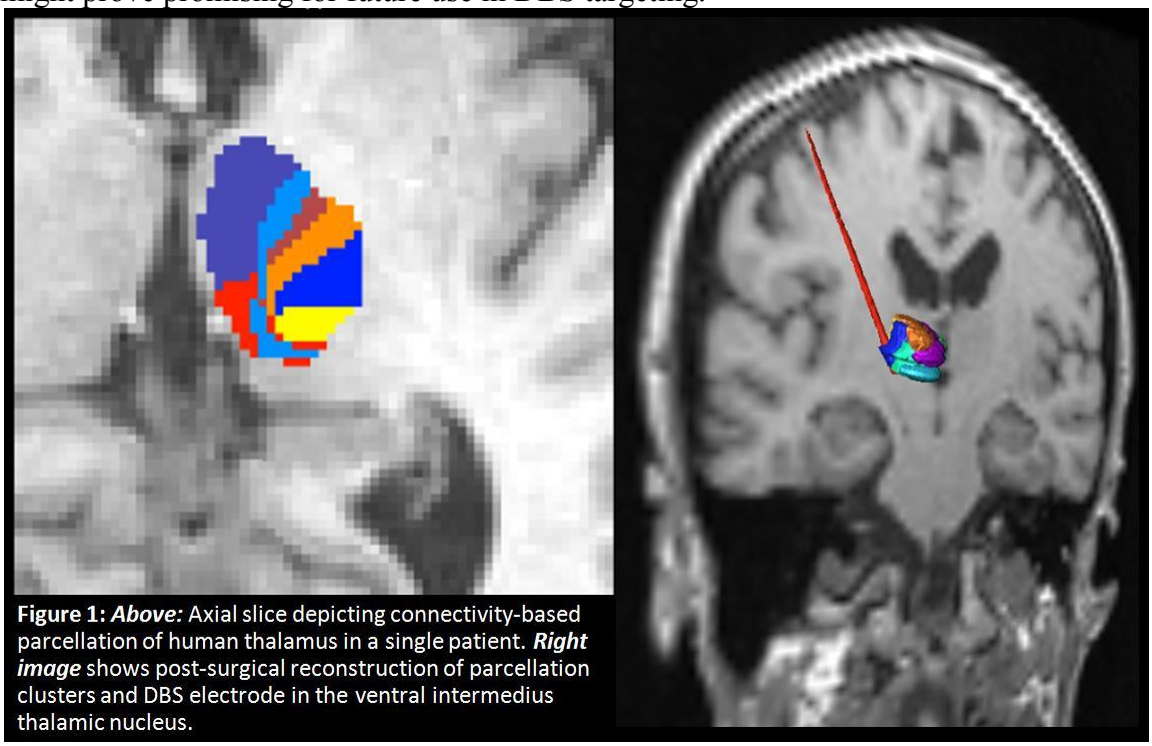


Figure 1: ***Above:*** Axial slice depicting connectivity-based parcellation of human thalamus in a single patient. ***Right image*** shows post-surgical reconstruction of parcellation clusters and DBS electrode in the ventral intermedius thalamic nucleus.

Disclosures: M. Beckmann, None; **A. Gharabaghi**, None; **L. Läer**, None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.6

Topic: D.18. Brain-Machine Interface

Support: NIH NINDS, 1RC1NS068396-0110

NIH NIBIB, P41 EB002030

Title: In vivo chronic cortical recordings using novel ultra-small carbon fiber based implantable microthread ultramicroelectrodes

Authors: *T. D. KOZAI[1], N. B. LANGHALS[1], P. R. PATEL[1], X. DENG[2], H. ZHANG[2], J. LAHANN[2], N. KOTOV[2], D. R. KIPKE[1];
[1]Biomed. Engin., [2]Chem. Engin., Univ. of Michigan, ANN ARBOR, MI

Abstract: Carbon fiber microelectrodes have been extensively used in the literature to record electrical and chemical neural activity. However, in chronic *in vivo* experiments their use has been limited by the electrode size necessary to achieve a sufficient signal-to-noise (SNR) ratio. An additional limitation has been the footprint of the insulation layer which typically consists of a large stiff glass or fused silica layer. Computer models and experimental studies of the probe-tissue interface suggest that flexible probes may help to minimize mechanical trauma caused by physiological motion between the probe and surrounding tissue. New fabrication techniques have also allowed advanced architectures with sub-cellular sized features demonstrating smaller reactive tissue response. Lastly, functional bio-coatings and anti-biofouling coatings, such as poly(ethylene glycol) (PEG), have shown promise of improving chronic neural interfaces. Here we have developed a multidisciplinary innovative strategy that uses leading-edge biocompatible polymers to make ultra-small (~4μm radius) neural probes that are flexible, yet durable, and that have advanced bioactive capabilities for controlling intrinsic biological processes.
Microthread electrodes (MTEs) were prepared with 3.5 μm radius carbon fibers coated with an 800nm insulation layer of parylene-N via chemical vapor deposition and then grafted with a 15 nm layer of functionalized parylene and PEG for improved biocompatibility. A 38.5 μm² carbon electrode site was exposed by cutting the parylene coated carbon. Poly(3,4-ethylene dioxythiophene) (PEDOT) with a poly(4-styrenesulfonate) (PSS) counterion was electrochemically deposited onto the exposed carbon to decrease recording site impedance. Presence of PEDOT/PSS was confirmed through cyclic voltammetry, electrochemical impedance spectroscopy, and scanning electron microscopy.
In vivo cortical recordings with PEDOT deposited, parylene insulated carbon fibers were able to record neural spikes in rat motor cortex. In all *in vivo* trials, the PEDOT coated MTEs were able to detect at least one neuronal action potential with an SNR greater than 1.1 up to 5.1. Conversely parylene insulated carbon fibers with a solely cut carbon exposed recording site were unable to record any discernable units. However, both variants of the MTEs were able to record local field potential (LFP) activity. Lastly, an uninsulated carbon fiber implanted into the cortex was able to record LFPs, but unable to discriminate any single unit spikes. Initial chronic *in vivo* recordings suggest the stability of these devices to record neural signals with sufficient SNR over time.

Disclosures: T.D. Kozai: None. **N.B. Langhals:** None. **P.R. Patel:** None. **X. Deng:** None. **H. Zhang:** None. **J. Lahann:** None. **N. Kotov:** None. **D.R. Kipke:** Ownership Interest; Neuronexus Technologies.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.7

Topic: D.18. Brain-Machine Interface

Support: NIH Grant R01NS055312

Title: Assessment of long-term neural recordings from rodents using MEMS based moveable microelectrodes

Authors: *A. SRIDHARAN[1], N. JACKSON[1], S. ANAND[1], J. SUTANTO[1], M. OKANDAN[3], J. MUTHUSWAMY[2];
[1]Sch. of Biol. & Hlth. Systems Engin., [2]Arizona State Univ., Tempe, AZ; [3]MEMS Sci. and Technol. division, Sandia Natl. Labs., Albuquerque, NM

Abstract: MEMS (Micro-electromechanical systems)-based moveable microelectrodes represent a unique technology to chronically sample neural signals in a spatiotemporal manner in vivo within the rodent brain. In this study, three independently-controlled microelectrodes are placed in an array with a typical interelectrode distance of 800 μm. The microelectrodes are independently moved in discrete microscale steps using either integrated electrothermal or geared electromechanical actuators that have been micromachined using the SUMMiTVTM (Sandia's Ultraplanar Multi-level MEMS Technology) process. In this study, the performance of these moveable MEMS based microelectrodes in long-term, chronic experiments in adult, male, Sprague Dawley rats is assessed. Multi-unit data was recorded in all of the experiments. Signal quality was assessed by determining the signal-to-noise ratio (SNR) and peak-to-peak amplitudes. Microelectrodes were moved at a rate of ~10μm/s when the average SNR of the multi-unit data was ≤ 10 dB. Spikes from single units in neural recordings were distinguishable above an SNR of 12 dB. The spike amplitudes for the first three weeks after implantation were 30-400 μV with an average RMS noise amplitude of 3 μV. Movement of microelectrode using electrothermal actuators significantly increased the average SNR from 14.61 ± 5.21 dB to 18.13 ± 4.99 dB during the first three weeks. Movement of microelectrodes beyond 3 weeks led to a significant increase in SNR from 11.88 ± 2.02 dB to 13.34 ± 0.919 dB in six of eleven instances. The average peak-to-peak signal amplitudes showed >100% improvement for the first three weeks with a modest 20% improvement beyond three weeks for up to 50 days post-implantation. The primary mode of failure in all devices was due to extensive tissue in-growth under the dental cement PMMA mount, leading to eventual biological rejection. In three devices, neural recordings were obtained for at least 76 days post implantation and strong neural spikes were observable in one device for up to 83 days. Current strategies to prevent biological rejection include modifications to surgical techniques such as periodic debridement of the skin tissue, novel device mounting strategies, and miniaturization and efficient packaging of the MEMS

based microelectrode array to reduce the device footprint and total implant weight.We conclude that moving the microelectrodes consistently improved the deteriorating neural signal quality in long-term experiments and is a viable strategy to sample distinguishable neural signals in long-term, in vivo experiments.

Disclosures: A. Sridharan, None; **N. Jackson**, None; **S. Anand**, None; **J. Sutanto**, None; **M. Okandan**, None; **J. Muthuswamy**, None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.8

Topic: D.18. Brain-Machine Interface

Support: NIH Grant R21EB008582

NIH Grant R01DC009643

NIH Grant R01NS054121

NIH Grant R01NS057287

Title: A novel technology for silicon-based neural prosthetics for the central nervous system

Authors: *M. HAN, K. YADAV, P. S. MANOONKITIWONGSA, V. PIKOV, D. B. MCCREERY;
Neural Engin. Program, Huntington Med. Res. Inst., PASADENA, CA

Abstract: We report on recent efforts by our research team to develop and validate silicon-based neural probe arrays for chronic implantation in the brain and spinal cord that offer an important set of capabilities for neural stimulating and recording. Our arrays are custom-designed for four specific targets: cochlear nucleus, inferior colliculus, subthalamic nucleus, and the sacral spinal cord. These chronic arrays combine the deep reactive ion etching (DRIE) technique and mechanical shaping of the probes' tip region, yielding a mechanically sturdy shank and a sharpened tip to reduce insertion force into the brain and spinal cord. The *in-vivo* longevity of the silicon arrays have been validated in the cochlear nucleus of cats. The overall insulation quality of the chronic device allowed chronically implanted devices to be fully functional for up to eighteen months. The microelectrode sites were electroplated with iridium oxide, which

markedly reduced the AC impedance and increased charge storage capacity by an order of magnitude, compared to the unplated gold sites. Extensive *in-vitro* and *in-vivo* electrochemical measurements were conducted. The functionality of the chronic array has been validated by stimulating in the cochlear nucleus while recording the evoked neuronal activity in the central nucleus of the inferior colliculus. Histopathology analyses of the implanted regions showed inflammation around the probe shanks at a level typical of chronically-implanted penetrating microelectrodes. Time-transient tissue response of rabbit cerebral cortex to DRIE-based thick silicon arrays showed a gradual transition from acute to a chronic status, consisted primarily of a glial sheath around the probe shank tracks after five months. We expect this type of array will find a variety of applications for long-term stimulation and recording in the central nervous system.

Disclosures: M. Han, None; **K. Yadav**, None; **P.S. Manoonkitiwongsa**, None; **V. Pikov**, None; **D.B. McCreery**, None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.9

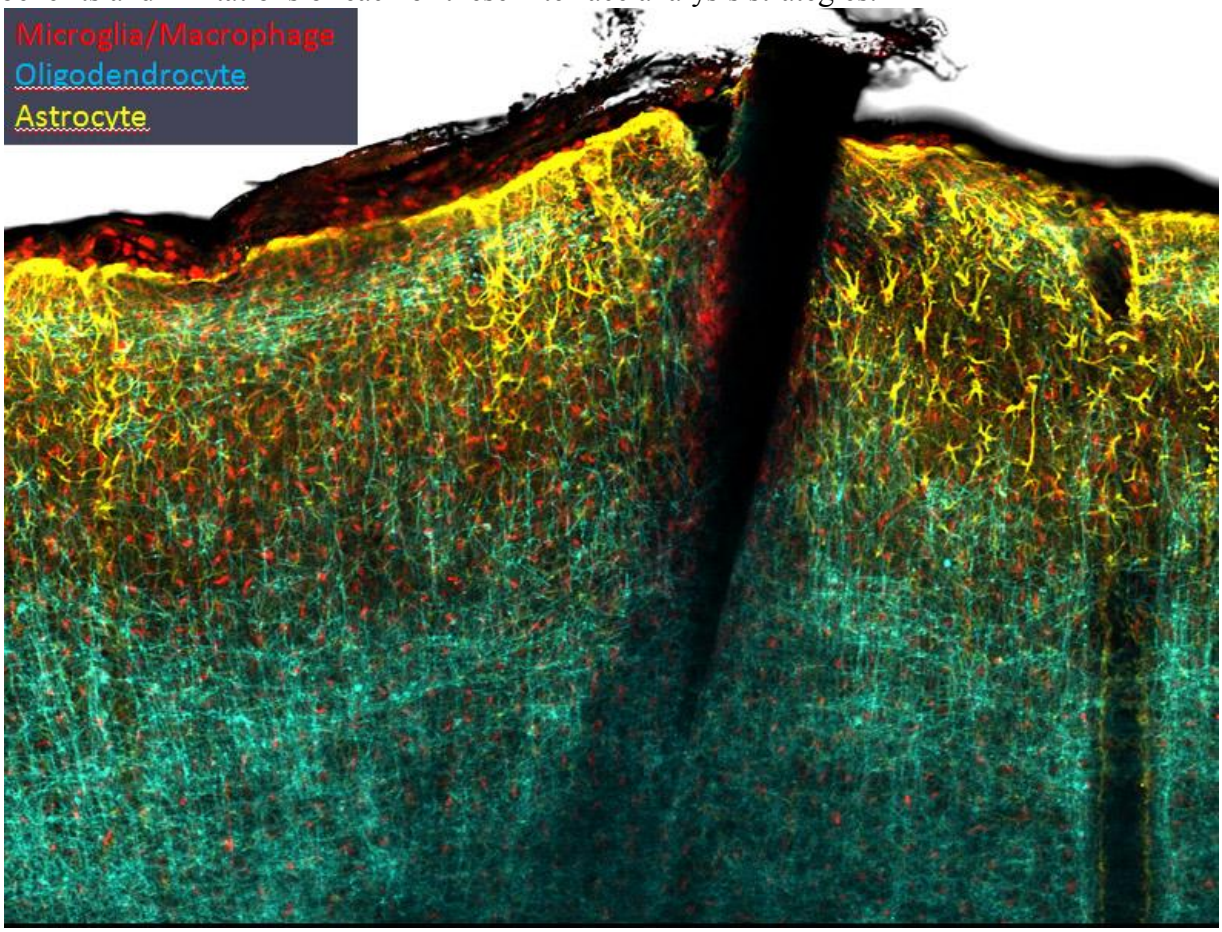
Topic: D.18. Brain-Machine Interface

Title: Characterizing tissue around intracortical microelectrode interfaces using imaging strategies which minimize morphological disruption

Authors: *A. J. WOOLLEY[1,2], H. DESAI[2], M. A. STECKBECK[2], N. PATEL[3], K. J. OTTO[2,3]; [1]Purdue Univ., LAFAYETTE, IN; [2]Biol. Sci., [3]Biomed. Engin., Purdue Univ., West Lafayette, IN

Abstract: Better imaging strategies to assess the progression of glial encapsulation around penetrating intracortical microelectrodes must minimize morphological disruption to provide a clearer understanding of the biological changes affecting physiological recordings over time. These characterization methods could then be employed to closely assess intervention strategies designed to maintain physiological recording at implanted microelectrode sites. We have developed an i*n situ* immunohistochemical analysis method in which electrode arrays are captured and the intact device/tissue interface is imaged, preventing morphological disruption to the tissue caused by explanting devices during histological processing. These fixed, multi-labeled tissue sections preserve depth information along the device and tissue displacement from device insertion, as well as other data vital to clearly interpreting the tissue response. Imaging the

in situ neural interface also allowed close assessment of fluorescently-tagged coatings applied to implanted devices, allowing characterization of coating stability. Finally, contrasting *in vivo* imaging of the neural interface with the *in situ* characterization method allows us to assess the benefits and limitations of each of these interface analysis strategies.



Disclosures: A.J. Woolley, None; **H. Desai**, None; **M.A. Steckbeck**, None; **N. Patel**, None; **K.J. Otto**, None.

Nanosymposium

227. Brain Machine Interface

Location: Room 5B

Time: Sunday, November 14, 2010, 1:00 pm - 3:30 pm

Program Number: 227.10

Topic: D.18. Brain-Machine Interface

Support: INCTSI grant #00014975

NSF CCLI-0728668

Title: Inhibition of MAPKAP Kinase 2-mediated cytokine release to reduce microglial encapsulation of chronic microelectrodes

Authors: N. ONUNKWO[1], A. PANITCH[1], *K. J. OTTO[2];
[1]Purdue Univ., W Lafayette, IN; [2]Purdue Univ., W LAFAYETTE, IN

Abstract: The reactive tissue response to chronically implanted intracortical microelectrodes continues to inhibit the long-term functionality of neuroprostheses. The general degradation in recorded signal quality is partly attributable to the inhibitory environment that is created by cortical injury. Following an injury, inflammatory cytokines are released at the implantation site. This response activates microglia, which migrate to the injury site in an attempt to remove the foreign implant, resulting in device encapsulation. A possible strategy to mitigating the reactive tissue response is to reduce the release of inflammatory cytokines following a brain injury. One pathway that facilitates inflammatory cytokine release is the mitogen-activated protein kinase-activated protein kinase 2 (MK2) pathway, which increases cytokine mRNA synthesis, stability and translation following activation. Therefore, inhibiting MK2-mediated cytokine release can reduce microglial activation, thus mitigating device encapsulation. Through *in vitro* studies, we demonstrate the ability of a cell-penetrating peptide (MK2i) to reduce MK2-mediated cytokine release. *In vitro* experiments modeled a brain injury by treating 7-10 day old E17 cortical tri-cultures (neurons, microglia, and astrocytes) with TNF-α, followed by MK2i treatment. Immunohistochemical stains verified tri-culture cell growth and showed healthy cell morphology following MK2i treatments. ELISA and cytotoxicity assays showed that 0.5, 1 and 3 mM treatment with the MK2i significantly lowers interleukin-6 and interleukin-1β production after TNF-α inflammation and is also non-toxic to cells. Results suggest MK2i may reduce cellular response to indwelling microelectrodes by reducing inflammatory cytokine production, resulting in lowered impedance and more reliable long-term neuronal recordings. Future *in vivo* experiments will also test this hypothesis.

Disclosures: N. Onunkwo, None; **A. Panitch**, None; **K.J. Otto**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.1

Topic: E.05. Stress and the Brain

Support: NIMH Grant MH07908

Title: Characterization of the physiological and genetic phenotype of crf-expressing neurons in the bed nucleus of the stria terminalis

Authors: *D. G. RAINNIE, J.-D. GUO, R. HAZRA, J. DABROWSKA, K. J. RESSLER; Dept Psychiatry, Emory Univ., ATLANTA, GA

Abstract: Corticotrophin-releasing factor (CRF) and its receptors are regarded as a major mediator of an organism's response to unexpected and stressful environmental challenges. Activation of neurons in the bed nucleus of the stria terminalis (BNST) plays a critical role in stress and anxiety-related behaviors. The BNST has a high density of CRF-expressing neurons, suggesting that activation of these neurons may play a critical role in the regulating the stress response. Little is known about the electrophysiological or genetic phenotype of CRF neurons in the BNST due to difficulties in indentifying this cell population in the in vitro slice preparation. We have overcome this obstacle by producing a transgenic mouse line expressing green fluorescent protein (GFP) driven by the CRF promoter. Here, we have combined patch clamp recording, immunohistochemistry, and single cell RT-PCR to examine the electrophysiological properties of CRF neurons in the BNST, and determine their genetic phenotype.
We first used scRT-PCR to validate that all GFP-positive BNST neurons expressed CRF mRNA transcripts. These neurons also co-express mRNA for GAD67, confirming that they are also GABAergic. Patch clamp studies showed that CRF-GFP neurons have a mean resting membrane potential (Vm) of -67.4 ± 0.4 mV, a mean input resistance (Rm) of 337 ± 20 MΩ, and mean time constant (τ) of 26.7 ± 1.6 ms. In response to hyperpolarizing current injection, these neurons exhibit a fast time-independent rectification that was indicative of activation of IK(IR), and a slower time-dependent depolarization which was indicative of activation of Ih. Significantly, the intrinsic membrane properties of CRF neurons in the mouse BNST closely resemble those previously described as Type III neurons in the rat BNST (Hammack et al 2007). In agreement with our physiological results, scRT-PCR studies showed that CRF neurons expressed mRNA transcripts for distinct ion channel subunits, including the IA channel α subunits Kv4.2 and Kv4.3, the Ih channel subunit HCN4, and the K(IR) channel subunits Kir2.1-2.3. Interestingly, we also noticed that the baseline excitatory input onto the CRF-expressing neurons shows a higher amplitude and frequency of spontaneous EPSPs than that observed in non-CRF neurons, suggesting that these neurons are under tighter excitatory control than the neighboring non-CRF neurons. These studies represent the first detailed examination of the physiological properties of central CRF neurons, which in combination with our genetic analysis, provide potential novel targets to modulate the excitability of BNST CRF neurons, and hence anxiety circuits.

Disclosures: D.G. Rainnie, None; **J. Guo**, None; **R. Hazra**, None; **J. Dabrowska**, None; **K.J. Ressler**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.2

Topic: E.05. Stress and the Brain

Support: NIH Grant MH072088 (SEH)

NIH Grant HD27468 (VM and KMB)

NIH Grant NS37179 (VM and KMB)

National Alliance for Research on Schizophrenia and Depression (NARSAD)

Molecular Biology Core Facility at the University of Vermont supported by NIH NCRR P20RR16435

Title: The role of pituitary adenylate cyclase-activating polypeptide (PACAP) in the bed nucleus of the stria terminalis (BNST) in mediating the behavioral changes associated with chronic stress

Authors: C. W. ROMAN[1], K. R. LEZAK[2], M. KOCHO-SCHELLENBERG[2], B. A. GRIMMIG[2], L. K. MICELI[2], W. A. FALLS[2], K. M. BRAAS[1], V. MAY[1], *S. E. HAMMACK[2]; [1]Anat. and Neurobio., Univ. of Vermont, Burlington, VT; [2]Psychology, Univ. of Vermont, BURLINGTON, VT

Abstract: Chronic stress has widespread behavioral effects in rodents including increased anxiety-like behavior and anorexia, and these behavioral changes have been associated with changes in neuroplasticity in specific brain nuclei that mediate emotional behavior. The bed nucleus of the stria terminalis (BNST) has been argued to mediate anxiety-like behavioral responding to long-duration anxiogenic stimuli, and neuroplasticity in this region is increased following chronic stress. Here we describe a series of studies investigating the role of BNST pituitary adenylate cyclase-activating peptide (PACAP) signaling in mediating the behavioral consequences of chronic stress. Chronic variate stress substantially and selectively increases transcript levels of PACAP and its cognate PAC1 receptor in anterolateral BNST tissue punches, and these increases are associated with increased PACAP immunoreactivity in the oval nucleus of the BNST, which is distinct from corticotrophin-releasing hormone (CRH) immunoreactivity in the same BNST subregion. PACAP infusion into the BNST produces an anxiogenic response on baseline acoustic startle responding, and also has a substantial anorexic effect that lasts for 24-hr following infusion. Hence, BNST PACAP infusion is sufficient to produce behavioral changes associated with chronic stress. Moreover, chronic PACAP antagonism using the antagonist PACAP(6-38) delivered via an osmotic minipump directly into the BNST attenuated

the stress-induced anorexia observed during the late phase of the week-long stress paradigm (days 4-7), but not the early phase (days 1-4), suggesting that when stress becomes chronic, the BNST is activated via a PACAP-dependent mechanism. These data suggest that some of the behavioral consequences of chronic stress are mediated by PACAP signaling in the BNST.

Disclosures: C.W. Roman, None; **S.E. Hammack**, None; **K.R. Lezak**, None; **M. Kocho-Schellenberg**, None; **B.A. Grimmig**, None; **L.K. Miceli**, None; **W.A. Falls**, None; **K.M. Braas**, None; **V. May**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.3

Topic: E.05. Stress and the Brain

Support: NIH MH52619

NIH MH065702

Title: Mechanisms of orexin A induced anxiety in the bed nucleus stria terminalis

Authors: *W. A. TRUITT[1], P. L. JOHNSON[2], A. MOLOSH[2], E. LUNGWITZ[3], R. C. DEAL[3], A. D. DIETRICH[3], P. E. MINICK[3], A. SHEKHAR[2];
[1]Anat. and Cell Biol., Indiana Univ. Sch. Med., INDIANAPOLIS, IN; [2]Psychiatry, [3]Anat. and Cell Biol., Indiana Univ. Sch. Med., Indianapolis, IN

Abstract: Panic response is a severe anxiety reaction characterized by sudden onset of anxiety, autonomic activation and escape behaviors. In a recent series of studies, we have demonstrated that the bed nucleus of stria terminalis (BNST) is a pivotal region regulating the anxiety component of a panic response. Furthermore, in rats made vulnerable to panic responses, the release of the neuropeptide orexin (ORX) in the BNST appears to be critical for the anxiety-like behavioral component of a sodium lactate evoked panic responses. Anxiety-like behavior responses to the sodium-lactate challenge were blocked when the ORX 1 receptor antagonist (ORX1r, SB3344867, 300 pmoles/100nl), but not vehicle (0.9% saline/100nl), was infused locally into the BNST prior to the sodium-lactate challenge. Additionally, direct injections of ORX-A into the BNST also increased anxiety in rats that were not panic vulnerable. Specifically, unilaterally injecting ORX A (300 pmol/100nl), but not vehicle (0.9% saline/100nl), into the BNST 30 min prior to testing increased anxiety-like behavior as measured by the social

interaction test. To elucidate the receptor mechanism by which ORX A injections into the BNST induces anxiety behavior, we first determined if the anxiety-like behavior is mediated by the ORX1r. This was demonstrated by unilaterally injecting the ORX1r antagonist (SB3344867, 300 pmoles/100nl) or saline vehicle into the BNST of rats 10 min prior to a subsequent BNST injections of ORX A (300 pmol/100nl) and assessing anxiety-like behavior via the social interaction test 30 min later. Next, considering the multitude of data supporting orexin's regulation of the glutamatergic systems, we also investigated the role of NMDA receptors in the BNST in ORX-induced anxiety. In the BNST, infusions of the NMDA receptor antagonist, AP5 (10 pmol/100nl), but not vehicle, 10 minutes prior to ORX A (300 pmol/100nl) infusion in to the BNST, blocked ORX-A-induced increased anxiety responses as measured by the social interaction test. This dose of AP5 by itself had no effect on social interaction time in the absence of ORX A, suggesting that ORX A effects require NMDA receptor activation. Experiments to determine the putative cellular mechanism by which ORX and NMDA receptors interact to elicit anxiety-like behaviors in the BNST are on going. Supported by RO1s MH52619 and MH065702.

Disclosures: W.A. Truitt, None; **P.E. Minick**, None; **A.D. Dietrich**, None; **R.C. Deal**, None; **A. Molosh**, None; **E. Lungwitz**, None; **P.L. Johnson**, None; **A. Shekhar**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.4

Topic: E.05. Stress and the Brain

Support: NIH Grant AA017668

ABMRF Young Investigator Grant

Title: Stress modulates kappa opioid receptor-mediated inhibition of gabaergic transmission in the bnst

Authors: *T. KASH[1,4], A. M. JIJON[2], C. LI[3];
[1]UNC- Chapel Hill, CHAPEL HILL, NC; [2]Pharmacol., UNC- Chapel Hill, Chapel Hill, NC; [3]UNC- Chapel Hill, Chapel hill, NC; [4]Pharmacol. and Bowles Ctr. for Alcohol Studies, UNC-Chapel Hill, Chapel Hill, NC, NC

Abstract: Strong evidence exists for endogenous stress and anti-stress systems in mammalian

organisms. Chronic exposure to stress is hypothesized to modulate the relative balance of activities of these systems within key circuitry in the brain, leading to dysregulated emotional behavior. The kappa opioid receptor (KOP) and its endogenous agonist, the neuropeptide dynorphin, are one such 'stress' system. Interestingly, dynorphin is expressed in the cell bodies and terminals of the bed nucleus of the stria terminalis (BNST), a brain region associated with anxiety and stress. This suggests that KOP activation in this region may play a role in the regulation of emotional behavior. The impact of KOP activation on synaptic transmission in this region, however, has not been characterized. Using whole-cell voltage clamp recordings in an ex vivo mouse brain slice preparation, we investigated the effect of KOP activation on inhibitory transmission in the BNST. We found that activation of KOP reduced GABAergic transmission. Using converging approaches we provide evidence that this inhibition is mediated presynaptically. We next examined if this form of modulation was influenced by genetic variation and a history of stress exposure. We found that the inhibitory effect of KOP activation on synaptic inhibition was significantly greater in DBA/2J mice compared to C57BL/6J mice. Further, we found that chronic, but not acute restraint, altered KOP modulation in C57BL/6J mice; while both acute and chronic restraint altered KOP modulation in DBA/2J mice. These findings suggest a mechanism by which KOP activation modulates output from the BNST, leading to altered recruitment of BNST targets, such as the lateral hypothalamus (LH), the locus coeruleus (LC), the paraventricular nucleus of the hypothalamus (PVN) and the ventral tegmental area (VTA).

Disclosures: T. Kash, None; **A.M. Jijon**, None; **C. Li**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.5

Topic: E.05. Stress and the Brain

Support: FONDECYT Grant 1070369

Millenium Science Initiative Grant Nº P06/008-F

Title: The bed nucleus of the stria terminalis exerts a CRF1 receptor-mediated excitatory influence on midbrain dopaminergic neurons

Authors: *M. FORRAY[1], A. P. MIRANDA[2], K. GYSLING[3];
[2]Fsrmacia, [3]Biología Celular y Mol., [1]Pont. Univ. Catolica de Chile, Santiago, Chile

Abstract: The bed nucleus of the stria terminalis (BNST) has been implicated in an excitatory regulation of the ventral tegmental area (VTA) dopaminergic neurons. It has also been proposed that corticotropin-releasing factor (CRF) central circuits including the BNST play a mayor role in stress-induced drug seeking behavior. The present study was aimed at evaluating the role of glutamate (GLU) and CRF from the ventral-BNST in mediating activation of VTA dopaminergic neurons in naïve and repeatedly stressed rats. Specifically, we studied whether the activation of the ventral BNST exerts GLU- and/or CRF-mediated excitatory regulation of VTA dopaminergic activity. To this end, we stimulated the ventral-BNST by infusing 0.25 μL of a solution containing GLU (100 μM) and bicuculline (10 μM), and studied GLU and DA extracellular levels in the VTA by *in vivo* microdialysis. To study the participation of CRF1 receptors on this effect we perfused a CRF1 receptor antagonist (NBI27914) through the microdialysis probe located in the VTA. Our results show that local stimulation of the ventral-BNST increases VTA dopamine extracellular levels in both naïve and repeatedly stressed rats, without affecting glutamate extracellular levels. Interestingly, a significantly greater increase of dopamine extracellular levels was observed in repeatedly stressed rats. The effect of ventral-BNST activation on VTA dopamine extracellular levels was blocked by intra VTA perfusion of NBI27914. Thus, our results indicate that ventral-BNST exerts a CRF1 receptor-mediated excitatory influence on DA neurons. Moreover, repeated stress sensitizes the response of VTA DA neurons to ventral-BNST activation.

Disclosures: M. Forray, None; **A.P. Miranda**, None; **K. Gysling**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.6

Topic: E.05. Stress and the Brain

Support: CIHR Grant MOP-79277

The Harry Botterell Foundation

The J.P. Bickell Foundation

Title: D1/Src tyrosine kinases-dependent long-term potentiation at GABA synapses contributes to cocaine reinforcement

Authors: M. KRAWCZYK, X. MASON, R. SHARMA, C. CHIANG, *E. C. DUMONT; Anesthesiol., Queen's Univ., Kingston, ON, Canada

Abstract: The bed nucleus of the stria terminalis (BST), especially its oval (ov) and juxtacapular subregions, receives a robust dopaminergic input from the periaqueducal, retrorubral, and ventral tegmental midbrain areas. Given the critical role of dopamine in motivated behaviours, we investigated whether and how dopamine in the ovBST contributes to the reinforcing properties of natural and pharmacological rewards. We combined behavioural testing of operant responding towards natural and pharmacological rewards, intra-cranial pharmacological manipulations, and brain slices patch-clamp recordings to test the hypothesis that dopamine modulates synaptic transmission in the BST and contributes to the reinforcing properties of cocaine. We observed that dopaminergic modulation of inhibitory transmission in the oval BST switched from a pre-synaptic D2-mediated reduction to a post-synaptic D1-mediated increase in $GABA_A$-inhibitory post-synaptic currents (IPSC) only in rats maintaining cocaine self-administration. This switch was specific to the reinforcing properties of cocaine during the maintenance phase of self-administration since dopaminergic modulation of $GABA_A$-IPSC was normal upon acquisition of cocaine self-administration, maintenance of operant responding for sucrose, or when cocaine infusions were not contingent upon lever pressing (yoke). Furthermore, intra-ovBST D1-dopaminergic receptor blockade inhibition reduced the reinforcing properties of cocaine, but not of sucrose, when rats were tested under a progressive ratio schedule of reinforcement. The change in direction and location of dopaminergic regulation of $GABA_A$-IPSC likely resulted from impaired pre-synaptic D2-mediated inhibition of adenylyl cyclase and *de novo* contribution of post-synaptic D1 receptors. Furthermore, we observed that D1-mediated increase in $GABA_A$-IPSC was sustained, sensitive to the Src-tyrosine kinases inhibitor PP2, but insensitive to G-protein, adenylyl cyclase or phospholipase C inhibition. Thus, altered dopaminergic regulation of inhibitory transmission in the BST specifically contributes in the maintenance of cocaine self-administration in rats, revealing a role in addiction for this neglected mesolimbic dopaminergic pathway of the brain. Furthermore, we observed Src tyrosine kinases-dependent long-term potentiation of $GABA_A$-IPSC by dopamine in cocaine-addicted rats, revealing a novel potential therapeutic target for psychostimulant addiction.

Disclosures: M. Krawczyk, None; **X. Mason**, None; **R. Sharma**, None; **C. Chiang**, None; **E.C. Dumont**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.7

Topic: F.03. Motivation and Emotion

Support: NIH Grant NS 15841

Title: Opposing regulation of norepinephrine and dopamine in the bed nucleus of the stria terminalis of freely moving rats by rewarding and aversive stimuli

Authors: *J. PARK, K. FONTILLAS, R. KEITHLEY, R. M. WIGHTMAN;
Univ. North Carolina, Chapel Hill, NC

Abstract: The bed nucleus of the stria terminalis (BNST), a component of the extended amygdala, regulates both stress and reward-related behaviors. Its subregions, the dorsolateral (*dl*BNST) and ventral BNST (*v*BNST) have dopaminergic terminals and the highest density of noradrenergic nerve terminals in the brain, respectively. Several studies have shown that catecholamine transmission in the BNST plays a critical role in stress responses and in mechanisms related to drug abuse and addiction. Despite this, little is known about how catecholamine signaling in the subregions of the BNST is regulated by aversive and rewarding stimuli due to the small size of this region in the rat brain and technical limitations. To study the involvement of norepinephrine and dopamine signaling, in real time, in the subregions of the BNST, we employed fast-scan cyclic voltammetry (FSCV) with carbon-fiber microelectrodes. The combination of neurochemical, immunohistochemical, anatomical, electrochemical and pharmacological results indicate that the main catecholamine responses monitored in the *v*BNST and *dl*BNST were norepinephrine and dopamine, respectively. In the present study, we investigated the role of the catecholamines in behavioral phenomena (reward and aversion processing). Here, we show for the first time that rewarding and aversive taste stimuli simultaneously evoke opposite patterns of norepinephrine and dopamine activity within milliseconds of presentation in the subregions of the BNST. Aversive stimuli induce activation of noradrenergic neurons and inhibition of dopaminergic neurons while rewarding stimuli inhibit noradrenergic signaling and activate dopaminergic signaling. Therefore, catecholamine input to the BNST reflects oppositional and simultaneous reward and aversion signals, providing insight into the roles of norepinephrine and dopamine in both stress and hedonic responses. In another set of studies using intracranial self-stimulation (ICSS), we demonstrate for the first time that dopamine release in the *dl*BNST was not only evoked by electrical stimulation, but also developed in a time-locked fashion to the associated audio-visual cue. In contrast, norepinephrine release in the *v*BNST was only evoked by the stimulation and did not develop to the cue. The results suggested that norepinephrine is not significantly correlated to cues while dynamic dopamine release in the *dl*BNST during ICSS responds to reward-predicting cues and subsequent behavior. This study will be very helpful in elucidating the role of norepinephrine and dopamine in regulation of different aspects of animal behavior.

Disclosures: J. Park, None; **K. Fontillas**, None; **R. Keithley**, None; **R.M. Wightman**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.8

Topic: C.17. Drugs of Abuse and Addiction

Support: DA019112

NS07491

Title: Orexin receptor antagonists attenuate yohimbine-induced impairment of extinction of cocaine conditioned place preference and excitatory transmission in the bed nucleus of the stria terminalis (BNST)

Authors: *K. L. CONRAD[1], A. DAVIS[1], H. J. G. MATTHIES[1], S. SALEH[2], C. W. LINDSLEY[2], D. G. WINDER[3];
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Abstract: Yohimbine is a widely used pharmacological stressor that is thought to act via its inhibition of noradrenergic autoreceptors (α2-adrenergic receptors, α2-ARs). Recent research has demonstrated that yohimbine 1) impairs extinction of cocaine conditioned place preference (cocaine CPP) in C57Bl/6J as well as α2a-AR knockout mice, 2) impairs extinction of cocaine self administration in rats, and 3) increases alcohol self administration. In acute brain slices containing the bed nucleus of the stria terminalis (BNST), yohimbine has been shown to depress excitatory transmission. However, these effects of yohimbine are intact in α2a-AR knockout mice and not fully mimicked by the more selective α2-AR antagonist atipamezole, suggesting that they are mediated by targets other than α2-ARs. Recent studies using yohimbine have proposed an interaction between the noradrenergic and orexinergic systems. Thus, the purpose of this study was to assess the potential role of orexin receptors in the actions of yohimbine on reward-related behavior as well as synaptic function in the BNST. Wildtype and α2a-AR knockout mice were trained in the cocaine CPP paradigm and the effects of the orexin receptor antagonist, SB-334867, on yohimbine-impaired extinction were investigated. The effects of SB-334867 as well as a newly synthesized specific orexin receptor antagonist, 2-{4-{5-methyl-2-(2H-1,2,3-triazol-2-yl)benzoyl]-1,4-diazepan-1-yl}quinazoline (MTBDQ), on yohimbine-induced depression of excitatory transmission in the BNST were also examined. Our results indicate that SB-334867 attenuated yohimbine-induced impairment of extinction. In addition, both SB-334867 and MTBDQ had no effect on excitatory transmission but blocked yohimbine-induced depression in BNST field potential recordings. Taken together, these results suggest that the orexin signaling system may be a direct target for the actions of yohimbine on reward related behaviors and excitatory transmission in the BNST.

Disclosures: K.L. Conrad, None; **A. Davis**, None; **H.J.G. Matthies**, None; **S. Saleh**,

None; **C.W. Lindsley**, None; **D.G. Winder**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.9

Topic: E.05. Stress and the Brain

Support: MILDT/INSERM/InCA 2006

Title: Nicotine self-administration but not passive nicotine infusion triggers LTP in vivo

Authors: *F. E. GEORGES[1], M. CADOR[2], O. MANZONI[1], S. CAILLÉ[2];
[1]CRI U862, INSERM, BORDEAUX, France; [2]Mouvement-Adaptation-Cognition, Univ. Bordeaux 2; CNRS UMR 5227, Bordeaux, France

Abstract: A current hypothesis is that the compulsive motivation for a drug or a natural reinforcement induces stable cellular changes similar to those involved in learning and memory processes. Motivated behaviors are largely mediated by the extended amygdala and the mesocorticolimbic dopaminergic (DA) systems. In addition, we recently disclosed a potent excitatory cortical influence on the Bed Nucleus Stria Terminalis (BNST), which in turn, projects to the ventral tegmental area (VTA). Altogether, these data suggested a key contribution of the BNST in motivational processes. Here, our aim is to investigate how nicotine, a drug of abuse and a potent regulator of midbrain DA neurons, could alter in vivo synaptic transmission and synaptic plasticity in BNST. Using an in vivo electrophysiological approach, we characterized a physiological form of synaptic plasticity in the BNST in rats trained for the intravenous nicotine, nicotine "yoked" or saline self-administration. Our results indicated that nicotine self-administration in rats, but not passive nicotine delivery, promotes NMDA dependent long-term potentiation (LTP) of cortical synaptic transmission onto BNST neurons projecting to VTA. The present study emphasizes the fact that active versus passive nicotine administration leads to different neuroadaptive modifications in the brain

Disclosures: F.E. Georges, None; **M. Cador**, None; **O. Manzoni**, None; **S. Caillé**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.10

Topic: E.05. Stress and the Brain

Support: MH47840

MH069056

MH080330

NARSAD Young Investigator Award

FACES Fellowship

Center for Behavioral Neuroscience Base Grant

Title: Pharmacological regulation of the bnsts role in sustained startle increases

Authors: *D. L. WALKER, K. SINK, L. MILES, M. DAVIS;
Dept. of Psychiatry and Behavioral Sci., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Previous findings in rats have revealed an important and apparently selective role for the BNST in the expression of sustained but not phasic startle increases to fear- and anxiety-evoking stimuli. Findings from human studies, using similar procedures, have suggested that sustained startle increases (i.e., versus phasic increases) are especially relevant to clinical anxiety, being particularly sensitive to established anxiolytics, and selectively increased in clinically anxious populations (i.e., in panic and PTSD patients - c.f., Neuropsychopharmacology 35:105). Here we describe results from a series of experiments in which we compared the effect of established (chlordiazepoxide, chronic fluoxetine), potential (corticotropin releasing factor receptor antagonist, calcitonin gene-related peptide receptor antagonist), or non-anxiolytic (acute buspirone, acute fluoxetine) treatments on sustained versus phasic startle increases. In general, the results suggest that sustained fear-associated startle increases in rats, as in humans, are more sensitive to established anxiolytics than are phasic increases, and that these increases might therefore be especially useful for screening new compounds for anxiolytic activity and, more generally, for exploring the neural substrates of clinical anxiety.

Disclosures: D.L. Walker, None; **K. Sink**, None; **L. Miles**, None; **M. Davis**, None.

Nanosymposium

228. The Bed Nucleus of the Stria Terminalis: Linking Stress, Addiction, and Affect

Location: Room 1B

Time: Sunday, November 14, 2010, 1:00 pm - 3:45 pm

Program Number: 228.11

Topic: E.05. Stress and the Brain

Support: NIH Grant MH59911

NIH Grant DK063922

Title: Collateralized inputs to the central nucleus of the amygdala (CEA) and bed nucleus of stria terminalis (BNST) in rats

Authors: *M. S. BIENKOWSKI, L. M. RINAMAN;
Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The CEA and BNST are highly interconnected limbic forebrain regions that coordinate behavioral, autonomic, and neuroendocrine responses to stressful stimuli. Afferents to both regions arise from cortex, diencephalon, pons, medulla, and several amygdala nuclei. The similar inputs to both the CEA and BNST support the theory that these limbic forebrain regions comprise a single functional unit known as the extended amygdala. A full characterization of the central sources of collateralized inputs to the CEA and BNST has not been reported, although previous studies have reported dual inputs arising within the posterior basolateral amygdala (BLAp), prelimbic/infralimbic and insular cortices, and ventrolateral medulla (VLM). To more fully analyze the anatomical organization of inputs to the CEA and BNST, we performed a dual retrograde tracing study using iontophoretic injections of Fluorogold and cholera toxin subunit B into the medial CEA (mCEA) and anterior ventrolateral BNST (vlBNST) in adult male Sprague-Dawley rats. One week after tracer injections, animals were perfused with fixative and brain tissue series were processed using immunoperoxidase for single labeling, or dual-immunofluorescence to localize both tracers simultaneously. Single peroxidase labeling revealed an extensive overlap of inputs to the CEA and BNST in alternate tissue sections from the same animals. The number of double-labeled neurons was then quantified in regions with a high degree of tracer overlap. Our results confirm previous observations of collateralized projections to CEA and BNST arising from cortex, BLAp, and VLM. In addition, several previously undescribed collateral projections were seen to arise from areas of the central visceral network, including the nucleus of the solitary tract, parabrachial nucleus, paraventricular thalamus, parasubthalamic nucleus, dorsolateral BNST, and other regions. Interestingly, we also identified regions of high tracer overlap that nonetheless lacked significant double-labeling, i.e., the arcuate nucleus of the hypothalamus. The widespread central distribution of neurons with collateralized inputs to the CEA and BNST supports the view that neural activation in these limbic regions is

modulated in a highly coordinated manner.

Disclosures: M.S. Bienkowski, None; **L.M. Rinaman**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.1

Topic: F.03. Motivation and Emotion

Support: Searle Scholars Program

NIH grant MH080833

NSF grant BCS0542694

Title: Emotional valence and arousal uniquely affect the neural connectivity predicting selective memory

Authors: *J. D. WARING[1,3], E. A. KENSINGER[2,3];
[1]Boston Coll, CHESTNUT HILL, MA; [2]Boston Coll, Chestnut Hill, MA; [3]Athinoula A. Martinos Ctr. for Biomed. Imaging, Charlestown, MA

Abstract: Emotionally arousing visual scenes are remembered better than neutral scenes, but not all portions of emotional scenes are remembered with the same accuracy. Although memory for emotional items is enhanced relative to neutral items within scenes, memory is often impaired for peripheral background information contained within emotional scenes, known as a trade-off effect. In this study, we examined the effective connectivity at encoding predicting a trade-off in memory for scenes containing emotional items compared to predicting successful memory for both item and background. In an event-related fMRI study, young adults viewed scenes containing high and low arousal positive and negative items, and neutral items placed upon neutral backgrounds. Later, outside the scanner, participants completed a surprise recognition memory test, with the items and backgrounds from the scenes presented independently. Subsequent memory design allowed us to measure the neural activity predicting a memory trade-off compared to later remembering both the emotional item and background from the scene. To better understand the pattern of neural activity associated with this effect, we modeled the effective connectivity between regions as a function of the four emotional scene types. Regions included in the anatomical model were identified in a whole-brain analysis comparing the neural

response to viewing all scene types versus a fixation cross, and selected for their theoretical relevance to encoding of emotional information. Results showed significant differences between the effective connectivity predicting a trade-off compared to remembering the item and background within the high and low arousal positive but not negative scenes. The trade-off in high and low arousal positive scenes is predicted by reciprocal negative connections between the amygdala and parahippocampus, and also stronger projections from the fusiform to the amygdala, compared to connectivity predicting remembering the item and its background. In contrast, connectivity between amygdala and anterior cingulate and between the parahippocampus and fusiform predicts memory for negative items across arousal levels, and regardless of whether the background is also remembered. These results suggest there are important differences in the effective connectivity predicting selective memory for emotional scenes depending upon scene valence and arousal characteristics.

Disclosures: J.D. Waring, None; **E.A. Kensinger**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.2

Topic: F.03. Motivation and Emotion

Support: VA Merit Review Award

NIH Grant MH076198

Title: Aberrant threat-sensitive amygdala-frontal interactions in returning combat veterans with ptsd before and after treatment

Authors: C. A. RABINAK[1], *K. D. PHAN[2,1];
[1]Research/Mental Hlth., VA Ann Arbor Healthcare Syst., Ann Arbor, MI; [2]Dept Psychiatry, Univ. Michigan, ANN ARBOR, MI

Abstract:
Background: Dysregulated fear is a core process in the etiology and maintenance of post-traumatic stress disorder (PTSD). Convergent and consistent evidence implicate aberrant amygdala reactivity and its interaction with frontal cortex when processing threat/fear in patients with PTSD. However, two major gaps still exist: 1) Would similar patterns of brain dysfunction exist in individuals recently traumatized by military combat; 2) Would

effective pharmacological therapies ameliorate these functional brain abnormalities?
Methods: Here, we couple functional magnetic resonance imaging (fMRI) with a clinical medication trial with an empirically-validated, first-line, FDA-approved medication - the selective serotonin reuptake inhibitor (SSRI) paroxetine. Thus far, 14 male U.S. veterans with PTSD related to their combat trauma from serving in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) and gender-, age-, SES-matched healthy controls have participated. All participants participate in two complementary tasks which probe amgydala and amygdala-frontal interactions: emotional face (threat [angry/fearful faces]/non-threat [happy faces]) matching and reappraisal-based regulation of negative affect. fMRI is first performed in untreated PTSD veterans and repeated after 12 weeks of paroxetine treatment.
Results: We report here between-group results from ($p<0.01$, $t>2.68$) preliminary fMRI analyses (SPM5, random effects model) using linear contrasts to test task effects and psychophysiological interaction (PPI) analyses to test amygdala-frontal connectivity directly contrasting threat vs. non-threat information on the emotional face matching task in unmedicated PTSD subjects prior to treatment vs. controls. Relative to controls, PTSD patients exhibit exaggerated bilateral amygdala reactivity and reduced ventral orbitofrontal (OFC) responses to social signals of threat. Moreover, patients exhibit less amygdala-OFC coupling than controls when processing threat; controls, but not patients, exhibit bilateral reciprocal interactions between amygdala and OFC. With larger samples, analyses of the emotion regulation task and pre-post treatment change brain function and connectivity are planned.
Conclusions: Disrupted amygdala function and amygdala-frontal interactions in response to social threat appear to mediate the maintenance of pathological anxiety in combat veterans returning from OEF/OIF with PTSD. Effective treatments appear to work by modifying these specific brain-based abnormalities, which could serve as targets to rationally test and refine treatments for this and other anxiety disorders.

Disclosures: C.A. Rabinak, None; **K.D. Phan**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.3

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01 MH080716

NIH Grant F31 MH090672

Title: Anxiety dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest

Authors: *M. J. KIM[1], D. G. GEE[2], R. A. LOUCKS[1], F. DAVIS[1], P. J. WHALEN[1]; [1]Psychological & Brain Sci., Dartmouth Col., HANOVER, NH; [2]Psychology, UCLA, Los Angeles, CA

Abstract: Anxiety is linked to compromised interactions between the amygdala and the dorsal and ventral medial prefrontal cortex (mPFC). Given that anxiety manifests as a sustained psychological state that does not require the presence of an anxiety-inducing stimulus, we hypothesized that anxiety would predict functional connectivity between these brain regions even during rest. Resting state fMRI scans and self-reported measures of anxiety were acquired from healthy subjects. Using a seed-based approach, we computed statistical maps of the brain for each subject, showing voxels that correlated with the intrinsic spontaneous low-frequency signals within the amygdala. At the group level, anxiety measures were included to generate maps of regions that covaried in connectivity with the amygdala. At rest, individuals with high anxiety were characterized by negatively correlated amygdala-ventral mPFC functional connectivity, while low anxious subjects showed positively correlated activity. Further, high anxious subjects showed amygdala-dorsal mPFC activity that was uncorrelated, while low anxious subjects showed negatively correlated activity. These data show that amygdala-mPFC connectivity at rest indexes normal individual differences in anxiety.

Disclosures: M.J. Kim, None; **D.G. Gee**, None; **R.A. Loucks**, None; **F. Davis**, None; **P.J. Whalen**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.4

Topic: F.03. Motivation and Emotion

Support: NIMH Grant 080716

Title: Neural responses to ambiguously valenced stimuli: Effects of explicit vs. implicit task demands

Authors: *M. NETA[1], W. M. KELLEY[1], M. J. KIM[1], D. G. GEE[2], P. J. WHALEN[1];
[1]Dartmouth Coll, HANOVER, NH; [2]Psychology, UCLA, Los Angeles, CA

Abstract: We have recently demonstrated that there is a range of individual differences in the way people interpret the valence of surprised facial expressions. While recent work has focused on the process of resolving ambiguity in the valence of surprised expressions, little is known about whether this processing of ambiguity is specific to face stimuli (surprise), or whether these effects represent a general process in response to valence ambiguity. As such, one goal of the present study was to determine if these effects generalize to other non-face emotional stimuli (i.e., IAPS scenes). A second goal of this study was to directly compare effects in the amygdala during explicit (valence) and implicit (gender) evaluations in response to ambiguous, as compared to clearly valenced stimuli. For both the faces and scenes, half of the stimuli had an ambiguous valence, and the other half were a combination of clearly positive and clearly negative images. We scanned 35 participants, and presented block of faces and blocks of scenes. The task in each block alternated, such that, for the faces, participants either rated the valence ("positive/negative") or gender ("male/female") of each stimulus. For the scenes, they either rated the valence ("positive/negative") or decided whether there was a human present or not in each image. As the manipulation of ambiguity was with respect to the valence of the stimuli, the valence judgment served as our explicit task, while the alternate judgment served as our implicit task.
We found that behavioral responses to ambiguous facial expressions (i.e., surprised) generalize to ambiguous non-face emotional stimuli (i.e., scenes). Further, when examining neural responses to the ambiguous stimuli, we found activity in regions comprising the task network (i.e., cingulo-opercular network) when participants made an explicit (valence) evaluation of each category of stimuli. Conversely, amygdala and prefrontal responses were observed for ambiguous faces during the implicit task. Thus, there is something special about the signals that faces seek to convey and this amygdala-prefrontal circuitry. This may suggest that, though some of the relevant processes that come online in the 'face' of ambiguity are similar across many categories of ambiguity (faces and scenes in the present work, semantic and visual motion ambiguity in previous work), there are other dedicated circuits for some specific biologically-relevant learning situations.

Disclosures: M. Neta, None; **W.M. Kelley**, None; **M.J. Kim**, None; **D.G. Gee**, None; **P.J. Whalen**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.5

Topic: F.03. Motivation and Emotion

Support: NIH grant DA007274

NIH grant MH080716

NSF grant 0746220

Dartmouth Brain Imaging Center

Title: Subcortical and cortical predictors of hypervigilant threat monitoring with greater trait anxiety

Authors: *L. H. SOMERVILLE[1], P. J. WHALEN[2], W. M. KELLEY[2];
[1]Sackler Inst., Weill Cornell Med. Col., NEW YORK, NY; [2]Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Though a key symptom underlying many anxiety disorders is hypervigilant threat monitoring, its biological bases in humans remain poorly understood. Animal models suggest that anxious processes such as hypervigilant threat monitoring are distinct from cued fear-like responses and mediated by the bed nucleus of the stria terminalis (BNST). Here we applied psychophysiological and neuroimaging methodologies sensitive to sustained arousal-based responses to test the role of the human BNST in mediating environmental threat monitoring, a potential experimental model for sustained anxiety symptoms. Healthy participants (n=50) with varying trait anxiety performed an environmental threat-monitoring task during fMRI scanning where a stimulus line continuously fluctuated in height, providing information relevant to subsequent risk for electric shocks. Skin conductance responses (SCRs) were collected in a separate cohort (n=47) to validate task-evoked modulation of physiological arousal. Results indicated that a forebrain region consistent with the BNST showed greater overall recruitment, and exaggerated tracking of threat proximity, in individuals with greater anxiety. The insular cortex and dorsolateral prefrontal cortex (DLPFC) tracked threat proximity across all participants and showed exaggerated threat proximity responding in individuals with greater trait anxiety. Further, the insular cortex showed enhanced recruitment when threat proximity was ostensibly controllable. Taken together, these findings indicate that activity in the BNST, insula, and DLPFC continuously monitor changes in environmental threat level, and also subserve hypervigilant threat-monitoring processes in more highly trait anxious individuals. This work bridges human and animal research informing the role of the BNST in anxious-related processes, and further suggests that continuous fMRI paradigms offer promise in elucidating the neural circuitries supporting sustained anticipatory features of anxiety.

Disclosures: L.H. Somerville, None; **P.J. Whalen**, None; **W.M. Kelley**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.6

Topic: F.03. Motivation and Emotion

Support: NIMH Grant 069315

NIMH Grant 080716

Title: Negative appraisal of ambiguous social cues: Subcortical responses to temporal unpredictability and the influence of genetic background

Authors: *C. DAVIS[1], M. NETA[1], J. KIM[1], A. R. HARIRI[2], P. J. WHALEN[1];
[1]Dartmouth Coll, HANOVER, NH; [2]Duke Univ., Durham, NC

Abstract: The human amygdala is sensitive to facial expressions of emotion, such as fear and anger, which predict negative events. Other expressions, such as surprise, have a variable reinforcement history since they predict positive and negative events. Because of this predictive ambiguity, contextual information has a strong influence on interpretations of surprised expressions. Previous research shows that unpredictable tones elicit enhanced amygdala activity, prime anxiety-like behaviors, and bias individuals toward negativity. Here, we use fMRI and facial electromyography to test whether temporal unpredictability prompts negative interpretations of surprised expressions as indexed by amygdala and corrugator responsivity. Given the importance of genetic background in mediating individual differences in behavioral and neural processing of emotions, we also examined the impact of a functional polymorphism biasing serotonin signaling on amygdala responsivity. We replicated previous findings showing that predictable surprised expressions elicited moderate but sustained amygdala responses. In contrast, unpredictable surprised expressions produced a pattern similar to negatively valenced fearful faces, namely, initially large amygdala responses that habituated. Interestingly, this effect was carried entirely by individuals with a genetic background conferring relatively increased serotonin signaling. Corrugator responses to unpredictable surprised faces were potentiated, similar to clearly negative expressions, while responses to predictable surprised faces were attenuated, similar to clearly positive expressions. The observed patterns of amygdala responsivity and corrugator activity suggest that temporal unpredictability induces an implicit state change that biases people toward negative interpretations of surprised expressions. The genetic data further suggest that individual differences in this bias reflect variability in serotonin signaling.

Disclosures: C. Davis, None; **M. Neta**, None; **J. Kim**, None; **A.R. Hariri**, None; **P.J. Whalen**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.7

Topic: F.03. Motivation and Emotion

Support: NWO grant 451.07.019

NWO grant 918.66.613

Title: Fear bradycardia and activation of the human periaqueductal grey

Authors: *E. J. HERMANS[1,2], M. J. A. G. HENCKENS[1,3], K. ROELOFS[4,5], G. FERNÁNDEZ[1,2];
[1]Radboud Univ. Nijmegen, Donders Inst., Nijmegen, Netherlands; [2]Radboud Univ. Nijmegen Med. Centre, Dept. of Neurol., Nijmegen, Netherlands; [3]Univ. Med. Ctr. Utrecht, Rudolf Magnus Inst. of Neurosci., Utrecht, Netherlands; [4]Leiden Univ. Inst. for Psychological Research, Clin. Psychology Unit, Leiden, Netherlands; [5]Leiden Inst. for Brain and Cognition (LIBC), Leiden, Netherlands

Abstract: Animal models of predator defense distinguish qualitatively different defense modes that are activated at increasing levels of predator threat. Freezing is a parasympathetically dominated reduction of locomotion and heart rate frequency (bradycardia) typically present at the intermediate (post-encounter) stage. This defense response is mediated by the (ventral) periaqueductal grey (PAG) and disappears when animals switch to a sympathetically dominated fight-or-flight response. In humans, freezing-like behavior and heart rate deceleration have been reported in response to aversive pictorial stimuli, but neural mechanisms underlying this autonomic response profile have not been identified. Here, we investigated in humans whether parasympathetic heart rate decelerations would correlate with PAG activity, and whether such an association would be independent of sympathetic autonomic activation.
Eighteen healthy males were scanned using functional MRI in a picture viewing paradigm. Decelerative heart rate responses were measured using finger pulse photoplethysmography. Sympathetic responses were indexed using pupil dilation measures. Functional MRI data were stereotactically normalized using a high-precision diffeomorphic anatomical registration technique (SPM8/DARTEL) and smoothed with a 4 mm FWHM gaussian kernel to retain sufficient resolution. Statistical analyses were performed with general linear models testing picture category effects and (hierarchical) linear regression of within-category trial-by-trial

fluctuations of physiological responses onto BOLD data.
As expected, we found robust decelerative heart rate responses (t=4.80, P<.0005) and pupil dilation responses (t=3.91, P<.005) to aversive (vs. neutral) pictures. Imaging data show responses to aversive (vs. neutral) pictures in the PAG (P<.05, whole-brain FWE corrected) and in commonly observed regions such as amygdala and inferotemporal cortex. Crucially, within the aversive picture category, PAG responses were negatively correlated with heart rate responses on a trial-by-trial basis, and this effect remained significant in a hierarchical regression analysis in which pupil dilation was partialled out (both P<.05, SVC).
These data suggest that the PAG response is associated with a mode in which the autonomic balance is dominated by parasympathetic activity. PAG responses have previously been observed with increasing threat imminence in humans. However, this study is the first to link the PAG with fear bradycardia responses akin to those observed in many vertebrate species, and may thus further the advancement of translational models of defense behavior.

Disclosures: E.J. Hermans, None; **M.J.A.G. Henckens**, None; **K. Roelofs**, None; **G. Fernández**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.8

Topic: F.03. Motivation and Emotion

Support: NIH Grant: P50 MH069315

NIH Grant: R01 MH043454-19

Title: Increased ability to sustain activity in fronto-striatal circuits is related to improved positive affect following treatment in major depression

Authors: *A. HELLER[1,2], T. JOHNSTONE[4], M. J. PETERSON[3], G. G. KOLDEN[3], N. H. KALIN[3], R. J. DAVIDSON[2,5];
[1]Waisman Lab. For Brain Imaging and Behavior, Madison, WI; [2]Psychology, [3]Psychiatry, Univ. of Wisconsin - Madison, Madison, WI; [4]Ctr. for Integrative Neurosci. & Neurodynamics Sch. of Psychology and CLS, Univ. of Reading, Reading, United Kingdom; [5]Waisman Ctr. for Brain Imaging and Behavior, Madison, WI

Abstract: Anhedonia, the inability to experience pleasure, is a cardinal symptom of major

depression. Previously, we demonstrated that compared with healthy controls, individuals with major depressive disorder displayed an inability to sustain activity and connectivity in the Nucleus Accumbens (NAcc) and fronto-striatal network, respectively, when up-regulating positive affect. Furthermore, individual differences in the ability to sustain NAcc activity was related to self-reported positive affect, such that those depressed patients better able to sustain NAcc activity across time reported higher levels of positive affect in their daily lives. Following the initial, pretreatment scan discussed above, the depressed patients began a trial of either venlafaxine or fluoxetine and were followed for a period of 24 weeks with scans at 8 and 24 weeks. Here, we report a follow-up analysis from those same subjects, 8 weeks into the trial. After 8 weeks of pharmacotherapy, the average HAMD score was 9.67 (SD=4.47), with 8 of 24 patients qualifying as 'remitted' (HAMD $\leq$ 7). This was significantly lower than baseline HAMD ($p < .001$). Compared with the scan at baseline, an fMRI scan at 8 weeks revealed that depressed patients were better able to sustain NAcc activity when up-regulating positive affect. We also observed a significant correlation between increases in the ability to sustain NAcc activity and higher levels of self-reported positive affect: Those individuals displaying the greatest improvement in the ability to sustain NAcc activity also showed the largest gains in self-reported positive affect. Further, using the NAcc as a seed region, connectivity analyses revealed a fronto-striatal network which was also correlated with the gains in self-reported positive affect. In addition to our previous report, these findings suggest that the ability to sustain activity in the fronto-striatal network may underlie symptoms of anhedonia in depression, and further that changes in the ability to sustain engagement of these networks underlies gains in positive affect following successful treatment.

Disclosures: A. Heller, None; **T. Johnstone**, None; **M.J. Peterson**, None; **G.G. Kolden**, None; **N.H. Kalin**, Astra Zeneca; Bristol-Myers-Squibb; CeNeRx Biopharma; Corcept; Cyberonics; Forest Laboratories; General Electric Corp; Jazz Pharmaceuticals, Ely Lilly; Neuronetics; Sanofi Syntholabs; Wyeth Pharmaceut, Consultant/Advisory Board; **R.J. Davidson**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.9

Topic: F.03. Motivation and Emotion

Support: MRC Grant G0701514

Title: Prefrontal-striatal activity during emotion regulation in a high risk group for depression: A longitudinal study

Authors: *C. MOUTSIANA[1], S. L. HALLIGAN[2], T. JOHNSTONE[1];
[1]Ctr. for Integrative Neurosci. & Neurodynamics, [2]Psychology Dept., Univ. of Reading, Reading, United Kingdom

Abstract: Research in the neuroscience of emotion regulation has lead to substantial progress in our understanding of the neural underpinnings of depression. However, research has primarily focused on currently depressed groups. To disentangle cause from symptoms, studies of the offspring of depressed parents have become established as means of investigating risk factors for depressive disorder.
In a unique longitudinal sample, we are examining the neural correlates of emotional responding and regulation in the adult offspring of postnatally depressed mothers, and a comparison group matched on sociodemographic characteristics and gender. Both groups have been extensively studied from birth. Exclusion criteria included any current depressive episode, assessed by administering the Structural Clinical Interview for DSM-IV. Here we report preliminary analyses from a sample of 42 participants (21 per group).
We use fMRI to measure prefrontal and subcortical brain activation while participants perform a standard emotion regulation task (cf. Johnstone et al, 2007). In a random order event-related task, participants view positively and negatively valenced pictures and are asked either to simply continue viewing the pictures, or to down/up-regulate their emotional responses using previously instructed reappraisal strategies.
Using FSL software, within and between-subject differences in the BOLD signal were modeled with a two stage mixed-effects GLM. At the first level, data were modeled with one regressor for each experimental condition as well as covariates to model BOLD signal variance due to motion. We compared the estimated BOLD responses in two contrasts of interest: decrease-attend for negative pictures, and increase-attend for positive pictures in the condition of interest. These contrast images were then compared between the 2 groups in a 2nd stage analysis. Here we present results specifically for the increase-attend condition for positive pictures.
We found that adult offspring of mothers with postnatal depression showed reduced activation of bilateral nucleus accumbens (NAcc) and the left ventrolateral prefrontal cortex (vlPFC) compared with controls when up-regulating emotional responses to positive pictures.
These preliminary findings suggest that individuals at risk for depression may lack the ability to effectively engage prefrontal cortical and subcortical regions involved in sustaining positive affect and reward. The results provide further evidence for the relevance of prefrontal-striatal interactions in anhedonia, a core feature of major depression (Heller et al., 2009).

Disclosures: C. Moutsiana: None. **S.L. Halligan:** None. **T. Johnstone:** None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.10

Topic: F.03. Motivation and Emotion

Title: Medial prefrontal-ventral striatum connectivity in major depressive disorder during emotional word processing

Authors: M.-J. VAN TOL[1,3], N. J. A. VAN DER WEE[1,3], D. J. VELTMAN[4], A. ALEMAN[5], F. G. ZITMAN[1], M. A. VAN BUCHEM[2,3], *T. JOHNSTONE[6,7];
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Abstract: Major Depressive Disorder (MDD) has been linked to a decreased capacity to regulate emotional information (Phillips et al., 2003), possibly owing to abnormal cross-talk between prefrontal and subcortical regions (Mayberg, 1997). We examined brain functional connectivity to test if frontal and subcortical connectivity is altered in MDD during performance of an emotional word processing task.
Methods: Twenty-five right-handed medication-free patients with a half-year diagnosis of MDD (16 f) and 25 right-handed healthy control (HC; 17 f) participants recruited through NESDA performed an event-related, subject paced emotional word evaluation task during functional magnetic resonance imaging (fMRI; Gradient echo, EPI, TE=30/28 ms,TR=2.3 sec,voxel size =2.29x2.29x3 mm, 35/39 slices, Philips 3T systems). Groups were matched on age (M=35.6, sd=10, in years) and education (M=13.4, sd=2.5, in years).
FMRI data were processed using FSL (www.fmrib.ox.ac.uk/fsl). Preprocessing involved: Motion correction, slicetime correction, non-brain removal, spatial smoothing (5 mm FWHM), and normalization to standard MNI-space. Next, data were analysed using dual regression - Independent Component Analyses (ICA) (Biswal et al., 2010), using eight template resting state networks (Smith et al., 2009). In this procedure, two independent stages of linear regression analyses are performed. 1) a time series is extracted for each template per subject; 2) a subject-specific weighted spatial map is created using the time series created in [1] as predictor. This spatial map represents un unbiased measure of the degree to which BOLD signal fluctuations correspond to the template time series. Next, individual spatial maps were used to evaluate between-group differences, with results reported at $p<.05$, corrected for Family Wise Error rate using permutation tests of cluster-mass (voxelwise threshold: z=2.3).
Results: MDD showed decreased correlation of the medial prefrontal cortex (PFC), ventrolateral PFC, and ventral striatum with a network involving the VLPFC, dorsolateral PFC, anterior cingulate gyrus, medial PFC, cerebellum, and cuneus. Within MDD, results were unrelated to illness severity. No other networks showed between-group differences.
Discussion: We showed decreased correlation of medial PFC and ventral-striatal regions within a task executive network during the performance of an emotional word task with the use of an ICA approach and resting state network templates. This decreased connectivity of frontal-striatal

regions confirms the hypothesis of a relative ventral-dorsal decoupling that may serve to explain abnormal emotional regulation in MDD.

Disclosures: M. van Tol: None. **N.J.A. van der Wee:** None. **D.J. Veltman:** None. **A. Aleman:** None. **F.G. Zitman:** None. **M.A. van Buchem:** None. **T. Johnstone:** None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.11

Topic: F.03. Motivation and Emotion

Support: NIH Grant U01 MH081902

Title: Neural substrates of emotion processing in the psychosis prodrome

Authors: D. G. GEE, K. H. KARLSGODT, A. M. JIMENEZ, T. A. LESH, L. KUSHAN, A. XU, J. TORRE, T. G. M. VAN ERP, M. D. LIEBERMAN, C. E. BEARDEN, *T. D. CANNON; UCLA, Los Angeles, CA

Abstract: Emotion processing abnormalities are core features of schizophrenia, with patients demonstrating particular deficits in emotion perception (Kohler et al., 2009). However, the extent to which such deficits are present prior to the onset of overt psychosis, and the role that they might play in its development, remain unclear. The present study uses functional magnetic resonance imaging (fMRI) to examine brain function associated with emotion processing in the psychosis prodrome. Participants were adolescents and young adults meeting criteria for a putative psychosis prodrome and group-matched neurotypical controls. Though data collection is ongoing, preliminary results are available with 8 patients and 8 controls. For each participant, we obtained a T2 image and 2 functional scans (127 volumes each; TR = 2500 ms). We used the affective labeling fMRI task (Hariri et al., 2000; Lieberman et al., 2007), during which participants judge which of two linguistic labels best identifies a target facial expression (Affect Label) and select which of two faces expresses the same emotion as a target face (Affect Match). Control conditions of Gender Label, Gender Match, and Shape Match allowed us to isolate unique effects of emotion processing. Region-of-interest analyses focus on amygdala and ventrolateral prefrontal cortical (vlPFC) activity, as well as task-dependent functional connectivity between them. Group analyses revealed that patients demonstrated different patterns of neural activity during emotion processing, compared with controls. Specifically, during emotion matching, patients exhibited decreased activation in lateral occipital cortex and fusiform

gyrus. During emotion matching relative to shape matching, patients demonstrated differentially increased activation in middle temporal gyrus and superior parietal lobule. ROI analyses revealed a trend toward decreased amygdala activation during emotion processing versus control (gender) conditions in patients. Our findings suggest that the high-risk syndrome is characterized by patterns of abnormal neural activity during emotion processing. These results are consistent with prior work in schizophrenia (Fakra et al., 2008) suggesting that patients adopt a more cognitive, feature-based approach to affect processing and show less activation in regions related to holistic face processing. These results have the potential to identify altered patterns of neural activity during facial affect processing prior to illness onset. Longitudinal studies on the development of such processes as they relate to conversion to psychosis are in progress.

Disclosures: D.G. Gee, None; **K.H. Karlsgodt**, None; **A.M. Jimenez**, None; **T.A. Lesh**, None; **L. Kushan**, None; **A. Xu**, None; **J. Torre**, None; **T.G.M. van Erp**, None; **M.D. Lieberman**, None; **C.E. Bearden**, None; **T.D. Cannon**, None.

Nanosymposium

229. Prefrontal-Subcortical Interactions in Health and Disease

Location: Room 2

Time: Sunday, November 14, 2010, 1:00 pm - 4:00 pm

Program Number: 229.12

Topic: F.03. Motivation and Emotion

Support: Smith Family Young Investigator Award

Sloan Foundation

Title: Sensory and reward integration during decision making: A role for the striatum

Authors: *A. Y. WANG[1,2], N. UCHIDA[2];
[1]Alice Wang, Cambridge, MA; [2]Harvard Univ., Cambridge, MA

Abstract: Selecting the best course of action requires the evaluation of multiple, oftentimes conflicting factors in one's environment. Therefore, a key to optimal decision-making requires the ability to integrate different sources of information such as sensory cues and expected values of actions. Previous studies showed that neurons in the striatum encode value of specific actions (Samejima et al., 2005; Lau and Glimcher, 2008) but exactly how they contribute to action selection remains to be clarified. Specifically, can striatal neurons integrate action values with other types of information such as sensory cues? Moreover, where in the striatum does such integration take place and does this occur at the single neuron level or at downstream areas

(Rorie and Newsome, 2010)?
In this study, we aimed to examine how the dorsomedial and ventral striatum are differentially involved in goal-directed action selection. To this goal, we recorded neural activity in these areas while rats performed a two-alternative forced choice task in which rats need to integrate an ambiguous odor cue with the amount of reward associated with each option. Specifically, psychophysical odor discrimination performance was obtained using binary odor mixtures with various ratios. In blocks of trials, we manipulated the value of the left and right reward ports by varying the amount of water delivered. As expected, rats biased their choices towards higher valued reward ports, particularly when given ambiguous odor mixtures.
By comparing firing rate distributions across different reward block conditions, we found striatal neurons encoding action value even before the onset of odor cue. Among these neurons, 60% encode relative value between leftward and rightward movements and 40% encode absolute value for either movement. Furthermore, the dorsomedial striatum contained more value encoding neurons than the ventral striatum in both quantity and quality (15% of ventral, 30% of dorsal neurons, Kolmogorov-Smirnov test based on ROC values, $p<.01$). We next examined whether striatal neurons integrate different sources of information (odor and reward value). During the odor presentation period, about 16% of striatal neurons showed odor-value interactions, where 70% of them are located in the dorsomedial striatum (10% of ventral neurons, 21% of dorsal). In all, neurons in the dorsomedial striatum demonstrate more sensitivity to value than the ventral striatum. Lastly, single neurons in the dorsomedial striatum not only hold memory about action value but also integrate this information with sensory cues, placing it as a likely structure to be involved in goal-directed action selection.

Disclosures: A.Y. Wang, None; **N. Uchida**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.1

Topic: G.05. Bioinformatics

Support: NIH 1R01MH084812-01A1

Title: Cognitive paradigm ontology: Experimental conditions and contrasts

Authors: *J. A. TURNER[1], A. R. LAIRD[2];
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Abstract: Background. The ability to share and mine publicly available data through automated methods is facilitated by formalized structures and definitions for different facets of experimental design, data collection, and analysis. A number of ontologies are being developed to address these different components of neuroscience research, such as neuroanatomical knowledge, imaging and other experimental methods, and the representation of some data transformations for analysis. Standardized terminologies for describing cognitive tasks used in fMRI or PET experiments have been previously developed by the BrainMap Project (www.brainmap.org) (Fox et al., 2005; Laird et al., 2005), but the definitions and structures as implemented in the BrainMap database are not amenable to automated reasoning. The Human Imaging Database (HID) developed by FBIRN (Keator et al., 2008) stores individual fMRI datasets in a structured, extensible hierarchy but does not include information regarding the cognitive paradigm used in the fMRI session. With the goal of mining data across BrainMap and BIRN, we aim to develop an ontology of cognitive paradigms.
Methods. This first version of the Cognitive Paradigm Ontology (CogPO) was developed as an OWL/RDF file, building off the Basic Formal Ontology (BFO) at the top-level and the Information Artifact Ontology (IAO) at the mid-level. The terminology of parsing experimental behavioral conditions in separate descriptions of *stimuli*, *response*, and *instructions*, originally developed by BrainMap, has been incorporated into CogPO. The proof of the ontological structure is in querying the BrainMap database and the FBIRN Human Imaging Database (HID) using the ontological terms.
Results. The ontological structure of CogPO version 1.0 has been established and validated in several cognitive tasks. Preliminary descriptions of auditory and visual oddball tasks have been developed in CogPO, with linked annotations of the appropriate ontological terms to the relevant fields in BrainMap and an instance of the HID. This provides a structure for implementing queries across a database of functional neuroimaging results (BrainMap) and distributed databases of raw imaging data (HID) using a standardized representation of cognitive paradigms.
Conclusions. While a diverse range of experimental paradigms can be implemented in cognitive neuroscience research, there is a basic structure to any given condition that can be formalized and parsed into three fundamental components. This structure that was first constructed in the BrainMap database is currently being refined and represented in logical formalizations for broader use in other applications.

Disclosures: J.A. Turner, None; **A.R. Laird**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.2

Topic: G.05. Bioinformatics

Support: Waitt Family Foundation

Title: The whole brain catalog and multiscale connectome browsing

Authors: *S. D. LARSON[1], C. APREA[2], J. MARTINEZ[3], D. A. PETERSON[4], R. CARLOZ[5], D. LEE[3], D. LITTLE[3], V. ASTAKHOV[5], H. S. KIM[6], A. MEMON[7], I. ZASLAVSKY[7], H. POIZNER[4], M. E. MARTONE[3], M. E. ELLISMAN[3];
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Abstract: A diverse array of behavioral functions provided by the mammalian brain relies on complex networks. The prospect of a complete wiring diagram of these networks, or "connectome", is being enabled by technological advances in wide-field electron microscopy, viral-based tract tracing, and genetic fluorescent labeling, each of which tend to reveal only pieces of a vast puzzle. An integrated view of this structural data would greatly facilitate our understanding of the structure and function of the mammalian brain. However, coherently assembling this information for easy use by a broad cross-section of the neuroscience community poses a daunting challenge. To address this we have developed the Whole Brain Catalog ("WBC"). The WBC is a free open-source web-based application that integrates brain data from multiple scales with a Google Earth-like interface. The WBC incorporates data sets from the mouse brain and retina, and is expanding to incorporate data from the zebrafish. It includes 1) a user interface for selecting, zooming to, and manipulating specific data sets in a 3D environment, 2) a digital brain atlas coordinated with services provided by the INCF Digital Atlasing Infrastructure task force that allows users to pull data from online sources such as the Allen Brain Institute 3) a knowledge management layer and interface to NeuroLex.org built in conjunction with the INCF Program on Ontologies of Neural Structures task force, and 4) a simulation service that allows users to upload, run, and animate results of NeuroML network models on a cluster running a parallelized version of the NEURON simulation engine. Recently the WBC has been expanded to include the Multi-scale Connectome Browser ("MCB"). MCB depicts 2D network maps based on inter- and intra-nuclear networks imported from standardized neuroanatomical databases via the Neuroscience Information Framework (NIF). Network projections are annotated with their excitatory/inhibitory and neurotransmitter characteristics. MCB users can seamlessly navigate between levels of detail and zoom in on their desired sub-networks. To our knowledge, MCB is the first simple means for interactively navigating systems-level diagrams of mammalian networks including not only cortical areas but also most of the prominent nuclei of the thalamus, basal ganglia, and brainstem. MCB allows diagram export for use in talks, posters, and journal publications, assisting neuroscientists who routinely build ad hoc graphical representations of brain networks. In conclusion, the Whole Brain Catalog enables connections between data and eases access to a diverse digital landscape of structural neuroanatomical knowledge.

Disclosures: S.D. Larson, None; **C. Aprea**, None; **J. Martinez**, None; **D.A. Peterson**, None; **R. Carloz**, None; **D. Lee**, None; **D. Little**, None; **V. Astakhov**, None; **H.S. Kim**, None; **A.**

Memon, None; **I. Zaslavsky**, None; **H. Poizner**, None; **M.E. Martone**, None; **M.E. Ellisman**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.3

Topic: G.05. Bioinformatics

Support: NIH Grant R01-12468

Title: Using ontologies to represent and reason over brain electrophysiology (ERP) data

Authors: *G. A. FRISHKOFF[1,2], R. M. FRANK[2], P. LEPENDU[4], S. S. NIKOLIC[1], H. LIU[3], D. DOU[3];
[1]Psychology, Georgia State Univ., Atlanta, GA; [2]NeuroInformatics Ctr., [3]Computer & Information Sci., Univ. of Oregon, Eugene, OR; [4]Stanford Ctr. for Biomed. Informatics Res., Stanford Univ., Stanford, CA

Abstract: We discuss recent progress in the development of Neural Electromagnetic Ontologies (NEMO; Ref. [1]) and the use of NEMO to support meta-analysis of event-related potentials (ERPs). ERP patterns are notoriously hard to identify and compare across datasets, due to spatiotemporal overlap within datasets, and variability in the timing and topography of patterns across datasets. To address these issues, we propose a novel approach (Figure 1) that combines data-driven (signal analysis) methods and knowledge-driven methods. Knowlege-driven methods include the use of formal semantics ("ontologies") to represent ERP spatial patterns and to describe how these patterns change over time and across experimental contexts, enabling semantically based storage, analysis, and integration of ERP data. To illustrate this approach, we present a case study in which these methods are applied to ERP datasets from the NEMO consortium. After extraction of ERP patterns, a set of features ("pattern attributes") is used as a summary of each pattern, and the relationships between these features are coded in owl/rdf and linked to NEMO ontologies. Clustering is then applied within and across datasets to derive classes that represent comparable ERP patterns. Finally, pattern attributes for these classes are subjected to statistical meta-analysis. We conclude with a discussion of efforts to coordinate the development of NEMO with related efforts in the bio- and neuro-ontology community.
[1] Frishkoff, G.A., Dou, D., e al. (2009). Development of Neural Electromagnetic Ontologies (NEMO): Representation and integration of event-related brain potentials. *Proc. Int'l Conf. on Biomedical Ontologies*. July 24-26, 2009. Buffalo, NY.

Acknowledgements: This research was supported by the National Institutes of Health (NIBIB), grant number R01-12468 (PI, Dou). We thank J. Turner, M. Martone, A. Laird, and F. Iman for helpful discussions.

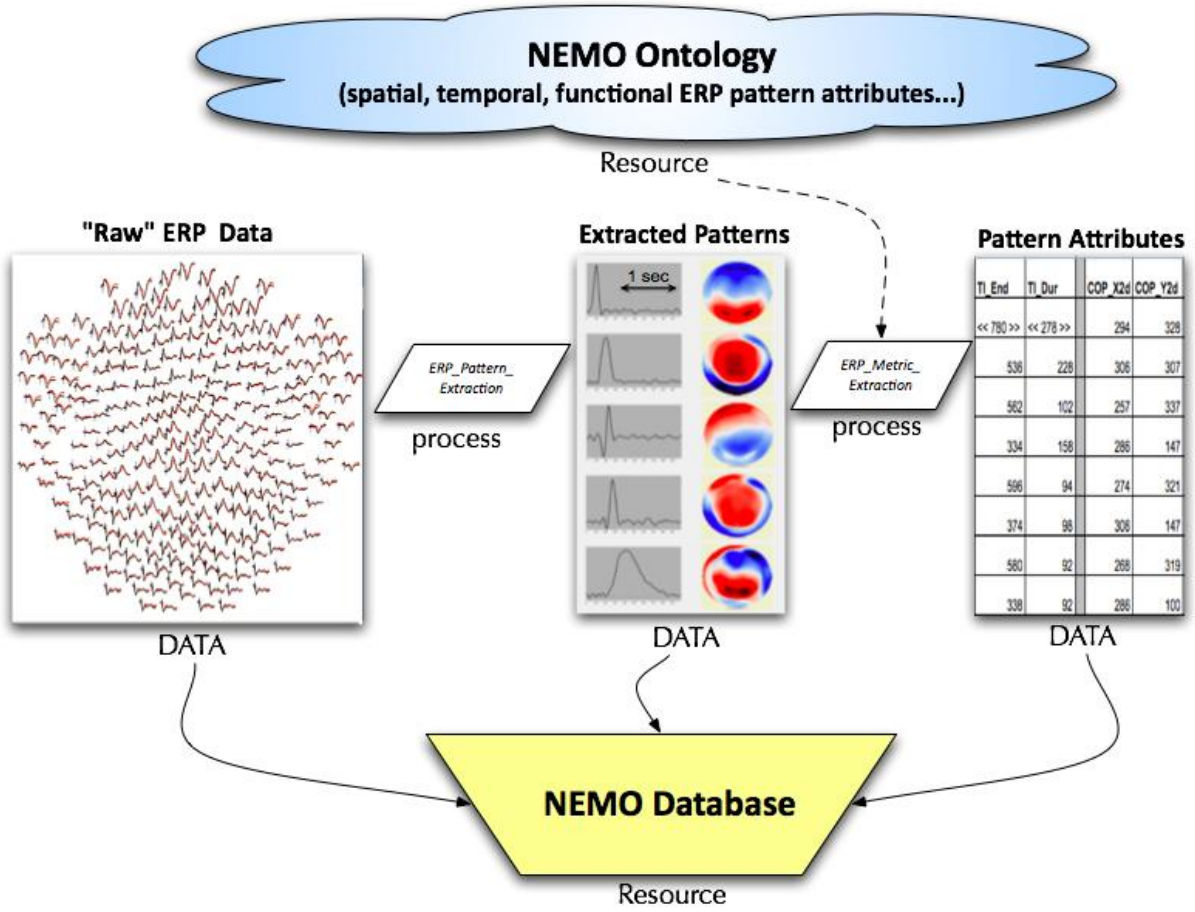

Figure 1. Outline of processes for linking ERP data to NEMO ontologies.

Disclosures: G.A. Frishkoff, None; **S.S. Nikolic**, None; **R.M. Frank**, None; **P. LePendu**, None; **H. Liu**, None; **D. Dou**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.4

Topic: G.05. Bioinformatics

Support: NIH Grant GRANT NIDA DA016602

NIH Grant NINDS RO1NS058296

Title: A knowledge based approach to matching human neurodegenerative disease and associated animal models

Authors: *S. M. MAYNARD[1], C. J. MUNGALL[2], S. E. LEWIS[2], M. E. MARTONE[1]; [1]UCSD, LA JOLLA, CA; [2]Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: Neurodegenerative diseases have a wide, complex range of biological and clinical features some of which are shared, yet have unique signatures. Models, key to translational research, only replicate a subset of related features. Pathology occurs across spatial and temporal scales and is expressed with varied vocabularies, so data mining approaches for comparisons among and between human disease and models are challenging.
We created the knowledge base and multiscale ontology, Phenotype Knowledge Base (PKB; http://ccdb.ucsd.edu/PKB/1.0/PKB.owl) and Neurodegenerative Disease Phenotype Ontology (NDPO; http://ccdb.ucsd.edu/NDPO/1.0/NDPO.owl) to address the critical need to match animal models to human disease. We use a knowledge based approach to compare phenotypes using a formal semantic representation using the Ontology of Phenotypic Qualities (http://purl.org/obo/owl/PATO), and Neuroscience Information Framework ontologies (NIFSTD; http://purl.org/nif/ontology/nif.owl). We drew human and nonhuman phenotypes from review and primary literature. We load phenotypes into the Ontology-Based Database (OBD; http://berkeleybop.org/pkb) for ontology based descriptions to find best matches for phenotypes and organisms using information content and semantic similarity statistical metrics.
In the past year, the system has matured as we refine the model and populate the knowledge base. We now express over 800 phenotypes of 11 human diseases and 13 model organism types primarily from genetically manipulated mice and flies. We added a significantly improved OBD user interface to display comparisons at the level of single phenotypes, e.g., organisms with aggregated alpha synuclein, and best matched organisms. The NIFSTD has added asserted and defined classes, for a more rich and expressive ontology. Our matching combines knowledge for individual phenotypes and statistical measures for overall similarity scores. NIFSTD provides the basis for identifying common high level entities between phenotypes. When retrieving phenotypes similar to a human with Parkinsons disease, a mouse overexpressing alpha synuclein with the phenotype “paracentral nucleus has extra parts cellular inclusion” matches phenotype “midline nuclear group has extra parts Lewy Body” due to the common subsumer: Thalamus has extra parts cellular inclusion. For overall similarity, groupings of organisms are based on their aggregate phenotypes. Rather than a rigid taxonomy of animal models and human diseases, our method facilitates identification of similar organisms based on their properties allowing for comparisons across models based on more granular features.

Disclosures: S.M. Maynard, None; **C.J. Mungall**, None; **S.E. Lewis**, None; **M.E. Martone**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.5

Topic: G.05. Bioinformatics

Support: NIH Neuroscience Blueprint HHSN271200800035C via NIDA

Title: An antibody registry for biological sciences

Authors: *A. E. BANDROWSKI, V. ASTAKHOV, M. MARTONE, A. Y. YELISETTY; UCSD, LA JOLLA, CA

Abstract: Recognition of entities inside of text is a difficult task for both machines and humans because both humans and machines often need much more information to identify an entity than is present in text. Herein, we propose a general solution to this problem for one type of entity, the antibody, that would allow for any simple text recognition software to accurately find any antibody in text.
The neuroscience information framework (NIF) as part of its mission to make scientific data and resources discoverable was tasked with a pilot project to determine the feasibility of identifying entities automatically in text and we were granted the ability to automatically index one full volume of the Journal of Neuroscience. Dr. Martone, an expert anatomist, generated a list of antibodies from the same volume of the Journal of Neuroscience, which served as a control dataset useful to compare human and machine efforts. In 8 articles, 106 (95 unique) antibodies were identified, and of those 52 references did not contain enough information to determine the catalog number, in addition only a few antibodies were identified with either a clone number or a catalog number, but supplier name and url were available in all but 26 cases. No antibodies had lot numbers associated. Automated systems were not deployed as their success is not possible, when a human expert, using papers (often going back to previous work of the authors) and company catalogs can identify less than 50% of the antibodies.
The solution to the problem requires a change in publishing practices, not software. To aid in this change, we have negotiated agreements for data download of a set of information for more than 800,000 commercially available antibodies including a unique identifier for each antibody that, we propose, should be included in any new publication of antibodies. The information includes the vendor, catalog number, clone id, antibody target, target subregion (where available), target modification, target species, raised in species, target Entrez id, clonality, name and comments. The data model was aligned with the model created by the EagleI project, tasked with cataloging non-commercial antibodies. In addition we have created an easy to use, web-accessible graphical interface, written in JAVA, which would serve as a search and registration tool for any scientist

to search for and add antibodies, now deployed at: http://nif-apps1.crbs.ucsd.edu:8080/Antibody4/antibodyform.html
With this extensive database, covering most commercial antibodies, and an easy to use registration tool scientists should be able to disambiguate antibodies within papers that any text mining software, or researcher can find.

Disclosures: A.E. Bandrowski, None; **V. Astakhov**, None; **M. Martone**, None; **A.Y. Yelisetty**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.6

Topic: G.05. Bioinformatics

Support: NIH/Neuroscience Blueprint contract HHSN271200800035C

Title: Nifstd 1.8: A comprehensive ontology for neuroscience

Authors: F. IMAM[1], S. LARSON[1], S. POLAVARAM[3], G. A. ASCOLI[3], G. M. SHEPHERD[4], J. S. GRETHE[1], *A. GUPTA[2], M. E. MARTONE[1];
[1]CRBS, [2]UCSD, LA JOLLA, CA; [3]Ctr. for Neural Informatics, Structure, & Plasticity, George Mason Univ., Fairfax, VA; [4]Biol. and Biomed. Sci., Yale Univ., New Haven, CT

Abstract: A critical component of Neuroscience Information Framework project (http://neuinfo.org), NIF Standard (NIFSTD) is a set of modular ontologies covering a comprehensive set of neuroscience terminologies. This poster will highlight the key features of NIFSTD and how NIF uses it to enable an effective concept-based search against a diverse collection of neuroscience resources.
NIFSTD is designed to collate existing neuroscience terminologies into a coherent set of orthogonal and interoperable modules. Closely following the best practices of Open Biological Ontology (OBO) community, NIFSTD is standardized to the same upper level ontologies for biomedical sciences. One of the largest roadblocks that we encountered was the lack of tools for the neuroscience community to contribute their knowledge into a formal ontology like NIFSTD. NIF has created NeuroLex (http://neurolex.org), a semantic wiki interface for the domain experts as an easy entry point to the NIFSTD contents. It has been extensively used in the area of neuronal cell types where NIF is working with a group of neuroscientists to create a comprehensive list of neurons and their properties. While the properties in NeuroLex are meant

for easier interpretation, the restrictions in NIFSTD are more rigorous and based on standard OBO-RO relations.
We have recently released NIFSTD v.1.8 (http://ontology.neuinfo.org) where the key feature is the inclusion of various cross-domain bridge modules. These modules contain necessary restrictions along with a set of defined classes to infer useful classification of neurons and molecules. These classifications include neurons in terms of their soma locations in different brain regions (e.g., Hippocampal neurons, Cerebellum neurons), neurons by their neurotransmitter (e.g., GABAergic neuron) and circuit roles (e.g., intrinsic neurons), classification of molecules and chemicals by their molecular roles (e.g., Drug of abuse, Neurotransmitter). Having the defined classes enabled us to have useful concept-based queries through the NIF search interface. For example, while searching for 'GABAergic neuron', the system recognizes the term as 'defined' from the ontology, and looks for any neuron that has GABA as a neurotransmitter (instead of the lexical match of the search term) and enhances the query over those inferred list of neurons.
The NIF project provides an example of how ontologies can be used to enhance search and data integration across diverse resources. As the project moves forward, we are using NIFSTD to build an increasingly rich knowledgebase for neuroscience that integrates with the larger life science community.

Disclosures: F. Imam, None; **A. Gupta**, None; **S. Larson**, None; **J.S. Grethe**, None; **M.E. Martone**, None; **G.A. Ascoli**, None; **S. Polavaram**, None; **G.M. Shepherd**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.7

Topic: G.05. Bioinformatics

Support: NIMH R01-MH074457

NIMH R01-MH084812

Title: Functional assessment of intrinsic connectivity networks

Authors: *A. R. LAIRD[1], P. M. FOX[2], S. B. EICKHOFF[3], J. A. TURNER[4], K. L. RAY[2], D. R. MCKAY[2], D. C. GLAHN[5], C. F. BECKMANN[6], S. M. SMITH[6], P. T. FOX[1];
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RWTH Aachen Univ., Aachen, Germany; [4]The Mind Res. Network, Albuquerque, NM; [5]Olin Neuropsychiatry Res. Ctr., Hartford, CT; [6]Ctr. for Functional MRI of the Brain, Univ. of Oxford, Oxford, United Kingdom

Abstract: Introduction Recently, independent component analysis (ICA) applied to peak coordinates from thousands of functional neuroimaging studies identified co-activation networks that matched similarly derived resting state networks (Smith et al., 2009). The functional significance of these intrinsic connectivity networks (ICNs) can be difficult to assess, given the task-independent nature of rest. However, characterization is possible using the BrainMap taxonomy. Here, we present results for extracting and mining this rich set of metadata as a means to delineate functional differences between ICNs, and propose this as a strategy for developing a cognitive ontology for functional neuroimaging.
Methods BrainMap 3D coordinates (locations of activation peaks) extracted from 8,108 functional neuroimaging experiments were smoothed to create pseudo-activation images. ICA was applied to this 4D dataset using FSL's MELODIC to decompose the images into 20 components. We computed a set of component x BrainMap metadata matrices that correspond to 14 metadata categories, including the cognitive process, paradigm, stimulus, response, and instructions. Hierarchical clustering analysis using a single linkage algorithm was performed on the 14 component x metadata matrices to determine groupings of similar components using 1 - r as the distance between clusters, where r is the Pearson's correlation coefficient.
Results Spatial maps for the ICNs derived from the 20-component decomposition demonstrated that a unique set of functional brain networks are captured by ICA. Normalized heat maps of the metadata matrices demonstrated a rich functional characterization of ICNs. Clustering quantified the co-occurrence of paradigms and mental operations within the context of ICNs and provided stratified groupings of similar behaviors. Confluence of brain and behavioral analyses resulted in greater segregation of our data than when behavior alone was considered, and associated specific cognitive operations with corresponding cognitive paradigms.
Conclusions The results of our analyses described the functional properties of the ICNs in a way not possible within resting state FMRI data. While behavioral domains and paradigm classes are crucial for understanding network functions, additional fields, such as stimulus, response, and instructions, may provide a more detailed assessment of the experimental differences across components. As predicted by Smith et al., 2009, we set forth a framework for deriving a neuroimaging-driven cognitive ontology and establish that this strategy is extremely powerful when pursued in the context of intrinsic connectivity.

Disclosures: A.R. Laird, None; **P.M. Fox**, None; **S.B. Eickhoff**, None; **J.A. Turner**, None; **K.L. Ray**, None; **D.R. McKay**, None; **D.C. Glahn**, None; **P.T. Fox**, None; **C.F. Beckmann**, None; **S.M. Smith**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.8

Topic: G.05. Bioinformatics

Support: INCF PONS/DAI Contract

Title: Brain connectivity at your fingertips: CoCoMac interfaced with the scalable brain atlas

Authors: R. BAKKER, G. BEZGIN, *R. KOTTER;
Donders Inst. Brain, Behaviour & Cognition, Radboud Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: The Scalable Brain Atlas (SBA, scalablebrainatlas.incf.org) is a web-based interactive brain atlas. It displays brain atlas templates (parcellations) for a variety of species and atlas providers. Brain regions can be selected to launch queries to other web-based resources or, websites can use the SBA to visualize sets of related brain regions.
CoCoMac (cocomac.org) is a database of anatomical connectivity for the Macaque brain, populated by manually collated data from more than four hundred peer-reviewed tracing studies. A web-interface to CoCoMac has been in place for many years, providing connectivity data to experts with knowledge of anatomical nomenclatures. The intuitive SBA interface (scalablebrainatlas.incf.org/cocomac) makes connectivity data available to a much wider audience, including fMRI and DTI experts who prefer to look at spatial patterns rather than textual, ontology-based statements.
The bottleneck in this process has been the mapping of CoCoMac brain regions to a common reference atlas: data in CoCoMac retains its original nomenclature, which has varied widely over time and research lab. Using literature statements which relate regions in different atlases to each other, we have mapped most of CoCoMac's brain regions to the F99 atlas surface available in Caret (1). Assuming a uniform cortical thickness, this surface is then transformed into a voxel-based space and sliced in coronal sections to be displayed in the SBA.
(1) Van Essen DC, Drury HA, Dickson J, Harwell J, Hanlon D, Anderson CH (2001). An Integrated Software Suite for Surface-based Analyses of Cerebral Cortex. Journal of American Medical Informatics Association, 8(5): 443-459.

Disclosures: R. Bakker, None; **R. Kotter**, None; **G. Bezgin**, None.

Nanosymposium

230. Neuroinformatics and Connectomics

Location: Room 7B

Time: Sunday, November 14, 2010, 1:00 pm - 3:15 pm

Program Number: 230.9

Topic: G.05. Bioinformatics

Title: Creation of a mouse brain transcriptome database from meta analysis of microarray data deposited at gene expression omnibus

Authors: *B. B. SAMAL[1,2], N. R. SAMAL[2];
[1]SMN, NIMH, NIH, BETHESDA, MD; [2]Samal, Rockville, MD

Abstract: Brain transcriptome databases such as Allen Brain atlas, GenePaint and GenSat have been created using the in situ hybridization data for different region of mouse and human brain. The work presented here is a different approach towards the creation of mouse and human brain transcriptome databases utilizing the microarray data deposited at data warehouses such as Gene Expression Omnibus (GEO). Processed microarray expression signal values from Affymetrix arrays for different brain regions of C57/BJ untreated (control) adult mouse were retrieved from GEO. Data from different labs were combined using Entrez GeneID as the common element. Expression values were normalized between different experiments (GSE) setting the expression value of G6PD at 100. Expression values were aligned with the latest information of mouse genes from NCBI gene website. Microarray data derived gene expression values have been correlated with the expression values as presented in Allen Brain Atlas.
At the website http://www.molecularbrain.org/ expression values of any gene across different brain regions could be visualized as a histogram by using search terms such as Entrez GeneID, gene symbol, synonym or description. Additional information of that gene, i.e. its expression values as reported in in-situ hybridization derived gene expression databases, its function, its regulation, interaction of its product with others as well as the sequence information of the gene, transcript and gene product could be accessed via links in a drop down menu format.

Disclosures: B.B. Samal, None; **N.R. Samal**, None.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.1

Topic: A.08. Transplantation and Regeneration

Support: NINDS

Wings for life

Adelson Medical Research Foundation

Craig Nelson Foundation

Title: Crosstalk between SOCS3 and PTEN regulated pathways in promoting optic nerve regeneration

Authors: *F. SUN, K. K. PARK, Z. HE;
Children's Hosp. Boston, Boston, MA

Abstract: The failure of injured axons to regenerate is a major reason for permanent functional deficits after CNS injury. We have recently identified two genes, PTEN and SOCS3, which when individually deleted in RGCs by a highly efficient AAV-Cre induced conditional knockout approach, promoted extensive axon regeneration after optic nerve injury in the adult mice. In the present study, crosstalk between the two pathways regulated by PTEN and SOCS3 in promoting axon regeneration is investigated.
We have previously demonstrated that axon regeneration after PTEN deletion was significantly reduced by mTOR inhibitor rampamycin, and gp130 knockout abolished axon regeneration induced by SOCS3 deletion. In the present study, in order to reveal interference between these two signal pathways, axon regeneration was examined in PTEN and gp130 double knockout mice, and in SOCS3 knockout mice treated with rampamycin or vehicle control. The extent of axon regeneration in PTEN gp130 double knockout mice was comparable to that of PTEN single knockout, while on the other hand, rampamycin treatment partially blocked axon regeneration induced by SOCS3 deletion. Since these two pathways are by large not overlapping in promoting axon regeneration, PTEN and SOCS3 double knockout mice were examined for potential combinatorial/synergistic effects. Indeed, PTEN SOCS3 double knockout mice exhibited striking long-distance axon regeneration, which was significantly longer than that seen in the individual knockouts; this effect was further enhanced by intraocular CNTF injection. Furthermore, in a more clinical relevant scenario, when PTEN and/or SOCS3 were deleted after optic nerve injury instead of before injury, extensive axon regeneration still existed. Remarkably, at 4 weeks after injury and delayed treatment, in PTEN SOCS3 double knockout mice with CNTF injection, many of the regenerating fibers grew into optic chiasm, and some fibers even extend into the brain.

Disclosures: F. Sun, None; **K.K. Park**, None; **Z. He**, None.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.2

Topic: A.08. Transplantation and Regeneration

Title: Hydrogen peroxide promotes peripheral sensory axon regeneration after epidermal injury

Authors: *S. RIEGER[1,2], A. SAGASTI[2];
[1]Los Angeles, CA; [2]Molecular, Cell and Developmental Biol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Peripheral sensory neurons regenerate spontaneously in response to injury, but regeneration can be limited, thereby often leading to incomplete functional recovery. In zebrafish, peripheral sensory neurons innervate the epidermis between 18 and 36 hpf. If a sensory axon is severed using laser axotomy during that developmental window it can reinnervate its former territory, but, if axotomy occurs after 48 hpf, regenerating axons avoid the denervated regions. The ability of zebrafish to regenerate tissues in response to injury prompted us to explore whether axon and tissue regeneration are related. At 78 hpf, when axotomized axons are normally no longer capable of reinnervating uninjured tissue, we amputated the caudal fin and used time-lapse confocal imaging to visualize the behavior of injured GFP-expressing Rohon-Beard (RB) sensory axons over 12h. We found that RB axons always regenerated into the wound site, suggesting that global fin injury can overcome growth inhibitors present at this stage. We further found that injury often promoted growth of all axonal branches, but only when the axon was injured close to the amputation site. To search for the source of regeneration signals, we laser damaged a few keratinocytes, followed by axotomy near the ablation site. Axotomized axons were indeed capable of regeneration. The ability of damaged keratinocytes to induce axon regeneration was not only restricted to the fin but also occurred in the head for trigeminal axons. To identify potential molecules mediating injury-dependent axon regeneration, we tested whether hydrogen peroxide (H_2O_2) was involved, as high concentrations of this reactive oxygen species are produced along the wound margins of the injured epithelium. Indeed, we observed that the addition of H_2O_2 to the media promoted peripheral sensory axon regeneration following axotomy in uninjured fins. Conversely, inhibition of H_2O_2 production via morpholino knockdown of *duox1* completely prevented axon growth following fin amputation. We also found that in *duox1* morphants, inhibition of IκB kinase (IKK) significantly rescued peripheral sensory axon regeneration, suggesting that H_2O_2 may be a negative regulator of IKK in this process. Together these findings suggest a novel function for H_2O_2 in promoting axon regeneration following epithelial injury.

Disclosures: S. Rieger: Employment; UCLA. **A. Sagasti:** UCLA.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.3

Topic: A.08. Transplantation and Regeneration

Support: Natural Science Foundation of China 90377013

Title: Neural signal regeneration and function rebuilding with microelectronic neural bridge between two separated toads 1000-km apart

Authors: *Z.-G. WANG[1], X.-Y. LÜ[2], W. LI[3], X. SHEN[3], Z. HUANG[3], X. ZHAO[3], L. DU[5], X. GAO[6], Z. JIANG[7], H. PAN[2], G. WANG[7], S. XIE[3], X. GONG[2], C. ZHU[4], L. QIU[3];
[1]Inst. of RF&OE-ICs, Southeast Univ., Jiangsu, China; [2]State Key Lab. of Bioelectronics, [3]Inst. of RF- & OE-ICs, Southeast Univ., Nanjing, China; [4]Southeast Univ., Institute of RF- & OE-ICs, China; [5]China Rehabil. Res. Ctr., Beijing, China; [6]Nanjing Med. Univ., Nanjing, China; [7]Key Lab. of Neural Regeneration of Jiangsu Province, Nantong Univ., Nantong, China

Abstract: In order to rebuild the function of an injured spinal cord, we invented a method of the microelectronic neural bridge (MNB). In this paper, we report the experiments between 2 separated spinal toads.
Fig. 1 shows the schema of the set-up. Two spinal toads lay in Beijing and Nanjing, respectively, about 1000-km apart. The sciatic nerves of 2 toads were connected by our MNB consisting of a hooked electrode array, a signal detecting circuit, 2 PCs accessing the 3G Internet, an FES circuit, and a cuff electrode array.
In the experiment, the left leg of Bejing's spinal toad was stimulated by one drop of 5% acetic acid. It withdrew immediately. The evoked neural signal was detected, amplified, transmitted to the FES circuit, and applied on the sciatic nerve of Nanjing's toad. Then, we observed that Nanjing's toad withdrew its left leg synchronously. The live videos were made in Beijing and Nanjing at the same time.
The neural signals monitored by the oscilloscopes at 2 sites are shown in Fig. 2. It is obvious that the signal monitored on the sciatic nerve of Nanjing's toad is similar to that detected from the sciatic nerve of Beijing's toad, except for some delay owing to the Internet. Thus, It is demonstrates that the signal in the sciatic nerve of Beijing's toad was successfully regenerated in the sciatic nerve of Nanjing's toad.
In addition, the experiment was also successfully complemented by the same set-up but in reverse direction on the right legs of the same toads.
The concept can be used for the healing training of paralytics.

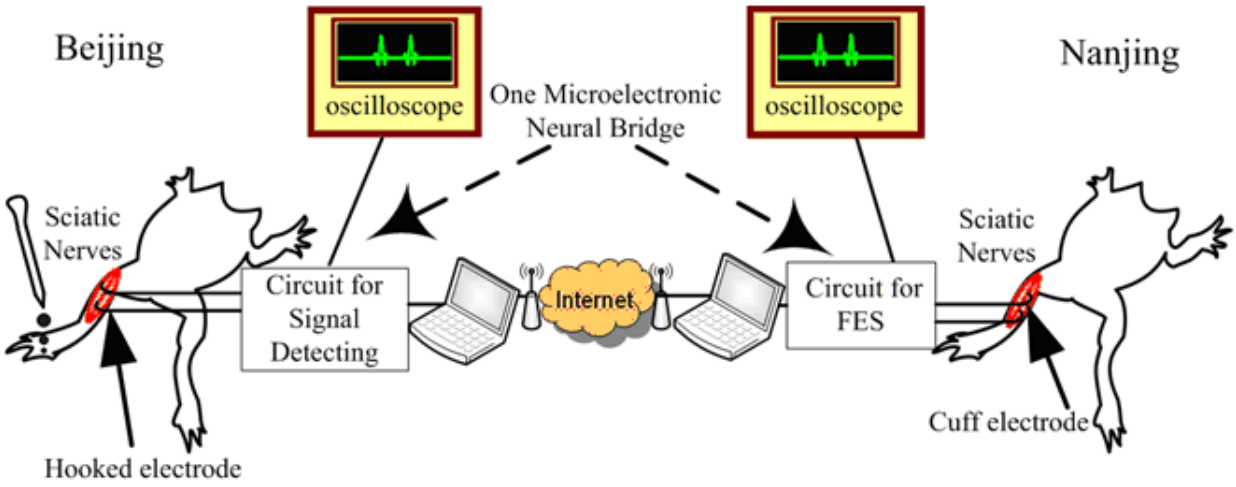

Fig. 1 Schema of the experiment set-up for neural function rebuilding between the sciatic nerves of 2 spinal toads lying in Beijing and Nanjing

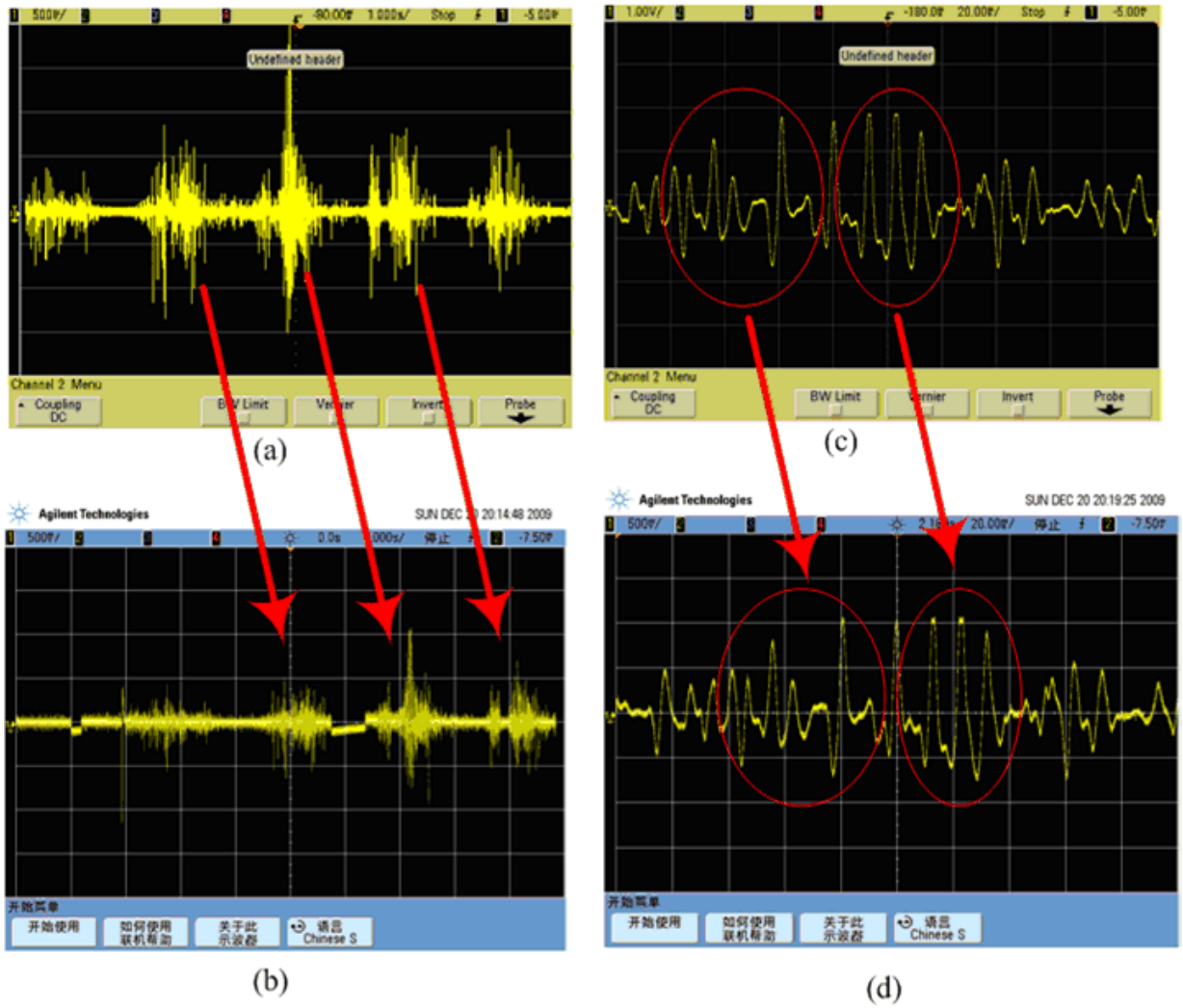

Fig. 2 (a) The detected neural signal from the sciatic nerve of Beijing's toad, (b) The signal monitored on the sciatic nerve of Nanjing's toad, (c) The expending waveform of one part of (a), (d) The expending waveform of the related part of (b)

Disclosures: **Z. Wang:** Research Grant; Natural Science Foundation of China. **X. Lü:** None. **W. Li:** None. **X. Shen:** None. **Z. Huang:** None. **X. Zhao:** None. **L. Du:** None. **X. Gao:** None. **Z. Jiang:** None. **H. Pan:** None. **G. Wang:** None. **S. Xie:** None. **X. Gong:** None. **C. Zhu:** None. **L. Qiu:** None.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.4

Topic: A.08. Transplantation and Regeneration

Support: NIH grant NS055295

NS58830 the Udall Center of Excellence in Parkinson's Disease Research at University of Cincinnati

The Gardner Family Center grant at the Univ. of Cincinnati

Title: Synergistic interactions between endogenous and transplanted neural precursor cells in the parkinsonian rat

Authors: *L. MADHAVAN[1], B. F. DALEY[1], R. L. BOUDREAU[2], B. L. DAVIDSON[2], A. COLE-STRAUSS[1], J. W. LIPTON[3], T. J. COLLIER[1];
[1]Dept Neurol, Univ. of Cincinnati, CINCINNATI, OH; [2]Dept. of Intrnl. Med., Univ. of Iowa, Iowa City, IA; [3]Translational Sci. & Mol. Med., Michigan State Univ., Grand Rapids, MI

Abstract: Neural stem/precursor cell (NPC) based therapeutic strategies for Parkinson's Disease (PD) are generally divided into two approaches: cell transplantation and endogenous cell stimulation. Realistically, future PD cell therapies will most likely involve combining these two approaches, a theme of our current research. Our previous studies in a 6-hydroxydopamine (6-OHDA) rat model of PD suggest a 'synergy' between transplanted and endogenous NPC actions (Madhavan et al, 2009; J. Comp. Neurol; Madhavan et al, Neuropharmacol, 2010). In particular, our work indicates that NPC implantation before the 6-OHDA insult can stimulate an endogenous NPC response and that this response is associated with nigrostriatal neuroprotection and amelioration of behavioral deficits (spontaneous paw placement in cylinder). Further, the transplanted NPCs expressed certain molecules [glial derived neurotrophic factor (GDNF), sonic hedgehog (SHH) and stromal derived factor 1 alpha (SDF1α)] providing a potential molecular basis for the observed phenomenon. Currently, we are investigating mechanisms underlying the phenomenon by examining the roles of (a) endogenous NPCs and (b) abovementioned graft-expressed factors. Specifically, we are studying the role of endogenous NPCs, by inhibiting host NPC proliferation and neurogenesis using cytosine-D-arabinofuranoside (Ara-C). Behaviorally, AraC infused animals which were subsequently grafted with NPCs performed significantly better on the cylinder task than sham controls, but were also significantly worse than NPC grafted animals which had received no prior AraC infusion. Tyrosine hydroxylase (TH) cell counts through the substantia nigra support the behavioral data. These results indicate that endogenous NPCs are contributing, in part, to the observed NPC-mediated neuroprotection. In parallel, the roles of graft-expressed GDNF and SHH are also being determined by using RNA interference (RNAi) techniques. NPCs in which either GDNF or SHH, or both have been silenced have been transplanted into host rats to determine whether or not they contribute to the observed NPC-mediated neuroprotection and endogenous response to transplantation. Overall, our studies will help determine some of the micro-environmental signals fundamental to the exogenous-endogenous stem cell synergism and neuroprotection, and will contribute towards the

development of novel stem cell based therapies for PD.

Disclosures: L. Madhavan: None. **B.F. Daley:** None. **R.L. Boudreau:** None. **B.L. Davidson:** None. **A. Cole-Strauss:** None. **J.W. lipton:** None. **T.J. Collier:** None.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.5

Topic: A.08. Transplantation and Regeneration

Support: Cariplo Foundation to EW, MPA and PI.

TRM-Award funded from the German Federal Ministry of Education and Research (BMBF, PtJ-Bio,0313909) to CH.

Grandi Attrezzature, Università di Milano-Bicocca to EW

Title: Functional regeneration of the meso-cortico-limbic dopaminergic system as a model to study novel neuro-reparative strategies

Authors: E. DOSSI[1], C. HEINE[2,3], L. COLOMBO[4], F. GULLO[1], A. MAFFEZZOLI[1], M. P. ABBRACCHIO[4], H. FRANKE[2], P. ILLES[2], *E. WANKE[1];
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Abstract: We characterized the developmental and regeneration features of a brain circuitry involved in working memory and reward processing, from the ventral tegmental area-substantia nigra [VTA-SN] fibers projecting to the prefrontal cortex [PFC] and the complementary glutamatergic pathways (Seamans and Yang, Prog. Neurobiol. 74:1-58). By utilizing a co-culture system (Heine et al., 2007, Neurosci., 149:165-181; Franke et al., 2003, Neurochem. Int., 42:431-439) adapted to multi electrode platforms (MEAs), we simultaneously recorded (and analyzed) from 60 electrodes, at brief (5-7 days in-vitro, div) and long-term (15-25 div) conditions, both spikes (bandwith 250-5000 Hz) and local field potentials (LFP, bandwith 1-200 Hz) activity (Gullo et al., 2009, J Neurosci. Methods, 181:186-198; Gullo et al., 2010, Front. Neural Circuits, 2010/04). After few days the co-cultures show a spontaneous activity in the form of bursts, trains of action potentials, and LFPs lasting ~0.3 and 1.5 s, respectively.

Surprisingly, the activity increased in parallel with the growth of new projections from one slice to the other and there was no need of evoking activity in VTA to observe activity in PFC. We characterized three types of spontaneous activities as follows: 1) bursts only in VTA-SN, while PFC was silent (VTA events, 59±3%), 2) bursts originating in VTA and rapidly reaching PFC (with a delay of ~80 ms), that is a cross-correlated activity (28±2%) and 3) bursts with synchronous activity in both regions (13±1%). This correlated activity is completely abolished if the new-born projections between the two areas are cut. Preliminary data suggest that the activity of the co-cultures is specifically modulated by dopaminergic, GABAergic and glutamatergic systems. We have also evaluated the damage induced by the application of 1h oxygen-glucose deprivation (OGD) to VTA-SN/PFC co-cultures, focusing on neurons and on the glial population (astroglia, microglia and oligodendroglia), in order to understand the damage extent and modification of the glial response after injury. Preliminary data suggest that, in the PFC, the number of astroglial cells is not affected by OGD, while an induction of microglial cells activation (approx. +50%) and an increased number of oligodendroglial cells (approx. +60%) were detected. Analysis of VTA-SN is under evaluation. We will exploit the ability of endogenous stem/precursor cells of brain parenchyma to sustain the regeneration-remodeling of damaged circuitries and to possibly differentiate to new-born neurons and glia able to replace irreversibly damaged cells.

Disclosures: E. Dossi: None. **C. Heine:** Research Grant; Translational Centre for Regenerative Medicine - Leipzig, University of Leipzig, Germany. **L. Colombo:** None. **F. Gullo:** None. **A. Maffezzoli:** None. **M.P. Abbracchio:** None. **H. Franke:** None. **P. Illes:** None. **E. Wanke:** None.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.6

Topic: A.04. Axon and Dendrite Development

Support: PVA Research Foundation Grant 2573

Title: Substrate stretch enhances axonal outgrowth but inhibits process branching and dendritic outgrowth of motor neurons

Authors: *J. M. COREY[1,3], M. K. LEACH[2], Z.-Q. FENG[4], Y. I. NAIM[1];
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Engin., Southeast Univ., Nanjing, China

Abstract: Since surface topography and mechanical forces play a role in neurite extension and neuronal morphology, we sought to investigate the effects of continual substrate stretching on the development of motor neurons *in vitro*. On stationary planar surfaces, development of cultured neurons has been well described (Dotti and Banker, 1988 J Neurosci). After adhering to a substrate, cultured neurons develop lamellipodia that condense into neurites. A major neurite then predominates to become the axon, and minor processes subsequently mature into dendrites over the course of several days; neurons mature further as neurite lengthening and branching continues. For neurons on stretched surfaces, we hypothesized that all neurites would be longer than those on planar surfaces. We also hypothesized that neurites emanating from the poles of each soma nearest the source of the stretch would experience a higher tensile force, making them more likely to become the axon. E15 rat spinal motor neurons were cultured on a polymer substrate subjected to continuous linear mechanical stretch at one of three rates. The substrate was a poly(lactic-co-glycolic) acid (PLGA) film coated in adsorbed poly-L-lysine. Stretching began 2 h after cell seeding and the substrate increased in length by 150% over the following 48 h period. Unstretched PLGA films and unstretched aligned PLGA fibers were used as controls. For all conditions, neurons expressed tau-1 in the major neurite at 3 DIV. By that time, the average major neurite length on fibers (152 μm) was 57% greater than that observed on unstretched film (97 μm), while stretching at the maximal rate doubled major neurite length (209 μm) compared to unstretched film. Major axons grew from areas of the soma near one of the poles of the soma nearest the source of the stretch. However, a very striking and unpredicted result was that as tension increased, the number and length of minor neurites, as well as overall neurite branching, decreased. This is similar to what is seen on nanofibers at 2 d of growth. These results suggest that while nanotopographical cues and mechanical stretch result in an increase in process length, mechanical stretch may facilitate axon extension at the expense of dendritogenesis.

Disclosures: J.M. Corey, None; **M.K. Leach**, None; **Z. Feng**, None; **Y.I. Naim**, None.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.7

Topic: A.04. Axon and Dendrite Development

Support: NIH R01 EY12873 (CBC)

Deutsche Forschungsgemeinschaft (CGB)

BBSRC (CW)

Wellcome Trust (TB, CGB)

Title: Degenerating tracts do not provide a strong guidance cue for regenerating optic axons: Observations in the astray/robo2 mutant

Authors: *C. WYATT[1], A. EBERT[1], M. M. REIMER[1], K. RASBAND[2], C. B. CHIEN[2], T. BECKER[1], C. G. BECKER[1];
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Abstract: During formation of the optic projection in *astray/robo2* mutant zebrafish, optic axons exhibit rostro-caudal projection errors, ectopic midline crossing and increased arbor termination area. Here we show that many of these errors persist into adulthood. Even when Robo2 function is conditionally reduced only during initial formation of the optic projection, rostral projection errors are retained in adults. Adult errors include massive ectopic optic tracts in the telencephalon. During optic nerve regeneration in *astray/robo2* animals, these tracts are not re-populated and ectopic midline crossing is reduced compared to unlesioned mutants. However, other errors, such as expanded termination areas and ectopic growth into the tectum, are recommitted by regenerating optic axons. Ubiquitous overexpression of Slit2 during regeneration does not elicit major pathfinding phenotypes.
This shows (1) the absence of an efficient correction mechanism for large-scale projection errors of optic axons during development, (2) that degenerating tracts do not provide a strong guidance cue for regenerating optic axons in the adult CNS, and (3) a reduced importance of Robo2 and its ligand, Slit2, for pathfinding of regenerating optic axons relative to development.
Supported by the Wellcome Trust and the BBSRC (to CW).

Disclosures: C. Wyatt, None; **A. Ebert**, None; **M.M. Reimer**, None; **K. Rasband**, None; **C.B. Chien**, None; **T. Becker**, None; **C.G. Becker**, None.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.8

Topic: A.08. Transplantation and Regeneration

Support: NIH/NINDS 1R01 NS054886

Craig H. Neilsen Foundation 161456

Title: Tetracycline-regulated lentiviral neurotrophin-3 gene delivery for axonal bridging after spinal cord injury

Authors: *S. HOU[1], L. NICHOLSON[1], M. TUSZYNSKI[1,2], A. BLESCH[1,3];
[1]Dept. of Neurosciences, UCSD, La Jolla, CA; [2]VA Med. Ctr., San Diego, CA; [3]Univ. of Heidelberg, Heidelberg, Germany

Abstract: Previous studies have shown that injured dorsal column sensory axons can bridge across a C3 lesion site filled with bone marrow stromal cells (BMSC) if axons are guided by a gradient of NT-3 rostral to the lesion site. In the present study we examined whether continuous NT-3 delivery is necessary to sustain regenerated axons beyond the lesion site using tetracycline-regulated (tet-off) NT-3 lentivirus or tet-off-GFP virus as control. In vitro, GFP and NT-3 expression was tightly regulated in 293 cells by doxycycline (Dox) treatment (1.04±0.17 ng (+Dox) vs. 12.13±2.29 ng (-Dox) NT-3/ml/10^6 cells). For in vivo experiments, virus was injected 2.5 mm rostral to a C3 dorsal column lesion filled with BMSC. Animals also underwent stimulation of regenerative cell body responses by conditioning lesions. Immunohistochemical labeling after tet-off-GFP lentivirus injection demonstrated strong GFP expression in the spinal cord of untreated animals (-Dox), whereas animals treated with Dox in the drinking water for 2 weeks showed very weak GFP expression. NT-3 ELISA of spinal cord segments injected with tet-off-NT-3 lentivirus showed significantly higher NT-3 levels (9.82±0.53 ng/g tissue) in the absence of Dox for 4 weeks whereas NT-3 levels in Dox-treated animals (1.11±0.12 ng/g tissue) were not significantly different from animals injected with tet-off-GFP virus (0.40±0.13 (+Dox) and 0.58±0.08 (-Dox) ng/g tissue). Regenerative responses were examined by transganglionic tracing with CTB. Axons regenerated into the cellular graft and beyond the lesion border only when NT-3 gene expression was turned on. In contrast, very few or no axons were found beyond the lesion when NT-3 gene expression was turned off or in animals that received injections of tet-off-GFP lentivirus. Significant differences in axon growth were detected up to 1200 μm rostral to the lesion site. These results demonstrate that NT-3 delivery can be efficiently regulated in vitro and in vivo thereby regulating axonal growth. Current experiments examine whether continuous neurotrophin delivery is necessary to sustain regenerated axons in the lesion site and beyond.

Disclosures: S. Hou, None; **L. Nicholson**, None; **M. Tuszynski**, None; **A. Blesch**, None.

Nanosymposium

318. Transplantation and Regeneration

Location: Room 25A

Time: Monday, November 15, 2010, 8:00 am - 10:15 am

Program Number: 318.9

Topic: A.08. Transplantation and Regeneration

Support: NIH Grant R01NS068128

NIH Grant R01NS054734

NIH Grant R01NS047718

Wings for life foundation

Title: Pten deletion rejuvenates the regenerative ability of adult corticospinal neurons

Authors: *K. LIU[1], Y. LU[1], J. LEE[2], R. SAMARA[2], K. PARK[1], I. SEARS-KRAXBERGER[3], R. WILLENBERG[3], A. TEDESCHI[1], B. CAI[1], B. XU[1], L. CONNOLLY[1], O. STEWARD[3], B. ZHENG[2], Z. HE[1];
[1]Neurol., Children's Hosp. Boston/Harvard Med. Sch., Boston, MA; [2]UCSD, San Diego, CA; [3]UCIrvine, Irvine, CA

Abstract: Despite the essential role of the corticospinal tract (CST) in controlling voluntary movements, successful regeneration of large numbers of injured CST axons beyond a spinal cord lesion has never been achieved. Here we demonstrate a critical involvement of PTEN/mTOR in controlling the regenerative capacity of corticospinal neurons. Upon the completion of development, the regrowth potential of CST axons lost and this is accompanied by a down-regulation of mTOR activity in corticospinal neurons. Axonal injury further diminishes neuronal mTOR activity in these neurons. Forced up-regulation of mTOR activity in corticospinal neurons by conditional deletion of PTEN, a negative regulator of mTOR, enhances compensatory sprouting of uninjured CST axons and even more strikingly, enables successful regeneration of a cohort of injured CST axons past a spinal cord lesion. Furthermore, these regenerating CST axons possess the ability to reform synapses in spinal segments distal to the injury. Thus, modulating neuronal intrinsic PTEN/mTOR activity represents a potential therapeutic strategy for promoting axon regeneration and functional repair after adult spinal cord injury.

Disclosures: K. liu, None; **Y. Lu**, None; **J. Lee**, None; **R. Samara**, None; **K. Park**, None; **I. Sears-Kraxberger**, None; **R. Willenberg**, None; **A. Tedeschi**, None; **B. Cai**, None; **B. Xu**, None; **L. Connolly**, None; **O. Steward**, None; **B. Zheng**, None; **Z. He**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.1

Topic: B.06. Neurotransmitter Release

Support: NIH Grant NS40296

Cleo and Paul Schimmel Fund

Title: Genetic analysis of synaptogyrin's role in the synaptic vesicle cycle

Authors: *R. J. STEVENS, Y. AKBERGENOVA, R. A. JORQUERA, J. T. LITTLETON; MIT, CAMBRIDGE, MA

Abstract: Synaptogyrin and synaptophysin are distantly related synaptic vesicle proteins and are members of the MARVEL domain family, which includes the myelin and lymphocyte (MAL) and occludin families. The MARVEL domain's four transmembrane-helix architecture is conserved across evolution and is putatively involved in vesicle trafficking and membrane apposition events, such as tight junction formation. Although synaptogyrin and synaptophysin were identified as abundant synaptic vesicle proteins over twenty years ago, the precise role of these proteins at the synapse is unknown. Both proteins have been implicated in several processes, including vesicle biogenesis and regulation of SNARE complex assembly. A knockout of synaptophysin and synaptogyrin in mice revealed minor defects in synaptic plasticity, possibly due to the functional redundancy of other gyrin and physin family members in mammals. To further elucidate the role of these proteins we have generated and characterized a *synaptogyrin* null mutant in *Drosophila*, whose genome encodes a single synaptogyrin and lacks a synaptophysin homolog. *Synaptogyrin* mutants are viable and fertile with no overt motor defects or decrease in lifespan. Evoked responses at the larval neuromuscular junction are of normal amplitude. However, during high-frequency stimulation, *synaptogyrin* mutant larvae have increased facilitation and augmentation, indicating abnormal regulation of vesicle fusion during strong stimulation. Ultrastructural analysis revealed no change in synaptic vesicle number. However, *synaptogyrin* mutants have an increased variability in synaptic vesicle size and an increase in average synaptic vesicle diameter. Furthermore, the resolution of endocytotic cisternae into synaptic vesicles following robust release induced by high potassium is defective in *synaptogyrin* mutants. While synaptogyrin is not required for basal synaptic transmission, our data indicate a role in the synaptic vesicle exo-endocytosis cycle that is manifest at high stimulation frequencies. Current efforts are underway to determine if synaptogyrin is required for normal synaptic vesicle reformation when the endosomal recycling pathway is triggered during elevated rates of vesicle fusion.

Disclosures: R.J. Stevens, None; **Y. Akbergenova**, None; **R.A. Jorquera**, None; **J.T. Littleton**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.2

Topic: B.06. Neurotransmitter Release

Support: A.D. is supported by a Boehringer Ingelheim Fonds PhD Fellowship.

S.O.R. is supported by a Starting Grant (FP7-NANOMAP) from the European Research Council.

Title: Synaptic vesicle recycling in vivo

Authors: *A. DENKER[1,2], I. BETHANI[1], K. KRÖHNERT[1], S. O. RIZZOLI[1];
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Abstract: To ensure reliable neuronal communication, chemical synapses use small neurotransmitter-containing organelles: the synaptic vesicles. Upon arrival of an action potential, the vesicles fuse with the plasma membrane, releasing neurotransmitter into the synaptic cleft and thereby stimulating the postsynaptic cell. To maintain membrane homeostasis, the vesicular membrane is then retrieved via endocytosis and refilled with neurotransmitter in what is termed vesicle recycling.
Synapses can contain from several hundred to nearly a million synaptic vesicles (as for instance in the frog neuromuscular junction; Rizzoli and Betz, 2005). However, in vitro stimulation at frequencies well above the physiological limit cause the recycling of only a fraction of these vesicles, indicating that even less vesicles might be required to support continuous recycling in vivo.
To investigate the use of vesicles in vivo, we injected a fluorescent marker into living animals, which were then returned to their environment (allowing them to eat, sleep, communicate or move). During this time, synaptic activity was monitored by dye uptake into recycling synaptic vesicles. After a specified amount of time, muscles of interest were dissected and fixed, followed by high-resolution microscopy to determine the number of vesicles used in vivo. To interpret the results in a broader (evolutionary) context, several different model organisms, ranging from insects and nematodes over fish and amphibians to mammals, were investigated.
For all organisms and conditions tested, only a minority (~1-5%) of vesicles were recycled over several hours. We therefore conclude that the majority of vesicles might not function in

neurotransmitter release.
References:
Rizzoli, SO, Betz, WJ (2005) Synaptic vesicle pools. Nat. Rev. Neurosci. 6, 57-69

Disclosures: A. Denker, None; **I. Bethani**, None; **K. Kröhnert**, None; **S.O. Rizzoli**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.3

Topic: B.06. Neurotransmitter Release

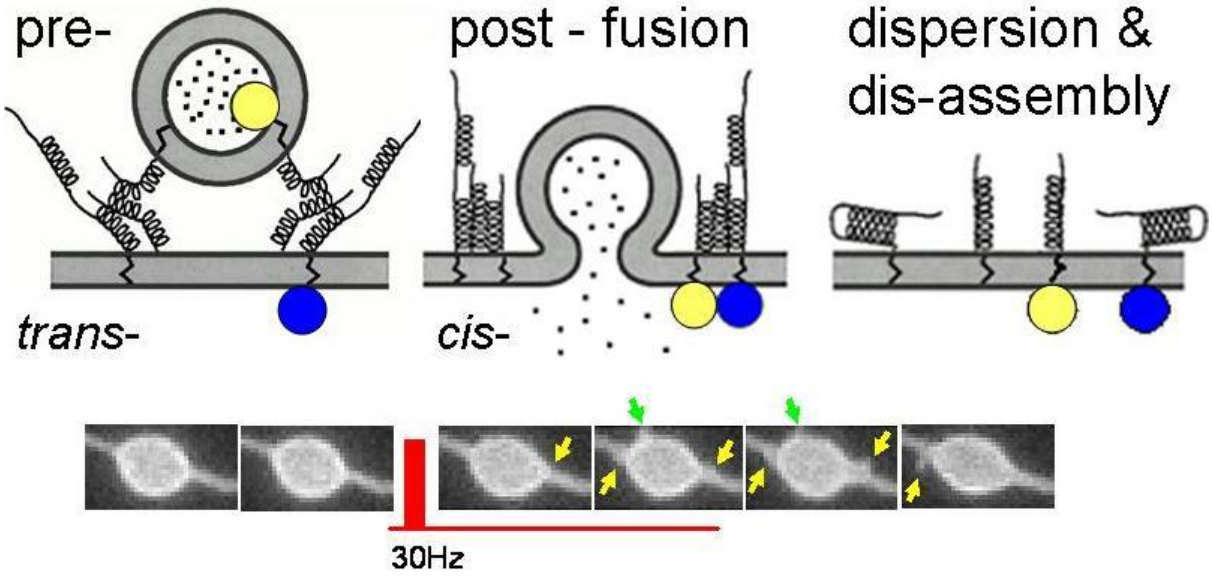
Support: 5R01NS054760-04

Title: Assessment of cis-SNARE complex formation and different modalities of syntaxin dispersion during synaptic transmission in hippocampal neurons

Authors: *V. DEGTYAR[1,2], R. S. ZUCKER[2];
[1]Bogomoletz Inst. Physiol, Kiev, Ukraine; [2]Mol. and Cell Biol., Univ. of California, Berkeley, CA

Abstract: Syntaxin1A and VAMP2 form *cis* SNARE complex upon completion of synaptic vesicle (SV) fusion at the presynaptic membrane. To study dynamics of their interaction and motion during synaptic transmission, we transiently expressed C-termini-labeled fluorescent constructs of syn1A-Cerulean and VAMP2-Citrine either alone or together in cultured hippocampal neurons. Efficiently transmitting synaptic boutons were identified by either FM4-64 destaining during stimulation or an increase of VAMP2-Cit fluorescence due to synaptopHluorin effect. Syn1A-Cer fluorescence decreased in active zones regions during stimulation and recovered afterwards. Dispersion of syn1A was seen as a transient increase of Cer fluorescence in areas adjacent to active zones, and it revealed notable heterogeneity in kinetics and spatial distribution. In addition to monotonic waves spreading in all directions consistent with diffusion, we observed transitions between quasi-steady levels of elevated fluorescence in loci adjacent to active zones that were non-uniform in different directions and sometimes expanded beyond boundaries of fluorescence seen before stimulation. The reduction in Cer fluorescence was stronger when syn1A-Cer was co-expressed with VAMP2-Cit, as compared to the Syn1a-Cer alone. The decrease remains if measured in larger areas that include dispersed probe, consistent with increased donor-quenching FRET between C-terminal Cer & Cit probes in *cis*-SNARE complexes. Without stimulation, repeated partial bleaching of VAMP2-Cit

in one synaptic bouton by 514-nm laser light resulted in repeated comparable increases in Syn1a-Cer fluorescence, consistent with donor de-quenching due to resting FRET. This protocol after stimulation produced an even stronger increase in donor fluorescence. Thus, we identify two processes causing a reduction of Cer fluorescence in active zones during synaptic transmission: dispersion of syn1A following SV fusion, and an increase in FRET between syn1A-Cer probe as a fluorescence donor and VAMP2-Cit as an acceptor.



Disclosures: V. Degtyar, None; **R.S. Zucker**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.4

Topic: B.06. Neurotransmitter Release

Title: Stochiometry of SNARE complexes sufficient for fast calcium-triggered exocytosis in central nervous system synapses

Authors: *R. SINHA, J. KLINGAUF;
Max-Planck Inst. For Biophysical Chem., Goettingen, Germany

Abstract: Assembly of low-energy SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein complexes drives synaptic vesicle (SV) fusion. But it is not

known, how many SNARE complexes are minimally needed for SV fusion in central nervous system synapses. We imaged single vesicle fusion events using the genetically encoded probe synaptopHluorin (spH), a pH-sensitive GFP fused to the luminal domain of the SV SNARE synaptobrevin 2. Quantitative single molecule experiments revealed that only 2-3 spH molecules are incorporated per SV. Surprisingly, when overexpressed on a genetic null background, this low spH copy number was sufficient to rescue evoked SV fusion. SVs expressing only one spH, however, were unable to rapidly fuse upon stimulation. Thus, two SNARE complexes are necessary and sufficient for SV fusion during fast synaptic transmission.

Disclosures: R. Sinha, None; **J. Klingauf**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.5

Topic: B.06. Neurotransmitter Release

Support: NARSAD YIA to PGUR

Title: Role of syntaxin 1 phosphorylation in neurotransmitter release: Implications for schizophrenia

Authors: H. MATTHIES[1], M. A. CASTILLO[2], A. GALLI[1], *P. G. ULERY-REYNOLDS[3]; [1]Mol. Physiol. and Biophysics and Ctr. for Mol. Neurosci., Vanderbilt Univ., Nashville, TN; [2]Neurol., [3]Neurol & Psychiatry, Univ. Texas Southwestern Med. Ctr., DALLAS, TX

Abstract: Syntaxin 1 (stx 1) is one of three pre-synaptic proteins that form a heterotrimer known as the SNARE complex, which is necessary for neurotransmitter release. While little is known about how stx 1 function is regulated, it has been shown that its interaction with MUNC18, another pre-synaptic protein necessary for neurotransmitter release, requires that stx 1 be in a "closed" conformation, and it also requires the N-terminus of stx 1. The N-terminus of stx 1 has been shown to be phosphorylated by the kinase ck2; however the role of phosphorylation in Stx 1 conformation and function remains largely unknown. We have recently shown that in the pre-frontal cortex of schizophrenia cases, both ck2 and phospho-stx 1 are decreased, and that lower levels of phospho-stx 1 correlate with reduced binding of Stx 1 to MUNC18, and with decreased SNARE complex formation. In the present study, we aimed to assess the role of stx 1 phosphorylation in neurotransmitter release, not only to gain insight into basic synaptic transmission, but to understand how a deficit in stx 1 phosphorylation may contribute to the

pathophysiology of schizophrenia.

Disclosures: H. Matthies, None; **M.A. Castillo**, None; **P.G. Ulery-Reynolds**, None; **A. Galli**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.6

Topic: B.06. Neurotransmitter Release

Support: CIHR Grant MOP-82827

Title: Cholesterol sensitive kinases regulate neurotransmitter release

Authors: *A. J. SMITH[1], S. SUGITA[1,2], M. P. CHARLTON[1];
[1]Univ. Toronto, Toronto, ON, Canada; [2]Toronto Western Res. Inst., Toronto, ON, Canada

Abstract: Changes in membrane cholesterol content can alter protein kinase activity, however it is not known if kinases regulating transmitter release are sensitive to membrane cholesterol content. Here we have used the cholesterol extracting agent methyl-β-cyclodextrin to measure the effects of acute cholesterol reduction on transmitter release from cultured cerebellar neurons. Cholesterol depletion increased the frequency of spontaneous transmitter release without altering the amplitude and time course of mEPSCs. Evoked transmitter release was decreased by cholesterol extraction and the paired pulse ratio was also decreased. Alterations in synaptic transmission were not associated with failure of action potential generation or changes in presynaptic Ca2+ signaling. Both the increase in mEPSC frequency and the change in paired pulse ratio were blocked by the broad spectrum protein kinase inhibitor staurosporine. The increase in mEPSC frequency was also sensitive to selective inhibitors of PKC and PKA. Our results therefore demonstrate that the activity of presynaptic protein kinases that regulate spontaneous and evoked neurotransmitter release is sensitive to changes of membrane cholesterol content.

Disclosures: A.J. Smith, None; **M.P. Charlton**, None; **S. Sugita**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.7

Topic: B.06. Neurotransmitter Release

Support: NIH Grant GM85791

Title: Complexins facilitate exocytosis and synchronize release by coupling vesicles and calcium channels

Authors: M.-Y. LIN[1], J. G. ROHAN[2], K. REIM[4], N. BROSE[4], C.-P. KO[1], *R. H. CHOW[3]; [1]Biol. Sci., USC, Los angeles, CA; [2]Keck USC Sch. Med., Los angeles, CA; [3]Keck USC Sch. Med., LOS ANGELES, CA; [4]Max Planck Inst. for Exptl. Med., Goettingen, Germany

Abstract: Complexins (Cplxs) proteins bind tightly and with 1:1 stoichiometry to the SNARE complex. There are four mammalian isoforms comprising two subfamilies (1, 2 vs. 3, 4). The role of complexin is controversial, some arguing for a facilitatory, some for an inhibitory, and some for a mixed role . Here, we examined the structure and synaptic transmission of neuromuscular junctions (NMJs) in transgenic mice lacking Cplx 1 (Cplx 1 KO) and performed parallel studies of exocytosis in Cplx 2 KO chromaffin cells.

We discovered that complexin 1 is the major isoform expressed at the mouse NMJ. In Cplx 1 KO mice, both the spontaneous (miniature end-plate potential, MEPP) and evoked (end-plate potential, EPP) vesicle release were significantly decreased. In addition to the decrease in the number of vesicles released, the timing of vesicle release was also greatly disrupted in Cplx 1 KO NMJs. We observed an increase in EPP width, greater variations in time to peak, and slower rise and decay times. High-frequency stimulation did not induce the EPP depression seen in control NMJs, but instead induced facilitation in Cplx 1 KO NMJs. We hypothesize that the desynchronization of vesicle release and facilitation upon high frequency stimulation results from a decrease in the number of vesicles co-localized with calcium channels, which constitute the immediately releasable pool (IRP). To test this hypothesis further, we monitored exocytosis in chromaffin cells using standard pulse protocols and found a decrease in IRP, relative to the total readily releasable pool (RRP), in chromaffin cells lacking Cplx 2, the only complexin isoform in chromaffin cells. The similarity of function between Cplx 1 and 2 was demonstrated by the successful rescue of IRP size with Cplx 1 in Cplx 2 KO chromaffin cells.

Our data indicate that lack of Cplx 1 reduces and desynchronizes vesicle release at the mouse NMJ. The decreased neuromuscular transmission supports the hypothesis that complexins positively regulate exocytosis. The desynchronization of evoked vesicle release and facilitation upon high frequency stimulation at the Cplx 1 KO NMJ further suggests that complexins might be involved in regulating IRP size, which is supported by the smaller IRP/RRP in Cplx 2 KO chromaffin cells.

Disclosures: M. Lin, None; **J.G. Rohan**, None; **K. Reim**, None; **N. Brose**, None; **C. Ko**, None; **R.H. Chow**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.8

Topic: B.07. Synaptic Transmission

Support: EMBO fellowship to I.M.

Title: Triggering clathrin uncoating is the major function of endophilin at neuronal synapses

Authors: *I. MILOSEVIC[1], S. GIOVEDI[1], X. LOU[1], A. RAIMONDI[1], S. PARADISE[1], O. CREMONA[2], P. DE CAMILLI[1];
[1]Yale Univ., New Haven, CT; [2]IFOM and Univ. Vita–Salute San Raffaele, Milano, Italy

Abstract: Retrieval of synaptic vesicle membranes from the plasma membrane by compensatory clathrin-mediated endocytosis is critical for maintaining synaptic transmission. Endophilin A (henceforth referred to as endophilin) is a bilayer binding protein and an interactor of the GTPase dynamin and of the PI(4,5)P_2 phosphatase synaptojanin, whose importance in synaptic vesicle recycling is well recognized. Yet, the precise function of endophilin at synapses is unclear. We have generated knock-out (KO) mice for three endophilins (1, 2 and 3), which are all expressed in neurons. Mice lacking single endophilins had no obvious pathological phenotype, animals lacking both endophilin 1 (the most abundant endophilin at synapse) and 2 (the ubiquitous isoform) had spontaneous seizures and failed to thrive, while the absence of all three endophilins caused perinatal lethality. Double and triple KO neurons showed strong defects in synaptic transmission and synaptic vesicle recovery, and had reduced synaptic vesicle number. Surprisingly, given the property of endophilin to bind dynamin and to assemble at coated pits upstream of dynamin, endophilin mutant neurons showed no increase in clathrin-coated pit number in nerve terminals. A striking accumulation of clathrin-coated vesicles was the major phenotype, which is reminiscent of defects observed at synapses lacking synaptojanin 1 (whose PI(4,5)P_2 phosphatase activity promotes shedding of the clathrin adaptors), or auxilin (a protein that cooperates with the ATPase Hsc70 in the disassembly of the clathrin lattice). Thus, the major function of endophilin in synaptic vesicle recycling at a mammalian synapse is clathrin uncoating. Most likely, functions of endophilin in fission are redundant with those of other proteins, while its uncoating function, likely mediated by the recruitment of synaptojanin, is rate

limiting.

Disclosures: I. Milosevic, None; **S. Giovedi**, None; **X. Lou**, None; **A. Raimondi**, None; **S. Paradise**, None; **O. Cremona**, None; **P. DE Camilli**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.9

Topic: B.05. Transporters

Support: A.P. Giannini Foundation

ABMRF

NARSAD

State of California for Medical Research on Alcohol and Substance Abuse through UCSF

NIDA K01DA026504

Title: Optogenetic glutamate corelease from dopamine terminals in the nucleus accumbens of adult mice is dependent on VGLUT2

Authors: *T. S. HNASKO[1], G. D. STUBER[3], J. P. BRITT[3], A. BONCI[3], R. H. EDWARDS[2]; [1]Physiol., [2]Neurol. & Physiol., Univ. of California, San Francisco, San Francisco, CA; [3]Ernest Gallo Clin. & Res. Ctr., Emeryville, CA

Abstract: Previous work has suggested that some midbrain dopamine neurons are capable of glutamate corelease, but this phenomenon remains poorly understood. Here, we expressed the light-activated cation channel Channelrhodopsin-2 (ChR2) in genetically defined midbrain dopamine neurons to stimulate exocytosis specifically from dopaminergic terminals in both the nucleus accumbens (NAc) shell and dorsal striatum of brain slices from adult mice. Optical activation resulted in robust glutamate-mediated excitatory postsynaptic currents in all medium spiny neurons examined in the NAc shell. In contrast, optically evoked glutamatergic currents were nearly undetectable in the dorsal striatum. The results provide definitive physiological evidence for glutamate release by mature dopamine neurons projecting to the NAc shell, but not

to the dorsal striatum. Further, we used a conditional knockout (cKO) mouse lacking the vesicular glutamate transporter VGLUT2, specifically in dopamine neurons, to determine whether VGLUT2 is required for the exocytotic release of glutamate from dopamine neurons. We find that the conditional knockout completely abolishes all optically evoked glutamate release. Additionally, cKO mice demonstrate a reduced locomotor response to cocaine, apparently due to a reduction in dopamine stores in the ventral striatum. Our findings suggest that the vesicular monoamine transporter VMAT2 and VGLUT2 colocalize to a population of synaptic vesicles. Vesicular glutamate transport acidifies these synaptic vesicles through a mechanism distinct from chloride, thereby promoting monoamine storage into these same vesicles. Thus VGLUT2 expression by dopamine neurons leads both to the presynaptic enhancement of dopamine quantal size and postsynaptic activation of glutamate receptors.

Disclosures: T.S. Hnasko, None; **G.D. Stuber**, None; **J.P. Britt**, None; **A. Bonci**, None; **R.H. Edwards**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.10

Topic: B.05. Transporters

Support: NIH NS15047

Title: Efflux of stored acetylcholine from intact synaptic-like microvesicles

Authors: *S. M. PARSONS[1], A. MULAKALURI[2];
[1]Univ. California, SANTA BARBARA, CA; [2]Neurosci. Res. Inst., Univ. California, Santa Barbara, CA

Abstract: The vesicular acetylcholine transporter (VAChT) takes up cytoplasmic ACh and stores it for exocytosis from nerve terminals as a quantum. Little is known about the dynamics of ACh while it is in storage. For example, does stored ACh leak from synaptic vesicles into cytoplasm, and is it exchanged with cytoplasmic ACh after the vesicle is full? These phenomena were investigated here with a preparation of synaptic-like microvesicles in postnuclear supernatant obtained from $PC12^{A1234.7}$ cells transfected with human VAChT. Uptake and release of [^{3}H]ACh in the presence of saturating ATP was characterized with filtration assays and the potent inhibitory drug aminobenzovesamicol (ABV). An ATP regeneration system was developed that sustains ATP in postnuclear supernatant for 60 min. Uptake of [^{3}H]ACh by the

microvesicles reached a constant amount by 30 min and was maintained for an additional 30 min. The amount of uptake at 30 min was approximately linear with respect to the concentration of postnuclear supernatant. It exhibited saturation as a function of the external concentration of [^{3}H]ACh. In subsequent experiments, uptake first was allowed to reach the maximal amount. In leakage experiments, an excess of ABV then was added and [^{3}H]ACh content was monitored for an additional 30 min. About two-thirds of the stored [^{3}H]ACh leaked. In exchange experiments, the suspension of vesicles was diluted 10-fold into the same concentrations of nonradioactive ACh and ATP to maintain ionic and electrical gradients, and [^{3}H]ACh content was monitored for an additional 30 min. Most of the stored [^{3}H]ACh was lost at a rate about twice that of leakage. The results indicate that in synaptic-like microvesicles, exchange of ACh through VAChT, leakage of ACh through a VAChT-independent pathway, and steady-state filling by ACh occur.

Disclosures: S.M. Parsons, None; **A. Mulakaluri**, None.

Nanosymposium

319. Presynaptic Mechanisms

Location: Room 6F

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 319.11

Topic: B.06. Neurotransmitter Release

Support: DK is supported by a research grant from the German Ministry for Education and Research (BMBF, Nanolive)

Title: High and low mobility stages in the synaptic vesicle cycle

Authors: *D. KAMIN[1], M. A. LAUTERBACH[2], V. WESTPHAL[2], S. W. HELL[2], S. O. RIZZOLI[1];
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Abstract: Synaptic vesicles move to the active zone to release their contents into the synaptic cleft, and are subsequently reformed and transported back onto the vesicle cluster. This classical synaptic vesicle cycle has been described in molecular detail; however, the general mobility of the vesicles is largely unknown. Here, we describe the vesicle mobility throughout the synaptic vesicle cycle using both conventional and subdiffraction-resolution stimulated emission depletion (STED) fluorescence microscopy. Video-rate STED imaging and subsequent tracking of fluorescently labeled single synaptic vesicles revealed that recently endocytosed vesicles were highly mobile, for a substantial period of time. Afterwards they underwent a maturation process

which resulted in their incorporation into pre-existing vesicle clusters, where the vesicles exhibited only little mobility. Despite the differences in mobility, fluorescence recovery after photobleaching (FRAP) measurements indicated that both recently endocytosed and mature vesicles were exchanged between synapses. Physiological stimulation did not appear to affect the mobility of either recently endocytosed or mature vesicles. However, blocking synaptic activity by use of tetrodotoxin decreased the mobility of recently endocytosed vesicles. After exocytosis, the vesicle material finds itself temporarily on the plasma membrane; the mobility of such vesicle components was found to be relatively limited, and it increased when higher quantities of vesicle material were fused onto the plasma membrane. Based upon these findings we suggest a new model of vesicle movement in the synaptic vesicle cycle, which relies on both high and low mobility states.

Disclosures: D. Kamin, None; **M.A. Lauterbach**, None; **V. Westphal**, None; **S.W. Hell**, None; **S.O. Rizzoli**, None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.1

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant AG00538

NIH Grant AG033069

Larry L. Hillblom Foundation Grant

Title: Site-specific denaturation and Raman spectroscopy reveal structural differences between the Aβ oligomers

Authors: *L. BREYDO[1], D. KUROUSKI[2], S. RASOOL[1], S. MILTON[1], J. WU[1], I. V. LEDNEV[2], C. G. GLABE[1];
[1]Mol. Biol. and Biochem., Univ. of California Irvine, Irvine, CA; [2]Chem., Univ. of Albany, Albany, NY

Abstract: Aggregation of Aβ peptides is a major contributor to Alzheimer’s disease. Oligomeric forms of these peptides are especially interesting due to their high neurotoxicity and potentially important role in disease progression. However, structures of Aβ oligomers are currently

unknown. Based on their reactivity with conformational antibodies, we have established that Aβ oligomers can be divided into at least two structural classes: prefibrillar (PFOs) and fibrillar (FOs) oligomers. Here we have compared site-specific conformational stability of Aβ40 fibrils and both classes of oligomers by introducing cysteine residues throughout the sequence of Aβ40, labeling them with acrylodan, and investigating their guanidine thiocyanate-induced denaturation.
We found that Aβ40 fibrils display cooperative unfolding with high stability towards denaturation and moderate water accessibility of the fluorophore consistent with their known structures. FOs also showed cooperative unfolding with lower stability and higher water accessibility supporting our earlier hypothesis that they represent fragments of protofibrils with β-sheet rich structure. Most tested residues in fibrils and FOs unfolded with $C_{1/2}$ values similar to those for global unfolding of these structures. Solvent accessibility and stability towards denaturation of PFOs were fairly low and varied with hydrophobicity of the peptide sequence suggesting more flexible, possibly molten globule-like structure. FTIR, CD and deep ultraviolet Raman spectra of oligomers and fibrils showed that secondary structures of FOs and fibrils were quite similar while PFOs contained much lower proportion of β-sheets. Structural differences between the oligomer classes observed in this study correlate with their differential recognition by conformational antibodies and may be related to differences in their neurotoxicity.

Disclosures: L. Breydo: None. **D. Kurouski:** None. **S. Rasool:** None. **S. Milton:** None. **J. Wu:** None. **I.V. Lednev:** None. **C.G. Glabe:** Consultant/Advisory Board; Kinexis.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.2

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant AG027818

granted the usage of NSF TeraGrid resources provided by Purdue University

Title: Elucidation of amyloid β-protein oligomerization pathways in the absence and presence of toxicity inhibitors: A multiscale computational study

Authors: *B. URBANC[1], B. BARZ[1], M. BETNEL[2], L. CRUZ[1], G. BITAN[3], D. B. TEPLOW[3]; [1]Physics Dept., Drexel Univ., Philadelphia, PA; [2]Physics Dept., Boston Univ., Boston, MA; [3]Neurosci., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Oligomers of amyloid β-protein (Aβ) play a central role in the pathology of Alzheimer's disease. Of the two predominant Aβ alloforms, Aβ40 and Aβ42, Aβ42 is more strongly implicated in the disease. We elucidated oligomerization of Aβ40 and Aβ42, and their Arctic mutants ([E22G]Aβ40 and [E22G]Aβ42), using discrete molecular dynamics (DMD) with a four-bead protein model, backbone hydrogen bonding, and residue-specific interactions due to hydropathy and charge. The characteristic oligomer size distributions of these four peptides agreed with prior experimental findings. Structural analysis revealed that the C-terminal region played a key role in Aβ42 oligomerization. The N-terminal region A2-F4 was involved in Aβ40, but not in Aβ42, oligomerization. The oligomer structures of both Arctic peptides resembled that of Aβ42 more than they did Aβ40, consistent with their potentially more toxic nature. Aβ40 and Aβ42 dimer conformers were further assessed for stability using all-atom MD with explicit water using NAMD (version 2.7b1), the CHARMM force field, and TIP3P water. Consistent with the DMD predictions, in Aβ42 dimers, the N-terminal region D1-H13 was significantly more disordered and exposed to solvent than in Aβ40 dimers. We then applied the DMD approach to study Aβ42 oligomer formation in the presence of three Aβ-derived C-terminal fragments (CTFs): Aβ(30-40), Aβ(31-42), and Aβ(39-42), which were previously shown to inhibit Aβ42 neurotoxicity in cell cultures. We showed that Aβ42 coassembled with CTFs to form Aβ42/CTF heterooligomers. In the process of assembly, CTFs inserted themselves in-between Aβ42 molecules, thereby reducing intermolecular contacts among Aβ42 molecules within heterooligomers in a concentration-dependent manner. The β-strand structure in Aβ42 was reduced by up to 3.4-fold, 2.8-fold, and 1.7-fold, in the presence of Aβ(31-42), Aβ(30-40), and Aβ(39-42), respectively. The solvent exposure of the region D1-D7 of Aβ42 within Aβ42/CTF heterooligomers was also reduced relative to pure Aβ42 oligomers, resembling the solvent exposure found in pure Aβ40 oligomers. These in vitro-driven computational results suggest that the increased β-sheet propensity of Ab42, or the solvent exposure of the D1-D7 region in Aβ42, relative to Aβ40, might be key structural elements mediating Aβ42 neurotoxicity.

Disclosures: B. Urbanc, None; **B. Barz**, None; **M. Betnel**, None; **L. Cruz**, None; **G. Bitan**, None; **D.B. Teplow**, None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.3

Topic: C.02. Alzheimer's disease and other dementias

Support: Conacyt Gran 59187

Title: Amyloid beta oligomers decrease hippocampal network activity: Involvement of the Integrin/FAK/Fyn/GSK3 signaling pathway

Authors: H. BALLEZA-TAPIA, A. MÁRQUEZ-RAMOS, A. HUANOSTA-GUTIÉRREZ, A. ADAYA-VILLANUEVA, F. CALVO-VANEGAS, *F. PENA;
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Abstract: There is growing evidence that early stages of Alzheimer's disease (AD) may be due to neuronal network dysfunction produced, at least in part, by the actions of soluble forms of amyloid beta protein (ABeta). Recently, we have shown that an oligomerized ABeta1-42 solution (osABeta) decreases hippocampal spontaneous network activity (hSNA), and that such effect correlates with a reduction in synaptic transmission. We have also demonstrated a reduction in hSNA produced by the short sequence ABeta25-35 which is not present in slices obtained from a Fyn knock out mouse. Here, we aimed to characterize the intracelular pathway as well as the membrane receptor associated with the reduction of hSNA induced by osABeta. To do so, we performed extracellular field recordings of spontaneous activity in the CA1 area of hippocampal slices and tested the effect of osABeta in the absence and presence of antagonist or inhibitors of membrane receptors ans intracellular kinases, respectively. First of all, we found that antagonizing integrins with echistatin prevents the osABeta-induced reduction in hSNA. In agreement to our previous finding, osABeta-induced reduction in hSNA was absent after blocking Fyn kinase with the src-family-kinases inhibitor PP2 or in slices obtained from Fyn knock out mice. Due to the fact that integrins can activate Fyn trough the recruitment of FAK kinase we tested whether or not an inhibitor of FAK migth prevent osABeta-induced reduction in hSNA and found that an specific inhibitor of such kinase (PF573228) abolishes osABeta-induced reduction in hSNA. Finally, downstream of Fyn kinse there are several targets including GSK3. Here, we found that inhibiting GSK3, either acutelly or chronically, prevents osABeta-induced reduction in hSNA. Moreover, indirect activation of GSK3, with wortmmanin induced a similar inhibición of hSNA as the one produced by osABeta. To suport these electrophysiological findings, we demonstrated that under identical conditions osABeta increase Fyn-mediated GSK3 tyrosine phosphorylation. In conclusion, our data show that Abeta oligomers disrupts hippocampal network through the activation of a signaling pathway including Integrins/FAK/Fyn/GSK3.

Disclosures: H. Balleza-Tapia, None; **F. Pena**, None; **A. Márquez-Ramos**, None; **A. Huanosta-Gutiérrez**, None; **A. Adaya-Villanueva**, None; **F. Calvo-Vanegas**, None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.4

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH grant P01AG03012801

Alzheimer's association grant ZEN-08-99900

University of Illinois-Chicago CCTS grant UL1RR029879

Anonymous foundation grant

Title: The effects of human apoE on Amyloid-beta pathology in a novel transgenic mouse model

Authors: *K. L. YOUMANS, K. LAXTON, L. M. JUNGBAUER, C. YU, M. LADU;
Univ. Of Illinois Chicag, CHICAGO, IL

Abstract: Alzheimer's disease (AD) is the most common form of dementia among the elderly. Apolipoprotein E (apoE) is the primary genetic risk factor for AD. Human apoE has 3 isoforms: apoE2, apoE3, and apoE4. ApoE2 decreases AD risk 2-4 fold versus apoE3, whereas apoE4 increases risk 4-8 fold. Causal factors for familial AD (FAD) are autosomal dominant mutations that increase the 42 amino acid isoform of amyloid-β peptide (Aβ42). Amyloid plaques and neurofibrillary tangles are pathological hallmarks of AD. However, recent studies show little correlation between plaques and dementia, while soluble oligomeric Aβ42 and/or intraneuronal accumulation of Aβ42 appear toxic. Although apoE4 increases extracellular Aβ42 plaque load, an apoE isoform-specific effect on the accumulation of soluble, oligomeric or intraneuronal Aβ42 remains unclear. Based on in vitro data, our hypothesis is that apoE4 acts synergistically with these toxic forms of Aβ42 to decrease neuronal viability. To test this hypothesis in vivo, we developed a novel mouse model that expresses the human isoforms of apoE (E2, E3 or E4) and significantly over-produces Aβ42. Preliminary results indicate: 1) This AD transgenic mouse model accurately recapitulates the regional development of Aβ42 pathology in humans, initiating in the subiculum and deep frontal cortex; 2) This process is apoE isoform-specific at 4 months: the greatest accumulation of Aβ2 develops with apoE2 whereas 3) intraneuronal Aβ42 is lowest with apoE2 and highest with apoE4; 4) Total Aβ42 and soluble Aβ42 increase in the brain specifically with apoE4; and 5) Loss of synaptophysin is greatest with apoE4 and does not correlate with Aβ42 accumulation. Results of this study will allow further investigation of the mechanism behind the relationship between apoE and Aβ42 and potentially lead to therapeutic interventions for AD.

Disclosures: K.L. Youmans, None; **C. Yu**, None; **K. Laxton**, None; **M. LaDu**, None; **L.M. Jungbauer**, None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.5

Topic: C.02. Alzheimer's disease and other dementias

Support: Alzheimer's Association Zenith Award

Title: Early accumulation of the amyloid-β peptide in human basal forebrain cholinergic neurons

Authors: S. VAHEDI[1], A. BAKER-NIGH[1], D. RIASCOS[1], *C. GEULA[2];
[1]Cognitive Neurol. and Alzheimer's Dis. Ctr., Northwestern Univ., Chicago, IL; [2]Cogn Neurol & Alzhei Dis Cent, Northwestern Univ. Med. Sch., CHICAGO, IL

Abstract: The presence and toxicity of pathologic proteins, such as the amyloid-β peptide (Aβ), in neurodegenerative disorders has been the subject of extensive experimental attention. However, the reason(s) for selective neuronal vulnerability in these disorders is unknown. The cholinergic neurons of the basal forebrain (BFCN) are characterized by early, selective and severe loss in Alzheimer's Disease (AD) and other neurodegenerative disorders that afflict the elderly. In the present set of experiments, we demonstrate accumulation of Aβ in the human BFCN early in the course of life. Immunohistochemical and Western blot analyses were carried out on fixed sections and basal forebrain homogenates, respectively. Polyclonal antibodies to Aβ, as well as antibodies 6E10, 4G8 and a specific antibody that recognizes Aβ 1-42 were used. All antibodies visualized Aβ immunoreactivity in the BFCN of young cases (<65 years) in tissue sections. Detectable Aβ was seen in the BFCN in the youngest cases studied (20- and 26-year old). The antibody to Aβ 1-42 demonstrated relatively selective presence of Aβ within the BFCN as cortical neurons displayed background staining only. Antibodies against the amyloid precursor protein (APP) from which Aβ is derived, visualized the same staining pattern and intensity in the basal forebrain and cortex, indicating that Aβ immunoreactivity in the BFCN in not due to differences in the levels of APP. Western blot analysis of basal forebrain homogenates using the same antibodies confirmed the presence of Aβ in young cases. Our preliminary observations also indicate sustained presence of Aβ within the BFCN in normal aged and AD cases. Qualitative Western blot analysis revealed that the basal forebrain in young individuals may contain higher levels of Aβ monomer and small oligomers when compared with normal old and AD and that the basal forebrain in AD may contain higher levels of larger Aβ oligomers. These results clearly demonstrate accumulation of Aβ within the human BFCN early in adult life. Given the known detrimental effects of Aβ, it is likely that sustained presence of Aβ oligomers within the BFCN is responsible, at least in part, for the selective vulnerability of these neurons to loss in neurodegenerative disorders that afflict the elderly.

Disclosures: S. Vahedi, None; **A. Baker-Nigh**, None; **C. Geula**, None; **D. Riascos**, None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.6

Topic: C.02. Alzheimer's disease and other dementias

Support: Vetenskapsrådet

Title: Suggestive evidence that diffuse Abeta deposits and senile plaques are generated by different mechanisms in AD

Authors: *L. N. NILSSON[1], O. PHILIPSON[1], A. LORD[2], L. LANNFELT[1], P. HAMMARSTRÖM[3], P. NILSSON[3], T. KLINGSTEDT[3];
[1]Publ. Hlth. & Caring Sci., Uppsala Univ., Uppsala, Sweden; [2]BioArctic Neurosci., Stockholm, Sweden; [3]Linköping Univ., Linköping, Sweden

Abstract:
Studies of familial Alzheimer´s disease (AD) suggest that misfolding and aggregation of amyloid-beta peptides initiate the pathogenesis which causes dementia. Among the evidence, one is the discovery of the Arctic amyloid precursor protein (APP) mutation which results in AD and facilitates Abeta protofibril formation. The Abeta-based neuropathology of AD may consist of amyloid deposits, but also of diffuse deposits which contain Abeta peptides but do not stain with Congo red. Amyloid deposits can be compact parenchymal plaques or amyloid angiopathy in vessels. From studies of postmortem brain, it has been suggested that diffuse deposits gradually mature and finally become amyloid plaques. But here we show that only the number of diffuse Abeta deposits, and not amyloid plaques, is increased if tg-ArcSwe mice synthesizing a low level of Arctic Abeta are crossed with plaque-depositing tg-Swe mice. The diffuse deposits of bitransgenic mice, which contain mainly wild type Abeta42, accumulate in regions both with and without transgene expression. The selective increase of diffuse parenchymal Abeta deposits suggests that different pathways of Abeta aggregation lead either to the formation of diffuse Abeta deposits or to the accumulation of amyloid plaques in the brain. We show that the selective raise in diffuse deposits is most likely due to direct physical interactions between Arctic and wild type Abeta42, and not to altered APP processing in young bitransgenic mice. *In vitro* studies demonstrate that a mixture of Arctic and wild type Abeta42 facilitates the formation of prefibrillar and fibrillar Abeta assemblies, but inhibits the further elongation of Abeta fibrils. The association of compact and diffuse Abeta deposits with amyloid-

associated proteins in bitransgenic mice is currently under investigation. Our findings likely have implications to the Abeta pathogenesis of AD in general, and specifically to patients who are heterozygous for the Arctic mutation. It also further illustrates how Abeta neuropathology can be manipulated *in vivo* in a manner reminiscent to prion disorders.

Disclosures: **L.N. Nilsson:** Consultant/Advisory Board; BioArctic Neuroscience. **O. Philipson:** None. **A. Lord:** Employment; BioArctic Neuroscience. **L. Lannfelt:** Ownership Interest; BioArctic Neuroscience. **P. Hammarström:** None. **P. Nilsson:** None. **T. Klingstedt:** None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.7

Topic: C.02. Alzheimer's disease and other dementias

Support: State of Illinois, IDPH

Title: Neuritic plaque (NP) and neurofibrillary tangle (NFT) densities are increased by compromised visual function in patients with dementia

Authors: *R. G. STRUBLE[1], N. WILKINS[2], K. SWONG[2], S. RANDALL[2], X.-X. YAN[3], B. E. MOORE[4], T. ALA[2];
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Abstract: Beta secretase (BACE) is the initial enzyme in the formation of beta amyloid (Aβ). Decreased brain metabolic rate can increase BACE activity and result in increased production of Aβ. Based on this relationship between metabolic decline and BACE activity, we hypothesized that those medical conditions that could decrease brain metabolic activity could increase Aβ deposition. To test this hypothesis we compared AD cases with visual deprivation (Vdep) to AD cases without. Vdep is known to suppress metabolic activity in striate and peristriate cortex of primary visual cortex. Therefore, we determined the density of NP and NFT densities in cases of AD with and without clinical evidence of visual deprivation. Seven clinically and neuropathologically diagnosed AD patients, with a definite clinical history of compromised visual dysfunction by chart review (Vdep), were compared to seven age- and sex-matched AD

cases without a history of Vdep. The Vdep patients included five with peripheral deficits (cataracts or retinopathy) and one each with optic ataxia and homonymous hemianopsia. We used a modified Bielschowski silver stain on paraffin sections for systematic counting of NP and NFT in striate and peristriate cortex. We found a two-fold increase in the density of NP in striate and peristriate cortex in the Vdep group ($p<0.01$). A ten-fold increase in NFT was found in Vdep patients ($p<0.02$). Peristriate cortex displayed more NP and NFT than did striate cortex. Supragranular cortex of peristriate cortex displayed substantially more NP than any other area.<br
In sum, we evaluated the density of NP and NFT in occipital cortex in naturally occurring cases of decreased visual cortex activity and found increased numbers in Vdep patients with AD. The results of this preliminary study suggest that brain metabolic activity could underlay the formation of Aβ deposition in neocortex. Specifically, the density of NP may represent the severity of brain metabolic decline. NFT also shows this pattern. We propose that identified risk factors for developing AD are the same as those causing brain metabolic declines. Hence, our hypothesis suggests that amyloid deposition, in the most common form of dementia, AD, may represent brain metabolic dysfunction.

Disclosures: R.G. Struble, None; **N. Wilkins**, None; **K. Swong**, None; **S. Randall**, None; **X. Yan**, None; **T. Ala**, None; **B.E. Moore**, None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.8

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant GM068596-05S1

NIH Grant NIA AG023012

Title: Functional interaction between β-amyloid precursor protein and DISC1: A link between schizophrenia and Alzheimer’s disease

Authors: V. MURESAN[1], B. T. LAMB[2], *Z. MURESAN[1];
[1]Pharmacol. and Physiol., UMDNJ - New Jersey Med. Sch., NEWARK, NJ; [2]Dept. of Neurosciences, Lerner Res. Institute, The Cleveland Clin. Fndn., Cleveland, OH

Abstract: The pathology of Alzheimer’s disease (AD) involves the 40-42 amino acid long

polypeptide, amyloid-β (Aβ), derived from the processing of Aβ precursor protein (APP) by two successive proteolytic cleavages. Aβ forms toxic oligomers that accumulate in neurons - particularly within their neurites, and become incorporated into neuritic plaques. Despite their relevance to AD, it is not known what causes the intracellular oligomerization of Aβ, or how plaques are formed. Along with Aβ, the intracellular Aβ accumulations and the plaques contain a variety of other components, which could participate in their formation. The neuronal cells (CAD) accumulate within their neurites oligomeric Aβ, similar to what is believed to occur in the AD-afflicted neurons in situ. Here we identify DISC1, a protein disrupted in schizophrenia, as part of the neuritic accumulations of Aβ in CAD cells, and of intracellular and extracellular Aβ deposits in a mouse model of AD. In differentiated CAD cells, DISC1 is normally concentrated in the neuronal soma, to a compact, pericentrosomal compartment that stains positive for the small GTPase, Rab11, a marker for the recycling endosome. Interestingly, this compartment also contains APP C-terminal fragments (CTFs) and Aβ, suggesting that it could function as the processing site of APP, and as sorting station for the generated APP fragments into transport vesicles. Within neurites, DISC1 is associated with transport vesicles, some of which also contain Aβ, but not full-length APP or CTFs. In the CAD cells that show neuritic accumulations of Aβ, DISC1 forms large deposits that colocalize with Aβ. These deposits likely contain oligomeric DISC1; indeed, Western blots of CAD cell lyzates show significant amounts of the ~ 150 kDa DISC1 dimer. We used RNAi to test whether DISC1 functions in deposition of Aβ in neurites. Remarkably, a 77% reduction of DISC1 levels increased the accumulation of the β-secretase cleavage product, CTFβ by 72%, but diminished the pool of Aβ by 90%. Furthermore, the treatment of CAD cells with DISC1-specific siRNA abolished the formation of intraneuritic deposits of Aβ. Consistent with a role of DISC1 in depositin of Aβ, immunohistochemistry of sections through brains from the transgenic mouse, R1.40, expressing an APPswe double mutant, show increased levels of DISC1 in the regions afflicted by Aβ pathology. These results indicate that DISC1 positively regulates the production and transport of Aβ, and facilitates the formation of neuritic Aβ accumulations. Our work suggests a cross talk between the machineries that lead to disease in AD and schizophrenia.

Disclosures: V. Muresan, None; **B.T. Lamb**, None; **Z. Muresan**, None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.9

Topic: C.02. Alzheimer's disease and other dementias

Support: PO1 AG09466

PO1 AG 14449

P30 AG10161

USF Honors College

Title: Structural neuroplasticity and neurodegeneration within the cortical connectome in mild cognitive impairment and Alzheimer's disease

Authors: *R. F. MERVIS[1,2], S. ARADI[3], V. LOZANO[3], A. R. LOZANO[3,2], R. A. LONG[3], J. D. KOTICK[4,2], A. WINKLER[5], M. SHAH[2,6], K. KASIMOS[3], J. J. MILLER[3,2], S. SCHEFF[7], E. J. MUFSON[8];
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Abstract: People with mild cognitive impairment (MCI), who present with memory loss, are at a higher risk for the development Alzheimer's disease (AD). The disruption of the neuronal "connectome", which represents the network of elements and connections underlying the neurostructural substrate of cognition and memory, plays a key role in the onset of dementia. Alterations in brain circuitry can be evaluated utilizing a morphometric assessment of dendritic branching and spines. Here, we characterized the earliest alterations in cortical circuitry associated with MCI; and, subsequently, any additional disruption of the connectome associated with onset of frank AD. We compared changes in layer II-III pyramidal cell morphology from 3 cortical regions: the inferior parietal cortex (IPC, Brodmann areas 39, 40), the inferior temporal cortex (ITC, area 21), and the superior frontal cortex (SFC, area 9). Formalin-fixed cortical tissue was harvested from individuals diagnosed antimortem with a clinical diagnosis of No Cognitive Impairment (NCI), MCI, or AD. Cortical tissue blocks were Golgi stained, all slides coded, and layer II-III pyramids were randomly selected for dendritic branching and spine analysis of the basilar dendritic arbor. A "Global Circuitry Index" (GCI) was devised to integrate changes of both dendritic parameters into a unitary component. Overall analysis revealed different patterns of alterations of brain circuitry with increasing loss of cognitive function depending upon the cortical region examined. In the MCI IPC, there was an initial 28% reduction of circuitry compared to NCI with essentially no additional loss in AD (total reduction of 29%). In MCI ITC, there was an initial 35.5% decrease in the connectome GCI followed by a additional 14.3% decrease in AD (total loss relative to NCI = 44.8%). The change in the GCI in the frontal cortex was uniquely different: In MCI, there was a striking 37% increase in the GCI frontal cortex layer II-III pyramids compared to NCI. This was followed by a 55.7% reduction of the index from MCI to AD. Overall, this left the AD SFC with an 18.7% loss in circuitry compared to normal NCI brain tissue. Thus, as opposed to the initial diminution of the circuitry index associated with MCI seen in IPC and ITC, frontal cortex pyramidal neurons display a remarkable compensatory neuroplastic response in MCI, which may be an attempt to rescue the cortical connectome and ,in turn, to maintain cognitive function prior to additional disruption of cortical circuitry in frank

AD.

Disclosures: R.F. Mervis, None; **S. Aradi**, None; **V. Lozano**, None; **A.R. Lozano**, None; **R.A. Long**, None; **J.D. Kotick**, None; **A. Winkler**, None; **M. Shah**, None; **K. Kasimos**, None; **J.J. Miller**, None; **S. Scheff**, None; **E.J. Mufson**, None.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.10

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH grant R21AG034283

Title: Characterization of a small molecule fluorophore that preferentially binds soluble beta-amyloid oligomers in the PDAPP transgenic mouse model of Alzheimer’s disease

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Abstract: In addition to the fibrillar form of beta-amyloid (Aβ), soluble oligomers have also been implicated in the early pathogenesis of Alzheimer’s disease (AD) and other amyloid-related disorders. Currently, there are several small-molecule imaging probes that bind the fibrillar form of Aβ, however none are known to specifically target soluble oligomers. This lack of probes that can differentiate between the two forms of Aβ has hindered studies that fully explore the potential role of oligomers in the pathogenesis of AD. Our group has previously reported the discovery of a small-molecule probe, named 68B-3, which preferentially binds soluble oligomers over fibrils *in vitro*. In the current study, our goal was to further evaluate 68B-3’s ability to function as an *in situ* labeling agent which could potentially be used as a tool to study, specifically, the role of soluble oligomers in AD pathogenesis. Therefore, histochemical analyses of brain sections from the PDAPP transgenic mouse model of AD were performed using 68B-3 as compared to fibril-specific probes Thioflavin S or PIB. Our results demonstrate that the amount of 68B-3 staining directly correlates with mouse age, suggesting that oligomer deposition increases with age. Using a two-step imaging approach, our results show that soluble oligomers and plaques are found both spatially distinct as well as co-located with each other, suggesting that soluble oligomers may well be indeed en route to becoming plaques. Taken together, our data demonstrate the *in situ* effectiveness of our soluble oligomer-specific probe,

68B-3, and lends support to this agent's potential use as an *in situ*, and potentially *in vivo*, imaging tool.

Disclosures: C. Tan Hehir: Employment; General Electric. **T. Tanner:** General Electric. **Z. Pang:** General Electric. **N. Barnhardt:** General Electric. **T. Murray:** General Electric. **J. Klimash:** General Electric.

Nanosymposium

320. Alzheimer's Disease: Abeta Assembly In Vitro and In Vivo

Location: Room 32B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 320.11

Topic: C.02. Alzheimer's disease and other dementias

Support: DFG Grant He3350/4-1,2

Title: Nitration of tyrosine 10 promotes amyloid β aggregation and plaque formation

Authors: *M. P. KUMMER[1], M. HERMES[1], T. HAMMERSCHMIDT[1], S. KUMAR[1], D. TERWEL[1], J. WALTER[1], H.-C. PAPE[2], S. KÖNIG[3], S. RÖBER[4], T. KLOCKGETHER[1], M. HENEKA[1];
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Abstract: Part of the inflammatory response in Alzheimer's disease (AD) is the upregulation of the inducible nitric oxide synthase (NOS2) resulting in increased production of NO. As a novel NO target we identified Aβ nitrated at tyrosine 10 ($3NTyr^{10}$-Aβ). Nitration of Aβ accelerated its aggregation and was detected in the core of Aβ plaques of APP/PS1 mice and AD brains. *In vivo*, we found that NOS2 deficiency and the NOS2 inhibitor L-NIL were able to revert cognitive impairment, increased amyloid β (Aβ) plaque deposition and deficits in synaptic plasticity in an AD mouse model (APP/PS1). Further, injection of $3NTyr^{10}$-Aβ into the brain of young APP/PS1 mice induced the formation of plaques. This suggest a disease modifying role for NOS2 in AD and therefore represents a potential therapeutic target.

Disclosures: M.P. Kummer, None; **M. Hermes**, None; **T. Hammerschmidt**, None; **S. Kumar**, None; **D. Terwel**, None; **J. Walter**, None; **H. Pape**, None; **S. König**, None; **S. Röber**, None; **T. Klockgether**, None; **M. Heneka**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.1

Topic: C.02. Alzheimer's disease and other dementias

Support: The Japan Society for the Promotion of Science (JSPS) #20599017

Alexander von Humboldt Foundation

Title: Role of neuronal STAT3 in the pathogenesis of Alzheimer’s disease

Authors: *T. CHIBA[1,2], M. YAMADA[2], S. AISO[2], B. GRONER[1];
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Abstract: Elevation of intracranial soluble amyloid-beta (Aβ) levels has been implicated in the pathogenesis of Alzheimer’s disease (AD). We have reported that phospho- (p-) STAT3 levels in hippocampal neurons are inversely correlated with the brain amyloid burden of AD mice and patients, suggesting that neuronal STAT3 inactivation plays a critical role in the pathogenesis of AD. To test this, we performed lenti-virus-mediated STAT3 knockdown experiment in primary neurons. Cell viability of the neurons with decreased STAT3 levels were significantly lower than those with control virus. In addition, cholinergic genes were substantially suppressed in STAT3 knocked down neurons. We further generated neuron-specific STAT3 knockout mice and prepared primary neurons from the mice. Primary neurons from neuron-specific STAT3 knockout mice were more vulnerable to cellular stress related to neurodegeneration such as Aβ, oxidative stress, and ER stress. This provides a novel insight into therapy for postmenopausal cognitive decline and AD.

Disclosures: T. Chiba, None; **M. Yamada**, None; **S. Aiso**, None; **B. Groner**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.2

Topic: C.02. Alzheimer's disease and other dementias

Support: K2009-67X-21128-01-3

European commission (EURAMY)

Mizutani foundation for glycoscience

Demensförbundet (Stockholm)

Stohnes Stiftelse (Bromma)

Title: Roles of heparan sulfate in Alzheimer's disease: Abeta depositon and cytotoxicity

Authors: *X. ZHANG[1], P. O'CALLAGHAN[1], E. SANDWALL[2], T. H. VAN KUPPEVELT[3], L. N. NILSSON[1], M. INGELSSON[1], B. T. HYMAN[4], H. KALIMO[5], U. LINDAHL[2], L. LANNFELT[1], J.-P. LI[2];
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Abstract: Amyloid-β (Aβ) is a central factor in the pathogenesis of Alzheimer's disease (AD). This cytotoxic peptide deposits into plaques, forming major neuropathological lesions in the brain of AD. Heparan sulfate (HS) and HS proteoglycan (HSPG) are found to be one of the pertinent components in the amyloid deposits. HS, negatively charged polysaccharide, interacts with a multitude of proteins including Aβ, and has been considered a limiting factor in the initiation of amyloid deposition. However, the pathophysiological implications of HS in development of AD remain unclear. We have been attempting to elucidate the functions of HS/HSPG in Aβ pathology through a number of approaches.
Examination of tissue sections from different types of AD brain demonstrated that HS preferentially accumulated around the Aβ40 dense cores of neuritic plaques, but was largely absent from diffuse Aβ42 plaques. We identified the membrane-bound HSPGs, glypican-1 (GPC1) and syndecan-3 (SDC3), in glial cells associated with Aβ deposits, proximal to sites of HS accumulation. In mouse primary glial cultures, we observed increased levels of GPC1 and SDC3 following Aβ stimulation. These results suggest that HS co-deposits with Aβ in neuritic plaques are mainly derived from glial cells.
The significance of the HS-Aβ interaction in Aβ uptake and its toxic effect was investigated in cell models. Wild-type Chinese hamster ovary (CHO-WT) cells showed loss of viability following exposure to Aβ40, whereas the HS-deficient cell line, pgsD-677, was essentially resistant. Immunocytochemical analysis revealed Aβ internalization by CHO-WT, but not by pgsD-677 cells. The effect of HS was further illustrated in human embryonic kidney cells

overexpressing heparanase (HEK293-hpa). The HEK293-hpa cells exhibited reduced Aβ40 toxicity, likely due to the extensive degradation of HS chains by heparanase. Finally, addition of heparin to human umbilical vein endothelial cells (HUVEC) prevented internalization of added Aβ40 and protected against Aβ toxicity.
These findings strongly suggest an active role of HS in Aβ pathology and future study to clarify the roles of HS may provide a new dimension for management of AD.

Disclosures: X. Zhang, None; **P. O'Callaghan**, None; **E. Sandwall**, None; **T.H. van Kuppevelt**, None; **L.N. Nilsson**, None; **M. Ingelsson**, None; **B.T. Hyman**, None; **H. Kalimo**, None; **U. Lindahl**, None; **L. Lannfelt**, None; **J. Li**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.3

Topic: C.02. Alzheimer's disease and other dementias

Support: Regione Toscana, Regional Health Research Program

Title: Glucose-oxygen deprivation and beta-amyloid toxicity in entorhinal cortex: the role of RAGE

Authors: *N. ORIGLIA[1], O. ARANCIO[2], S. YAN[2], L. DOMENICI[1,3];
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Abstract: Hypoxic condition may be the cause of progressive neuronal alterations in Alzheimer’s disease (AD). However, a causal relationship between oxygen deficiency and AD at the cellular and molecular level has not been established. We evaluated the effect of acute transient hypoxia on fEPSP recorded in acute slices containing enthorinal cortex (EC), a brain region primarily affected in AD. EC slices were exposed to oxygen-glucose deprivation (OGD) for a short period (10 min), a well known model of in vitro ischemia.We raised the hypothesis that acute oxygen deprivation activates the Receptor for Advanced Glycation Endproducts (RAGE) in different cell targets, resulting in synaptic dysfunction. RAGE functions as a cell surface binding site for several ligands, including beta-amyloid (Aβ), and its expression is altered in neuronal and non-neuronal cells under ischemic conditions. We first investigated the effects of OGD on synaptic transmission and plasticity in: i) EC slices treated with blocking antibodies against RAGE, ii) EC slices from RAGE null mutant (RAGE-/-) or transgenic (Tg) mice in which the dominant negative form of RAGE lacking the cytosolic domain of the receptor, is

expressed selectively in neurons (DN-RAGE) or in microglial cells (DNMSR). In EC slices exposed to 10 min OGD, we observed a fast depression of fEPSPs that continues and slowly recover after re-introduction of oxygenated ACSF resulting in steady-state synaptic depression. Absence of RAGE in EC slices from RAGE null mutant mice resulted in a faster and complete recover of fEPSPs amplitude following OGD. The same protective effect was achieved in EC slices from Tg DNMSR mice but not in slices from Tg DN-RAGE mice. To test whether Aβ enriched environment increases synaptic dysfunction induced by oxygen-glucose deprivation, the effect of acute OGD on neuronal impairment was evaluated in EC slices treated with different concentrations of soluble oligomer amyloid peptide (Aβ42). We found that the effects of OGD were enhanced in Aβ perfused slices. Importantly, the absence of RAGE or selective deficiency of RAGE in microglia (DNMSR) protected from synaptic impairment induced by either Aβ alone or Aβ coupled to OGD. These results indicate cell-specific contribution of RAGE to OGD effects on synaptic function in Aβ enriched environment. These results suggest that RAGE expressed in neuronal and non-neuronal cells may be viewed as part of a neural system that controls synaptic responses in vascular pathology and AD.

Disclosures: N. Origlia, None; **O. Arancio**, None; **S. Yan**, None; **L. Domenici**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.4

Topic: C.02. Alzheimer's disease and other dementias

Support: NIA (PO1AG17490)

Alzheimer's Association

Title: Early deficits in synaptic mitochondria in an Alzheimer’s disease mouse model

Authors: *H. DU[1], L. GUO[2], S. YAN[2], A. A. SOSUNOV[2], G. M. MCKHANN[2], S. YAN[2]; [1]Columbia Univ., NEW YORK CITY, NY; [2]Columbia Univ., New York, NY

Abstract: Synaptic dysfunction and the loss of synapses are early pathological features of Alzheimer’s disease (AD). Synapses are sites of high energy demand and extensive calcium fluxes; accordingly, synaptic transmission requires high levels of ATP and constant calcium fluctuation. Thus, synaptic mitochondria are vital for maintenance of synaptic function and transmission through normal mitochondrial energy metabolism, distribution and trafficking, and

synaptic calcium modulation. To date, there has been no extensive analysis of alterations in synaptic mitochondria associated with amyloid pathology in an amyloid beta (Aβ)-rich milieu. Here, we identified differences in mitochondrial properties and function of synaptic versus nonsynaptic mitochondrial populations in transgenic mouse brain that overexpresses the human mutant form of amyloid precursor protein (mAPP) and Aβ. Compared to nonsynaptic mitochondria, synaptic mitochondria showed a greater degree of age-dependent accumulation of Aβ as well as mitochondrial alterations. The synaptic mitochondrial pool of Aβ was detected at an age as early as 4 months, and well before the onset of nonsynaptic mitochondrial and extensive extracellular Aβ accumulation. Aβ insulted synaptic mitochondria revealed early deficits in mitochondrial function as shown by increased mitochondrial permeability transition, decline in both respiratory function and activity of cytochrome c oxidase, and increased mitochondrial oxidative stress. These results demonstrate that synaptic mitochondria, especially Aβ-rich synaptic mitochondria, are more susceptible to Aβ-induced damage highlighting the central importance of synaptic mitochondrial dysfunction relevant to the development of synaptic degeneration in AD.
This study is supported by grants from NIA (PO1AG17490) and the Alzheimer's Association.

Disclosures: H. Du, None; **L. Guo**, None; **S. Yan**, None; **A.A. Sosunov**, None; **G.M. McKhann**, None; **S. Yan**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.5

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH R01 NS056049-01

P50 AG008702-21

Title: Role of Phospholipase D2 and phosphatidic acid signaling in Alzheimer's disease-linked synaptic dysfunction and cognitive deficits

Authors: *T. G. OLIVEIRA[1,2], R. B. CHAN[1], H. TIAN[1], M. LAREDO[1], A. STANISZEWSKI[1], H. ZHANG[1], L. WANG[1], T.-W. KIM[1], K. E. DUFF[1], M. R. WENK[3], O. ARANCIO[1], G. DI PAOLO[1];
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Abstract: Lipid signaling has been recently implicated in Alzheimer's disease (AD) pathogenesis. While phospholipases A2 (PLA2) and C (PLC) have been recently shown to mediate key actions of amyloid β-peptide (Aβ) through a dysregulation of arachidonic acid and phosphatidylinositol-4,5-bisphosphate metabolism, respectively, the role of phospholipase D (PLD), which produces phosphatidic acid (PA), has so far remained elusive. Here we show that oligomeric Aβ enhances PLD activity in cultured neurons and that this stimulatory effect does not occur upon ablation of PLD2 via gene targeting. Aβ fails to suppress long-term potentiation (LTP) in PLD2-deficient hippocampal slices, suggesting that PLD2 is required for the synaptotoxic action of this peptide. Further supporting this idea, Pld2 ablation rescues memory deficits in a transgenic model of AD (Tg2576 or "Swedish APP mutant") despite a significant Aβ load. Consistent with a rescue of cognitive function, synaptic protein levels are restored back to normal in the forebrain of Tg2576 mice upon ablation of PLD2. Mass spectrometry-based lipid analysis of Pld2 mutant brains in the presence or absence of the Swedish APP transgene unmasks striking crosstalks between different PA species. This lipid analysis shows an exquisite acyl chain specificity and plasticity in the perturbation of PA metabolism, with the notable elevation in the Swedish APP brains of a pool of PA previously linked to degeneration. Moreover, a broader analysis of multiple lipid classes uncovered potential lipid signaling pathways through which PLD2 ablation may confer protection. Collectively, our results point to specific molecular species of PA as key modulators of AD pathogenesis and identify PLD2 as a novel potential target for therapeutics.

Disclosures: T.G. Oliveira, None; **R.B. Chan**, None; **H. Tian**, None; **M. Laredo**, None; **A. Staniszewski**, None; **H. Zhang**, None; **L. Wang**, None; **T. Kim**, None; **K.E. Duff**, None; **M.R. Wenk**, None; **O. Arancio**, None; **G. Di Paolo**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.6

Topic: C.02. Alzheimer's disease and other dementias

Support: NIA (PO1AG17490)

Alzheimer's Association

Title: Cyclophilin D deficiency protects against Aβ-induced impairment in axonal mitochondrial

trafficking

Authors: *L. GUO[1], H. DU[2], S. YAN[2];
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Abstract: The activity of synapses demands functional mitochondria to provide ATP and modulate calcium homeostasis in situ. Accordingly, axonal transport of mitochondria is of essential importance in maintaining neuronal function. Axonal mitochondria undergo intermittent anterograde and retrograde movement; and thus mitochondria transit constantly between movable and stationary status. The distribution and movement of axonal mitochondria are damaged by many pathological factors, for example, amyloid beta (Aβ) and accompanies synaptic failure. As a result, to maintain axonal mitochondrial density and movement in normal fashion has positive impact on sustaining neuronal homeostasis against deleterious insults. In this study, we adopted cultured hippocampal neurons and demonstrated that low concentration of Aβ down-regulated both axonal mitochondrial anterograde and retrograde movement, decreased axonal mitochondrial density and increased axonal mitochondrial fragmentation. Notably, the Aβ suppression of mitochondrial anterograde movement was more predominant than its effect on mitochondrial retrograde movement. In contrast, genetic depletion of cylclophilin D (cypD) rescued mitochondrial trafficking in axon as shown by the preserved axonal mitochondrial distribution, normal mitochondrial length and active mitochondrial movement. In parallel, the absence of CypD maintained axonal calcium homeostasis and preserved synaptic vesicle release. Thus, CypD-dependent mitochondrial trafficking in axon could be an important mechanism underlying Aβ-induced synaptic stress and the synaptic degeneration. CypD might be a therapeutic target for halting and preventing Alzheimer's disease.
This study is supported by grants from NIA (PO1AG17490) and the Alzheimer's Association.

Disclosures: L. Guo, None; **H. Du**, None; **S. Yan**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.7

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant AG023084

Title: Chronic increase in VEGF along with amyloid-beta in brain induce anti-angiogenic effect in mouse model of Alzheimer's Disease

Authors: *I. SINGH[1], R. S. LOVE-KASISCHKE[1], R. DEANE[1], H. H. MARTI[2], B. V. ZLOKOVIC[1];
[1]Univ. Rochester, ROCHESTER, NY; [2]Univ. of Heidelberg, Heidelberg, Germany

Abstract: Alzheimer's Disease (AD) is associated with progressive accumulation of amyloid-beta peptide into brain, chronic neuroinflammation and neuronal dysfunction. AD brain also shows chronic increase in vascular endothelial growth factor (VEGF), one of the most potent angiogenic growth factors. However, both AD individuals and mouse models of AD show a significant reduction in vascular volume, capillary length and blood flow. Impaired blood flow leads to hypoxic conditions in brain, which further augments VEGF production. We developed a mouse model (TgAPP-VEGF) of AD expressing VEGF in neurons by crossing TgAPP $sw^{+/-}$ mice with transgenic mice overexpressing neuronal VEGF (TgVEGF). In vivo multiphoton studies of double transgenic TgAPP-VEGF mice showed a pronounced decrease in cerebral vascular diameter, length and density compared to either TgAPP $sw^{+/-}$ or TgVEGF mice. Further, TgAPP-VEGF mice had reduced cerebral blood flow and increased blood brain barrier permeability compared to TgAPP $sw^{+/-}$ or TgVEGF mice. Behavioral studies by operant learning, novel object recognition and novel object location showed a significant impairment of cognitive functions in TgAPP-VEGF mice. In addition, both in vivo studies with these mice and in vitro studies with human brain endothelial cells showed a marked increase in oxidative stress accompanied with downregulation of VEGFR2, a VEGF receptor that play major role in angiogenic signaling. These findings suggest that chronic overexpression of VEGF in neurons along with increase in amyloid-beta production suppresses angiogenesis contributing to microvascular reductions and progressive decline in cognitive functions.

Disclosures: I. Singh, None; **R.S. Love-Kasischke**, None; **R. Deane**, None; **H.H. Marti**, None; **B.V. Zlokovic**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.8

Topic: C.02. Alzheimer's disease and other dementias

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ)

Instituto Nacional de Neurociência Translacional (INNT)

Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP)

NIH grant 5SC1NS063950-04

NIH grant 3SC1NS063950-03S1 (ARRA)

Title: D1/D5 receptor activation protects neurons from synaptic dysfunction induced by Alzheimer's Aβ oligomers

Authors: *S. JURGENSEN[1], L. L. ANTONIO[2], G. MUSSI[3], E. R. GARRIDO-SANABRIO[4], E. A. CAVALHEIRO[2], S. T. FERREIRA[3];
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Abstract: Soluble oligomers of the amyloid-beta peptide (AβOs) accumulate in the brains of Alzheimer's disease (AD) patients and are increasingly recognized to be responsible for synapse dysfunction and early memory loss in AD. AβOs have been shown to inhibit long-term potentiation (LTP), facilitate long-term depression (LTD), and induce endocytosis of AMPA and NMDA receptors. Recent studies have shown that activation of dopamine D1/D5 receptors reinforces excitatory transmission via phosphorylation of AMPA and NMDA receptor subunits (GluR1 and NR1, respectively) by protein kinase A (PKA). Phosphorylation of GluR1 and NR1 by PKA favors its insertion into extra-synaptic and synaptic sites, respectively, rendering the receptors more resistant to endocytosis. We show that treatment of mature hippocampal neurons in culture with AβOs (400 nM for 4h) significantly decreased surface levels of GluR1 and NR1 in dendrites, while total cellular levels of both proteins remained unchanged. A selective D1/D5 receptor agonist, (±)-6-Chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine hydrobromide (SKF81297), blocked the endocytosis of both GluR1 and NR1 induced by AβOs. On the other hand, the protection exerted by SKF81297 was abrogated by (R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-te trahydro-1H-3-benzazepine hydrochloride (SCH23390), a selective D1/D5 receptor antagonist, further substantiating the involvement of D1/D5 receptors. Phosphorylation of AMPA receptor subunit GluR1 at Ser845 (pS845-GluR1) is required for synaptic insertion of the receptor during LTP; conversely, dephosphorylation at this specific site by calcineurin increases receptor endocytosis and is required for the induction of LTD. We found that AβOs induce a decrease in pS845-GluR1 levels. SKF81297 prevented the reduction in pS845-GluR1 levels, providing a mechanism by which SKF81297 blocks AβO-induced loss of surface AMPARs. SKF81297 further prevented the impairment of LTP by AβOs in acute hippocampal slices, indicating the functional relevance of these findings. Results suggest that specific stimulation of D1 receptors may provide a novel pharmacological approach to prevent synapse failure and memory decline in AD.

Disclosures: S. Jurgensen, None; **L.L. Antonio**, None; **G. Mussi**, None; **E.R. Garrido-Sanabrio**, None; **E.A. Cavalheiro**, None; **S.T. Ferreira**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.9

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH R01 DK 077106

Title: Administration of soluble beta-amyloid 1-42 oligomers to the amygdala causes acute impairment of fear-motivated learning and insulin signalling

Authors: A. BYRNE, J. PEARSON-LEARY, *E. C. MCNAY;
Behavioural Neurosci., Univ. At Albany, ALBANY, NY

Abstract: Alzheimer's Disease (AD) is associated with increased amyloid plaques in both the hippocampus and amygdala, both of which are involved in formation and retrieval of memories with affective components. Prior to plaque formation, beta-amyloid 1-42 monomers form into oligomeric diffusible ligands (ADDLs) which we and others have shown to have potent neurotoxicity within the hippocampus via attenuation of insulin signaling, leading to marked cognitive deficits. However, little or no study has been made of amyloid actions within the amygdala. Here, we aimed to determine (i) the cognitive and metabolic effects of intra-amygdalar ADDL administration and (ii) whether amyloid acts within the amygdala via attenuation of endogenous insulin signalling, as seen in the hippocampus. Male Sprague-Dawley rats received ADDLs into the basolateral amygdala prior to testing on three behavioral tasks (elevated plus maze, open field task, and inhibitory avoidance [IA]) known to be amygdala dependent. ADDL administration did not alter anxiety, indicated by similar open field and elevated maze performance to controls. However, ADDL-treated rats had markedly lower retention in the IA task, suggesting an impairment in consolidation of salient information; we used a separate cohort of animals receiving ADDLs post-training in the IA task to confirm this conclusion. In addition, ADDL treated animals had decreased amygdala pAKT and plasma membrane associated GluT4, both downstream targets of insulin signaling mediating local metabolism.
Our results show that acute effects of beta-amyloid on cognitive and metabolic processes extend beyond the hippocampus to include the amygdala, and suggest that interference with endogenous insulin signalling may be a common motif of amyloid's actions across brain regions. Moreover, these data may provide a mechanism to explain the clinical finding of impaired affective processing in AD.

Disclosures: A. Byrne, None; **E.C. McNay**, None; **J. Pearson-Leary**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.10

Topic: C.02. Alzheimer's disease and other dementias

Support: UCSC

MIUR (PRIN)

Title: Role of the redox state of methionine 35 in the synaptotoxicity of amyloid-β peptide

Authors: *C. RIPOLI[1], E. RICCARDI[1], R. PIACENTINI[1], P. MAITI[2], G. BITAN[2], C. GRASSI[1];
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Abstract: Alzheimer's disease (AD), the most common neurodegenerative disorder of elderly, is characterized by marked alterations of the synaptic function that precede neuronal loss in hippocampus and cortex. Numerous experimental evidence linked abnormal accumulation of amyloid-β peptide (Aβ) to synaptic failure underlying the cognitive decline observed in AD patients. We previously demonstrated that the redox state of the methionine residue in position 35 (Met35) plays a critical role in Aβ toxicity. When Met35 is in the reduced state (WT), Aβ42 exerted a marked neurotoxic action that was dependent on intracellular Ca^{2+} homeostasis dysregulation and the consequent activation of pro-apoptotic pathways. Conversely, Aβ42 harboring oxidized Met35 ($A\beta42^{Met35(O)}$), a form that has been found in amyloid plaques of AD brain, only slightly reduced cell viability, and this effect was independent of intracellular Ca^{2+} overload (Piacentini et al., 2008).
To determine whether oxidation of Met35 also influences Aβ synaptotoxicity we investigated the effects of $A\beta42^{WT}$ and $A\beta42^{Met35(O)}$ on synaptic transmission and plasticity.
In primary cultures of rat hippocampal neurons that form autapses, 48-72 hour treatment with 200 nM $A\beta42^{WT}$ significantly reduced the frequency of spontaneous miniature excitatory postsynaptic currents (from 9.1±1.1 to 4.4±1.0 Hz; n=20; P<0.005) and the amplitude of excitatory postsynaptic currents evoked by action potentials (from 6.7±0.7 to 4.8±0.6 nA; n=20; P<0.05). The spontaneous vesicular release rate and the vesicular release probability were also

significantly reduced in neurons exposed to $A\beta42^{WT}$ ($P<0.05$). None of the studied parameters were significantly modified by cell treatment with $A\beta42^{Met35(O)}$.
Long-term potentiation (LTP) protocols were used to study synaptic plasticity in the CA3-CA1 region of hippocampal brain slices from C57BL/6 mice. Slices were exposed to either vehicle or 200 nM Aβ for 20 min before theta burst simulation and changes in field excitatory postsynaptic potential (fEPSP) were evaluated 60 min later. Under control conditions, fEPSP amplitude (A) and slope (Slp) were increased by 138±18% and 132±15% of baseline, respectively (n=12). This potentiation was significantly lower after $A\beta42^{WT}$ treatment (A: +72±10%; Slp: +68±10%, n=9; $P<0.001$) whereas no significant changes in LTP were observed in slices exposed to $A\beta42^{Met35(O)}$ (A: +130±14%; Slp: +134±21%; n=9).
Our findings suggest that oxidation of Met35 markedly affects the synaptotoxicity of Aβ42.

Disclosures: C. Ripoli, None; **E. Riccardi**, None; **R. Piacentini**, None; **P. Maiti**, None; **G. Bitan**, None; **C. Grassi**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.11

Topic: C.02. Alzheimer's disease and other dementias

Support: MGH ADRC 2010 Pilot Project

Title: Are some human brains able to tolerate the insult of beta-amyloid?

Authors: *T. GOMEZ-ISLA, O. DOLS, T. SCOTTON, B. DA ROCHA-SOUTO, P. SANCHEZ-FERRER, A. SERRANO-POZO, M. FROSCH, J. GROWDON, B. HYMAN; Massachusetts Gen. Hosp., BOSTON, MA

Abstract: Background: Numerous amyloid plaques (SPs) may be present in the brain of nondemented individuals matching the regional distribution seen in Alzheimer's Disease (AD). This raises the possibility that some people may tolerate the insult of Aβ accumulation in their brains and remain cognitively normal. Whether or not Aβ deposition in the human brain is inevitably associated with derangement of neuronal/synaptic integrity remains largely unknown. Alternatively, as it has been recently suggested, SPs, rather than being directly toxic themselves, may lead to another species, soluble oligomeric Aβ, that could play a much larger role in synapse/neuronal damage. **Objectives**: To investigate whether neuronal populations in some human brains are resistant to the insult of Aβ. **Methods**: Series of cases from the MGH ADRC

Brain Bank were assessed for SPs, neurofibrillary tangles (NFTs) and gliosis. A subset of outliers that met NIA-RI criteria for high “likelihood” of AD, despite unusually modest concomitant gliosis, were identified. Detailed quantitative neuropathological assessments were conducted on the outliers (N=6), age-matched nondemented controls free of SPs and NFTs (N=8) and demented individuals with definite AD (N=12). Stereologically-based counts of neurons, NFTs and astrocytes were performed in immunostained sections (NeuN, PHF-1 and GFAP) in the region that forms the superior temporal sulcus (STS). Amyloid burden was quantified using Aβ immunostaining (10D5). **Results**: The outlier group had comparable amounts of SPs and NFTs in the STS to the AD group. As expected, the number of neurons was dramatically decreased in the AD group (by 50%) in comparison to controls ($p<0.001$). However, no significant neuronal loss was found in the outlier group despite very robust amyloid deposition and neurofibrillary changes. A very significant increase in the number of astrocytes in the STS was found in AD compared to controls ($p<0.001$), while the astrocytic load in the outlier brains did not differ from control brains ($p=0.38$). **Conclusion**: The neuronal populations in the temporal neocortex of some subjects may tolerate the insult of Aβ deposition. The striking divergence between abundant amyloid and tau alterations and a scarce glial cell reaction to those changes might explain individual differences in neuronal vulnerability to AD pathology. Alternatively, a differential Aβ phenotype (oligomeric vs. fibrillar Aβ) may explain the different impact of Aβ on anatomical integrity. This latter is being investigated and the clinical information available on the outliers carefully reviewed.

Disclosures: T. Gomez-isla, None; **O. Dols**, None; **T. Scotton**, None; **B. Da Rocha-Souto**, None; **P. Sanchez-Ferrer**, None; **A. Serrano-Pozo**, None; **M. Frosch**, None; **J. Growdon**, None; **B. Hyman**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.12

Topic: C.02. Alzheimer's disease and other dementias

Title: Increased brain vulnerability to systemic inflammation in Alzheimer disease mouse model

Authors: *S. TAKEDA[1], N. SATO[1], H. RAKUGI[2], R. MORISHITA[1];
[1]Dept. of Clin. Gene Therapy, Osaka Unuversity, Suita, Osaka, Japan; [2]Dept. of Geriatric Medicine, Grad. Sch. of Medicine, Osaka Univ., Osaka, Japan

Abstract: Neuroinflammation with overexpression of inflammatory cytokines is now recognized

as a prominent feature in Alzheimer's pathology. It has been reported that activated microglia which are primed by Alzheimer amyloid pathology could produce excessive inflammatory cytokines. On the other hand, systemic inflammation caused by infectious disease such as pneumonia or urinary tract infections often spreads into central nervous system, which could cause cognitive impairment in older people. So, we hypothesized that microgrial cells in Alzheimer disease brain might be more sensitive to systemic inflammation and cause more severe neuroinflammation in several infectious disease.
In this study, using novel microdialysis system, which allowed the consistent recovery of several brain peptides, we evaluated the dynamic changes in the concentrations of brain beta-amyloid peptide and inflammatory cytokines (interleukin-6 and tumor necrosis factor-α) during lipopolysaccharide(LPS)-induced septic shock in wild-type and Alzheimer APP transgenic mice (11~12 week). Intraperitoneal administration of LPS (10mg/kg) induced acute elevation of plasma interleukin-6 and tumor necrosis factor-α, followed by a gradual increase in these cytokines in brain interstitial fluid dialysate which was associated with microglial infiltration into brain parenchyma. Although there was no significant difference in the severity of systemic inflammation (plasma cytokine levels) between wild and APP-Tg mice, APP-Tg mice showed more severe increase in brain interleukin-6 level in dialysate than wild-type mice. Then, we evaluated the blood-brain barrier permeability in this experimental model using FITC-albumin leakage assay, and observed a tendency for higher leakage level in APP-Tg mice relative to wild-type mice, although this change did not reach statistical significance.
These findings may suggest that Alzheimer disease brain could be more prone to inflammation during systemic infection. The increased vulnerability of Alzheimer disease brain to systemic inflammation could have an important clinical implication.

Disclosures: S. Takeda, None; **N. Sato**, None; **R. Morishita**, None; **H. Rakugi**, None.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.13

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Training Grant GM08136

Probiodrug AG

Owens Family Foundation

Title: Pyroglutamate-modified β-amyloid amplifies tau-dependent cytotoxicity of conventional β-amyloid

Authors: J. NUSSBAUM,[1], H. CYNIS[2], S. SCHILLING[2], H.-U. DEMUTH[2], *G. S. BLOOM[3]; [1]Dept. of Biol., Univ. of Virginia, Charlottesville, VA; [2]Probiodrug AG, Halle (Saale), Germany; [3]Dept Biol, Univ. Virginia, CHARLOTTSVLLE, VA

Abstract: Impairment of cognition and memory in Alzheimer's disease (AD) is accompanied by neuronal cell death and accumulation of extracellular amyloid-β (Aβ) plaques and intraneuronal neurofibrillary tangles made from tau. A growing body of evidence points to Aβ oligomers that act through a tau-dependent mechanism as a principal toxic agent responsible for the neuronal cell death, and impaired cognition and memory associated with AD. Conventional Aβ most commonly comprises 40 or 42 residues (Aβ1-40 and Aβ1-42), and constitutes the bulk of both soluble and insoluble amyloid in AD brain, but N-terminally truncated Aβ species are also strongly associated with AD. Most notable are those that have been truncated immediately prior to E3 or E11, and have undergone a cyclization by the enzyme, glutaminyl cyclase, yielding pEAβ3-40, pEAβ3-42, pEAβ11-40 or pEAβ11-42. It has recently been shown that in vivo inhibition of glutaminyl cyclase significantly reduces cognitive impairment, as well as both pEAβ and conventional amyloid plaque load in APP transgenic mice (Schilling et al. 2008. Nature Med 14: 1106-1111). These data suggest that pEAβ acts as a seed that facilitates the accumulation and toxicity of conventional amyloid.
Using synthetic pEAβ and conventional Aβ to treat primary cortical neurons in culture, we now report that apparent dimers and trimers of pEAβ3-42 are cytotoxic at an ~5-fold lower concentration than conventional Aβ1-42, and that incubation of pEAβ3-42 with a 19-fold molar excess of Aβ1-42 during oligomer formation yields amyloid cocktails that are strongly cytotoxic at 100 nM total peptide, which is 10-50-fold more cytotoxic than either peptide individually. This enhanced cytotoxicity was not observed when pEAβ3-42 and Aβ1-42 oligomerized independently and were mixed together immediately before being added to cell cultures. Furthermore, primary cortical neurons from tau knockout mice were insensitive to oligomers made from either pure pEAβ3-42 or mixtures of pEAβ3-42 and Aβ1-42 under conditions in which comparably treated WT neurons were killed within 24 hours.
These data suggest that pEAβ either confers toxicity upon conventional Aβ during oligomerization, or that mixed oligomers of the two peptides are dramatically more toxic than oligomers consisting of either peptide alone. Both possible conclusions support the goal of pursuing treatments that focus on alleviating AD pathology by reducing pEAβ content.

Disclosures: J. Nussbaum,: Other Research Support; Research support from Probiodrug AG. **H. Cynis:** Employment; An employee (Propject Leader) of Probiodrug AG, a private German Biotech company, active in drug discovery to combat neurodegeneration. He possesses stock options in the company. **S. Schilling:** An employee (Department Head) of Probiodrug AG, a private German Biotech company, active in drug discovery to combat neurodegeneration. He possesses stock options in the company. **H. Demuth:** An employee (CSO) of Probiodrug AG, a private German Biotech company, active in drug discovery to combat neurodegeneration. He possesses stock and stock options in the company. **G.S. Bloom:** Other Research Support; Research support from Probiodrug AG.

Nanosymposium

321. Alzheimer's Disease: Abeta Toxicity and Downstream Effectors

Location: Room 23A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 321.14

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH P01 HD29587

NIH R01 EY05477

NIH P01 ES016738

NIH P30 NS057096

Title: Amyloid beta mediated glutamate release from astrocytes

Authors: *S. SANZ-BLASCO, J. PINA-CRESPO, M. TALANTOVA, G. CAO, S. A. LIPTON;
Del E. Webb Neuroscience, Aging & Stem Cell Res. Ctr., SANFORD-BURNHAM MEDICAL RESEARCH INSTITUTE, SAN DIEGO, CA

Abstract: Excitotoxicity is caused by excessive exposure to glutamate or overstimulation of its membrane receptors. Several lines of evidence indicate that synaptic damage and neuronal loss can result from excessive NMDA-type glutamate receptor (NMDAR)-dependent Ca2+ entry. Dysregulation of intracellular Ca2+ homeostasis and glutamate-dependent excitotoxic injury has been suggested to underlie amyloid beta-peptide (Abeta) toxicity in Alzheimer's disease (AD), but the exact mechanism remains unknown.
We used the glutamate sensing fluorescent reporter (SuperGluSnFR) developed by Roger Tsien's laboratory to perform quantitative optical measurements of glutamate release in response to oligomerized Abeta peptide in cultures of rodent astrocytes.
Astrocytes are known to modulate neuronal excitability and synaptic transmission. We extend these findings here by showing that astrocytes can release toxic levels of glutamate in response to oligomerized Abeta.
The main objective of this project was to directly measure and quantify glutamate release in from purified rat cortical astrocytes in response to Abeta oligomers.
We performed Foerster resonance energy transfer (FRET) microscopy to image glutamate release using the glutamate "sniffer" SuperGluSnFR. This probe provides a sensitive fluorescent readout of glutamate concentration by FRET-dependent changes in the CFP/YFP emission ratio.

We co-transfected HEK 293T cells with SuperGluSnFr and Neuroligin, and then co-cultured these ‘glutamate-sensing cells’ with purified rat cortical astrocytes. We used the protein Neuroligin, which is known to induce functional synaptic contacts between cells, to allow the ‘probe’ HEK cells to come into close contact with astrocytes that were suspected of releasing glutamate. Abeta oligomers, as well as Abeta in its non-oligomerized form as a control, were applied during FRET measurements.
As monitored with the glutamate “sniffer” SuperGlu, nanomolar concentrations of Abeta oligomers induced glutamate release from astrocytes at concentrations of a few hundred micromolar. These levels of glutamate are known to cause synaptic loss and excitotoxic damage in neurons.
Taken together with prior studies, this work suggests that the toxic effects of Abeta oligomers on neurons may be mediated at least in part by local release of glutamate from astrocytes.

Disclosures: S. Sanz-blasco, None; **J. Pina-Crespo**, None; **M. Talantova**, None; **G. Cao**, None; **S.A. Lipton**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: European contract, FP7 NEUGENE

Title: Tissue-specific targeting of lentiviral vectors for CNS applications

Authors: *A. DELZOR[1,2], I. NASCIMENTO-FERREIRA[3], N. DUFOUR[1,2], G. AUREGAN[1,2], N. DÉGLON[1,2];
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Abstract: Tissue targeting is of major interest to study the contribution of cellular subpopulations in neurodegenerative diseases. Recent data in Huntington’s disease (HD) show that not only neuronal but also glial mutant huntingtin contribute to the neuropathology. However, methods to further investigate the contribution of these cell types in vivo are limited. In the present study, we evaluated the impact of viral envelopes on the tropism and evaluated various promoters to achieve cell-specific expression of the green fluorescent protein. Neuronal promoters of prion (PrP), synapsin I (SYN), neuron-specific enolase (NSE), glutamic

acid decarboxylase of 67 kDa (GAD67), dopaminergic receptor 1 (D1R), glutamate receptor 1 (GluR1), preprotachykinin 1 (TAC1), enkephalin (ENK), homeobox dlx5/6 (dlx5/6-CMVmin) were compared with the ubiquitous phosphoglycerate kinase 1 (PGK) promoter. Astrocytic promoters of glial fibrillary acidic protein (GFA2, 2 kb ; GFA-ABC1D, 681bp) and glutamine synthetase (GS) were studied in parallel to the excitatory amino acid transporter 1 (EAAT1) promoter.
Stereotaxic injection of the vectors was done in the striatum and hippocampus, two structures implicated in HD and Alzheimer's disease, respectively. Rats were sacrificed after three weeks. Tissue-specificity and transgene expression levels were analyzed by measuring the total GFP intensity and the number of infected cells.
Preliminary results indicate that VSV-G pseudotyped viral vector with D1R, ENK, GluR1 and PGK promoters lead to a strong neuronal expression of the transgene while the GS promoter with a Mokola envelope leads to a partial glial tropism (more than 50% and 90% of stained astrocytes in striatum and hippocampus, respectively).
Further analyses are ongoing with D1R, TAC1, NSE and GS promoters in GLAST-DsRed and GLT1-GFP transgenic mice.

Disclosures: A. Delzor, None; **I. Nascimento-Ferreira**, None; **N. Dufour**, None; **G. Auregan**, None; **N. Déglon**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NeRF, Région Ile-de-France, doctorate fellowship to LG

Title: The striatal marker Dclk3 is neuroprotective against mutant huntingtin

Authors: *E. P. BROUILLET[1], L. GALVAN[1], M.-C. GAILLARD[1], G. LIOT[2], M. DE CHALDÉE[3], G. AURÉGAN[1], N. DUFOUR[1], M. GUILLERMIER[1], M. CHAIGNEAU[1], F. PETIT[1], C. MALGORN[1], J.-M. ELALOUF[3], S. HUMBERT[2], N. DÉGLON[1];
[1]CEA, MIRCen, URA CEA-CNRS 2210, Fontenay-aux-Roses, France; [2]Insitut Curie, UMR3306 CNRS, U1005 Inserm, Orsay, France; [3]CEA, IBITec, SBIGem, Saclay, France

Abstract: Although mutant "huntingtin" (Htt) (mHtt) is expressed ubiquitously throughout the brain, the striatum is found preferentially affected in Huntington's disease (HD). This particular

vulnerability may result from the expression of a particular set of transcripts in striatal neurons. To explore this hypothesis we carried out a high-throughput analysis of the transcriptome of different brain territories in mice and identified novel transcripts that are highly enriched in the striatum. We report here on the study of one of these striatal markers, the kinase Dclk3 (double cortin like kinase 3). In normal animals, we found that Dclk3 is mainly expressed in the adult striatum in rodents. Expression of Dclk3 was found reduced in the R6/2 transgenic mouse model of HD. We developed lentiviral vectors expressing Dclk3 or a selective siRNA targetting Dclk3 mRNA. We first validated the vectors for their efficacy to over-express or knock down Dclk3 in vivo. We next studied the effects of the Dclk3 (or siRNA) vectors when injected with lentiviral vectors coding an N-terminal fragment of mutant Htt. Results showed that overexpression of Dclk3 in the mouse striatum protected against the toxicity of mutant Htt. Similar results were obtained in primary culture of striatal neurons. On the opposite, reducing Dclk3 expression increased toxicity of the mutant Htt fragment in vivo. We also identified characteristics of Dclk3 (subcellular localization, protein partners, cleavage…) indicating that the neuroprotective effect of Dclk3 may involve Ca^{2+} and cytoskeleton regulation. The present study points to Dclk3 as a novel potential therapeutic target to slow striatal degeneration in HD.

Disclosures: E.P. Brouillet, None; **L. Galvan**, None; **M. Gaillard**, None; **G. Liot**, None; **M. de Chaldée**, None; **G. Aurégan**, None; **N. Dufour**, None; **M. Guillermier**, None; **M. Chaigneau**, None; **F. Petit**, None; **C. Malgorn**, None; **J. Elalouf**, None; **S. Humbert**, None; **N. Déglon**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS

CHDI

NIA intramural program

Title: The role of deregulated miR-200a expression in mutant huntingtin-induced neuronal dysfunction

Authors: *J. JIN[1], Y. CHENG[1], Y. ZHANG[5], W. H. WOOD, III[5], Q. PENG[1], M. JIANG[1], J.

FU[1], O. PLETNIKOVA[2], J. C. TRONCOSO[2,3], C. A. ROSS[1,3,4], K. G. BECKER[5], W. DUAN[1]; [1]Dept. of Psychiatry and Behavioral Sci., [2]Dept. of Pathology, [3]Neurol., [4]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [5]Gene Expression and Genomics Unit Res. Resources Br., NIA, NIH, Baltimore, MD

Abstract: MicroRNAs (miRNAs) are ~20 nucleotides-long non-coding RNAs that act as post-transcriptional regulators of gene expression by binding to 3'UTR region of mRNA targets. There is emerging evidence showing that miRNAs may play an important role in neuronal survival and the pathogenesis of neurodegenerative disorders. Huntington's disease (HD) is caused by an expanded CAG repeat in the exon 1 of *huntingtin*. The molecular mechanisms responsible for the dysfunction and death of selective striatal and cortical neurons in HD remain largely unknown. Our previous microRNA array study revealed that miR-200 family was significantly altered in the cortex of a transgenic mouse model of HD. We further confirmed the deregulation of miR-200a in both cortex and striatum of HD mice, as well as HD cell models by using real time RT-PCR method. More interestingly, we found that miR-200a was significantly increased at the onset and early stage of disease progression in N171-82Q HD mice, suggesting that the alteration of miR-200a might contribute to disease pathogenesis of HD. The expression of miR-200a was also altered in the striatum of HD patients. We are performing gain of function and loss of function studies in cell models of HD and aim to understand the role of miR-200a in the HD pathogenesis. These studies might reveal novel molecular target(s) for developing therapeutic approaches for HD.
Financial support: NINDS (WD, CAR), CHDI (WD), NIA intramural program (KB)

Disclosures: J. Jin, None; **Y. Cheng**, None; **Y. Zhang**, None; **O. Pletnikova**, None; **J.C. Troncoso**, None; **K.G. Becker**, None; **W.H. Wood**, None; **Q. Peng**, None; **M. Jiang**, None; **J. Fu**, None; **C.A. Ross**, None; **W. Duan**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NSC98-2321-B-001-008

NSC97-2321-B-001-012

Title: Treatment of Huntington's disease using a novel dual-function adenosine compound

Authors: *Y. CHERN[1], J.-T. LIN[1], J.-F. CHEN[2], H.-M. CHEN[1];
[1]Inst. Biomed Sci., Taipei 11529, Taiwan; [2]Boston Univ. Sch. of Med., Boston, MA

Abstract: Huntington's disease (HD) is a neurodegenerative disease caused by a CAG trinucleotide expansion in exon 1 of the Huntingtin gene. We describe the purification and characterization of a novel adenosine analogue (T1-11), which has beneficial effects on several major symptoms of HD. T1-11 was originally isolated from a Chinese herb (*Gastrodia elata)*. Of 208 major receptors and signaling proteins, binding analyses and functional assays demonstrated that T1-11 is an agonist of the A_{2A} adenosine receptor and an inhibitor of the adenosine transporter. Molecular modeling analyses showed that T1-11 binds to the adenosine pockets of the A_{2A} adenosine receptor and to a major adenosine transporter (ENT1) in the brain. A single intraperitoneal administration of T1-11 allowed it to enter the brain in 10 min, suggesting a potential use of T1-11 to treat neuronal diseases. Indeed, chronic treatment of a transgenic mouse model (R6/2) of HD with T1-11 resulted in beneficial effects including improvements in motor deterioration, formation of mutant Htt aggregates, and a reduced level of a neurotrophic factor (brain-derived neurotrophic factor). The dual functions of T1-11 enable it to effectively activate the adenosinergic system and subsequently delay the progression of HD. This is a novel therapeutic strategy for HD.

Disclosures: Y. Chern, None; **J. Lin**, None; **H. Chen**, None; **J. Chen**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: a Milton Wexler Award and a fellowship from the Hereditary Disease Foundation

NIH Grant NS39074

NIH Grant NS45491

NIH Grant AG022074

the Taube-Koret Center

Title: Proteastasis of polyglutamine varies among neurons and predicts proneness to degeneration

Authors: *A. TSVETKOV[1,3], M. ARRASATE[2], P. SHARMA[2], S. FINKBEINER[3,4];
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Abstract: In several polyglutamine diseases, only certain partially overlapping groups of neurons die, despite widespread expression of the offending protein. How the same aggregation-prone protein selectively kills some neurons without apparently affecting others is unclear. Polyglutamine expansion may stabilize such proteins and induce neurodegeneration by impairing proteastasis pathways that prevent or mitigate catastrophic protein misfolding and aggregation. Protein aggregation and toxicity confound conventional measurements, obscuring the effect of polyglutamine expansion on protein stability. Using a novel optical pulse-chase method, we measured the effect of polyglutamine expansions on the half-life of a fragment of huntingtin - the protein that causes Huntington's disease - in live neurons. Here we show that polyglutamine expansion destabilizes mutant huntingtin. The ability of a neuron to destabilize huntingtin varied and predicted the duration of survival. Cortical neurons cleared mutant huntingtin faster and lived longer than striatal neurons, and the extent of destabilization and longevity correlated significantly. Thus, cells differ in their capacity to clear the same polypeptide. These differences may play a role in cell susceptibility, and efforts to bolster proteastasis could be broadly neuroprotective.

Disclosures: A. Tsvetkov, None; **M. Arrasate**, None; **P. Sharma**, None; **S. Finkbeiner**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Live imaging of mutant huntingtin oligomerization in cell cultures

Authors: *F. HERRERA[1], S. TENREIRO[2], T. F. OUTEIRO[2];
[2]Cell and Mol. Neurosci. Unit, [1]Inst. De Medicina Mol., Lisbon 1649, Portugal

Abstract: Huntington´s disease is an inherited neurodegenerative disorder caused by the misfolding and subsequent aggregation of a mutant form of huntingtin, an ubiquitous protein with a yet undefined function. Aggregation of mutant huntingtin starts with the association of a few misfolded huntingtin monomers in small, soluble oligomeric structures. These structures become larger and more insoluble as more misfolded monomers are added and incorporated. Growing evidence suggests that the solubility of the aggregates is directly proportional to their toxicity, oligomers being the most toxic species. Many experimental models of Huntington´s disease allow the visualization and study of the largest aggregates, and they provided very relevant information about the mechanisms involved in huntingtin misfolding and aggregation. However, little is still known about the first steps of aggregation, especially because of the lack of suitable models for their study. Here, we developed a cellular model for the visualization and study of dimers and oligomers of mutant huntingtin in living cells. Our model is based on a GFP-based protein complementation assay. We generated two different constructs that carried complementary portions of the Venus fluorescent reporter protein fused to the exon-1 of mutant huntingtin (103Q glutamine tract). When the exon-1 of mutant huntingtin dimerizes inside the cells both complementary halves of Venus reconstitute the functional fluorophore and emit green fluorescence, which can be easily visualized and measured. Oligomer generation and toxicity was evaluated over time and confirmed by different methods. We used similar fusion constructs with wild-type huntingtin exon-1 (25Q glutamine tract) as a negative control for oligomerization and toxicity. Additional controls included the transfection of cells with only one of the constructs, with the two halves of the Venus protein without huntingtin, or with huntingtin exon-1 without the Venus halves. The robustness of our model is being tested by analyzing the effect of known modifiers of aggregation, such as heat shock proteins, on our system. Our preliminary results indicate that this model can be a powerful tool for the identification of new molecular targets for the treatment of Huntington´s disease.

Disclosures: F. Herrera, None; **S. Tenreiro**, None; **T.F. Outeiro**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Rhes, implicated in huntington’s disease, induces and binds to endoplasmic reticulum (er) stress markers, pdi and perk

Authors: *S. SUBRAMANIAM[1], N. SHAHANI[2], M. R. ROBBERT[1], S. H. SNYDER[1];

[1]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Mol. Psychiatry,, Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Huntington's Disease (HD) is an autosomal dominant disorder caused by an expansion of glutamine repeats in the gene encoding the protein huntingtin (Htt). Htt and mutant Htt (mHtt) are ubiquitously expressed throughout the brain and peripheral tissues; yet HD is associated with highly selective degradation of the striatum, to a lesser extent cortex and with no notable alterations in peripheral tissues. Rhes (Ras homolog enriched in striatum), a member of the Ras family of small G proteins, is highly enriched in striatum. We recently reported that Rhes binds mHtt selectively and with high avidity to enhance mHtt sumoylation, with Rhes acting as an apparent E3 ligase for mHtt (Subramaniam et al Science 324: 1327, 2009). Sumoylation of mHtt leads to its disaggregation and augmented neurotoxicity. Recently, we reported that Rhes enhances cross-sumoylation of basic SUMO machineries, E1 and Ubc9 (Subramaniam et al J. Biol. Chem. in press). Here we demonstrate that Rhes is localized to the endoplasmic reticulum (ER) and induces ER stress markers, phosphodisulphide isomerase (PDI) and PERK (protein kinase-like endoplasmic reticulum kinase). Rhes binds strongly to PERK and moderately to PDI. This interaction of Rhes with ER stress proteins may play a pathogenic role together with mHtt, which may lead to selective striatal neuronal loss in HD.

Disclosures: S. Subramaniam, None; **N. Shahani**, None; **M.R. Robbert**, None; **S.H. Snyder**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Tenovus Scotland

Ataxia UK

Title: The MID1 / protein phosphatase 2A protein-complex regulates the translation of mRNAs with CAG-repeats

Authors: *S. SCHWEIGER[1], S. KRAUSS[2], D. RUTSCHOW[1], E. JASTRZEBSKA[2], C. ACHMUELLER[3], E. WANCKER[4], R. SCHNEIDER[3];
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Abstract: Huntington's Disease (HD) is a devastating neurodegenerative disorder that is caused by the expansion of a CAG repeat in its first exon. CAG repeats are found frequently in genes mainly expressed in the central nervous systeme. Although the physiological role of these repeats is unknown, their expansion is a common feature of neurodegenerative disorders. We show here that (i) CAG-repeats mediate the binding of mRNAs to a protein complex containing the ubiquitin ligase MID1, protein phosphatase 2A (PP2A), its inhibitory subunit α4 as well as several other factors, which together regulate protein translation, (ii) binding of CAG sequences to the MID1 protein complex as well as protein synthesis from CAG repeat containing mRNAs increases with repeat size and (iii) knock-down of MID1 as well as of α4 reduces protein translation of mRNAs with expanded CAG-repeats in a PP2A dependent manner, while translation of mRNAs with physiological repeat sizes is significantly less affected. Our data indicate that CAG-repeat stretches regulate protein translation and suggest the MID1-complex as a promising target for the development of therapeutic strategies for HD and other CAG repeat expansion disorders.

Disclosures: S. Schweiger, None; **S. Krauss**, None; **D. Rutschow**, None; **E. Jastrzebska**, None; **C. Achmueller**, None; **R. Schneider**, None; **E. Wancker**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant R01NS064138

HDF Research Grant to D.D.R.

Title: RNA< RNA-binding protein and the pathogenesis of Huntington's and Huntington's disease-like 2

Authors: *D. D. RUDNICKI[1], J. L. PRUITT[2], R. L. MARGOLIS[2];
[1]Psychiatry, Johns Hopkins Univ., BALTIMORE, MD; [2]Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Huntington's disease-like 2 (HDL2) is pathologically and clinically very similar to Huntington's disease (HD). The causative mutation is a CTG/CAG repeat expansion that falls, in the CTG orientation, in an exon of junctophilin-3 (JPH3) on chromosome 16q24.3. Our previously published data suggests that expression of junctophilin-3 transcript with an expanded CUG repeat is neurotoxic and may account, at least in part, for HDL2 pathogenesis. The toxicity is rescued by muscleblind-like protein 1 (mbnl1), an RNA binding protein (RBP) involved in the pathogenesis of myotonic dystrophy type 1. Given the great similarity between HD and HDL2, we have hypothesized that mbnl1 may also be involved in HD pathogenesis. Using transient and stable HD cell models, we now demonstrate that mbnl1 can rescue huntingtin-induced toxicity. The rescue is not dependent on the capacity of mnbl1 to bind to the huntingtin RNA CAG repeat. We have further hypothesized that other RBPs may also modify huntingtin toxicity. To test this, we have established a cell-based huntingtin RNA pull-down/mass spectroscopy assay to search for novel huntingtin RBPs. One of the identified proteins is ataxin-2 (atx2), a polyglutamine (polyQ) tract-containing RBP. Expansion of the ATX2 repeat tract leads to spinocerebellar ataxia type 2. Overexpression of atx2 with a normal repeat length enhances mutant huntingtin toxicity in cell models of HD. The effect is not dependent on the atx2 polyQ region, as an atx2 version without the polyQ tract also enhances htt toxicity. We are currently exploring the role of atx2 in cell models of HDL2 toxicity. We propose that in HD, as in HDL2, interaction of the mutant RNA with specific RBPs contributes to neuropathogenesis.

Disclosures: D.D. Rudnicki, None; **J.L. Pruitt**, None; **R.L. Margolis**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01 NS65874

Title: Over-expression of PGC-1α alleviates huntingtin protein toxicity by restoring mitochondrial activity and enforcing the ROS defense system in vitro and in vivo

Authors: T. TSUNEMI, *A. R. LA SPADA;
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Abstract: Huntington's disease (HD) is caused by the expansion of a CAG repeat in the huntingtin (htt) gene, yielding a htt protein with an expanded polyglutamine (polyQ) tract that

misfolds and is resistant to proteasomal degradation. Important clues to selective neuronal vulnerability in HD have been mitochondrial dysfunction and oxidative stress in the striatum. Another key feature of HD pathogenesis is the production of polyQ-expanded htt peptide fragments that localize to the nucleus and there disrupt transcription. While an interplay may exist among these features, the exact mechanistic basis of HD striatal degeneration remains to be elucidated. Previous studies have shown that mutant htt interferes with transcriptional programs co-activated by PPARγ co-activator 1α (PGC-1α), a key regulator of mitochondrial biogenesis. To test if restoration of PGC-1α function is sufficient to ameliorate neurological disease, we crossed HD N171-82Q transgenic mice with Rosa26-del-rtTA mice, and then with TRE-pCMV-PGC-1α mice to develop "triple" transgenic mice. Induction of PGC-1α expression significantly improved neurological function in HD triple transgenic mice. Analysis of striatum and cortex revealed that mitochondrial activity was significantly increased in the triple transgenic mice, and oxidative stress in the striatum was significantly reduced due to activation of reactive oxygen species (ROS) response genes by PGC-1α. Interestingly, improvement in the neurological phenotype in the HD triple transgenic mice was accompanied by a marked decrease in htt aggregate formation in the brains, including oligomeric insoluble species on filter trap assays. When we tested if striatal-like cells expressing wild-type htt (ST-Hdh-Q7/Q7) or mutant htt (ST-Hdh-Q111/Q111) could handle oxidative stress brought on by hydrogen peroxide exposure, we found that ST-Hdh-Q111/Q111 cells exhibited significantly increased cell death, which could be rescued by PGC-1α over-expression. We also found that oxidative stress accelerated mutant htt aggregate formation and cell death in Neuro2a cells transfected with htt expression constructs. Co-transfection of a PGC-1α expression construct similarly reduced mutant htt aggregate formation and caspase-3 dependent cell death in this system. Taken together, our data suggest that PGC-1α alleviates htt toxicity by activating mitochondria to improve bioenergetics status, and by inducing expression of the ROS defense system to reduce htt protein oligomerization. Thus, therapies aimed at restoring PGC-1α function hold great promise for treating HD in human patients.

Disclosures: T. Tsunemi, None; **A.R. La Spada**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Health Research Council of New Zealand

Neurological Foundation of New Zealand

Matthew Oswin Memorial Trust

Auckland Medical Research Foundation

University of Auckland

Title: Variable pattern of cortical interneuron loss in the human brain in Huntington's disease correlates with symptom profiles

Authors: *E. KIM[1], D. C. V. THU[1,3], A. L. NANA[1], D. E. OORSCHOT[4], B. J. SYNEK[5], V. M. HOGG[2], L. J. TIPPETT[2], H. J. WALDVOGEL[1], R. L. M. FAULL[1];
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Abstract: Huntington's disease (HD) is characterized by variable symptomatology (choreiform movements, mood and neuropsychological change) and variable neuropathology in the basal ganglia and cerebral cortex. Our recent studies on the primary motor and anterior cingulate cortex have shown that the variable pattern of pyramidal cell loss in the two regions correlates with the variable motor and mood symptomatology in HD, respectively *(Thu et al., Brain, 133: 1094-1110, 2010)*. We are now extending these studies to determine whether there is a variable pattern of cortical interneuron loss in the motor and cingulate cortices which also correlates with the variation in HD symptom profiles. A double-blind study was conducted in 13 HD and 14 matched control cases using unbiased stereological cell counting methods to quantify three major types of cortical GABAergic interneurons immunoreactive for - calbindin-D28k, calretinin, and parvalbumin - in the primary motor (BA4) and anterior cingulate (BA24) cortices. Detailed data on symptomatology of HD patients was collected from family members and clinical records as previously described *(Tippett et al., Brain, 130: 206-221, 2007)*. The HD cases were categorized into 3 groups according to their main symptom profile ("motor", "mood", or "mixed" [motor/mood] symptoms).
The results demonstrated a marked but variable loss of interneurons in the two cortical regions in HD cases compared to control cases. Most interestingly, the results showed a significant association between the pattern of symptomatology and interneuron loss in HD. The HD cases with predominantly "mood" symptoms showed a major significant cell loss in all three interneuronal populations in the anterior cingulate cortex (71% loss of calbindin-D28k; 60% loss of calretinin; and 80% loss of parvalbumin positive cells), but no cell loss in the primary motor cortex. By contrast, the HD cases with predominantly "motor" symptoms showed a selective loss of only calbindin-D28k positive interneurons (57% loss) in the primary motor cortex, but no interneuron cell loss in the anterior cingulate cortex. The HD cases with "mixed" [motor/mood] symptoms showed a significant loss of calretinin (45% loss) and parvalbumin (56% loss) positive cells in the anterior cingulate cortex, but no interneuron cell loss in the primary motor cortex. These findings show that there is a major heterogeneity in the pattern of interneuron loss in the cerebral cortex which correlates with the symptom profiles in HD.

Disclosures: E. Kim, None; **D.C.V. Thu**, None; **D.E. Oorschot**, None; **V.M. Hogg**, None; **L.J. Tippett**, None; **B.J. Synek**, None; **H.J. Waldvogel**, None; **R.L.M. Faull**, None; **A.L. Nana**, None.

Nanosymposium

322. Huntington's Disease I

Location: Room 10

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 322.12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NSERC

Title: Proteomic analysis of redox sensitive proteins in a Huntington's disease cell model reveals Peroxiredoxin-1 as a target for thiol-based antioxidants

Authors: *R. C. CUMMING, A. PITTS, K. DAILEY;
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Abstract: Numerous studies have shown a link between oxidative stress and various neurodegenerative diseases, including Huntington's disease (HD). Although reactive oxygen species (ROS) have traditionally been viewed as agents that cause non-specific damage to DNA, lipids and proteins, recent evidence has shown that ROS can act as second messengers and selectively target proteins leading to a change in their activity or function. High ROS levels promote the oxidation of protein cysteine sulfhydryl groups to covalent disulfide bonds or higher oxidized species such as sulfinic or sulfonic acids. In an effort to determine the spectrum of disulfide-bonded proteins that are specifically altered in an HD context, protein extracts from PC12 cells that inducibly express an amino terminal fragment of the Huntingtin (Htt) protein with either a 25 (non-pathogenic) or 103 (pathogenic) polyglutamine repeat were resolved by a novel two-dimensional SDS-PAGE technique (Redox 2D-PAGE) followed by MALDI-TOF mass spectrometry analysis. Several antioxidant and mitochondrial proteins were identified that exhibited changes in disulfide bonding only in Htt-103Q expressing PC12 cells. In particular, the antioxidant protein Peroxiredoxin 1 (Prx1) exhibited an overall decrease in protein levels and disulfide bonding in response to mutant Htt expression whereas the levels and redox status of Prx1 in Htt-25Q expressing cells remained unchanged. The loss of disulfide-linked Prx1 following mHtt expression correlated with the appearance of an overoxidized (sulfonylated) monomeric form of the protein. Ectopic expression of Prx1 in PC12 cells attenuated mutant Htt-induced toxicity. Furthermore, treatment of mutant-Htt expressing cells with thiol based

antioxidants suppressed toxicity and aberrant disulfide bonding. Interestingly, of all the compounds tested, dimercaptopropanol (DMP) strongly suppressed mutant-Htt-induced toxicity and restored the levels and redox state of Prx1. DMP was previously shown to have some clinical efficacy in treating HD patients and was also identified as a strongly neuroprotective compound in a HD cell model following a blind screen of over 1000 FDA-approved drugs. Our studies reveal for the first time that pathogenic Htt can promote aberrant disulfide bonding of antioxidant and mitochondrial proteins; an event countered by specific thiol-based compounds. These findings should provide a catalyst to explore the use of thiol-based antioxidants as potential therapies in HD.

Disclosures: R.C. Cumming, None; **A. Pitts**, None; **K. Dailey**, None.

Nanosymposium

323. Genetic Approaches to Addiction

Location: Room 31C

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 323.1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Addiction neurobiology: Support from human gene variants identified by moderate p values in each of multiple independent samples

Authors: *G. R. UHL, T. DRGON, C. JOHNSON, D. WALTHER, M. NINO;
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Abstract: Conventional, “template” analyses of human addiction vulnerability genome wide association data seek individual SNPs that are associated with substance dependence with “genome wide” 10-8 levels of significance in each of multiple independent samples, and find little evidence for such effects. We have identified genes that are identified by clusters of SNPs with more modest, nominally significant p values in each of multiple independent samples. Genes identified in this way that encode putative cell adhesion molecules that have been identified in 13 and 10 independent samples studied using pooled or individual genotyping, cadherin 13 and cub sushi multiple domains 1. At least 20 other genes are identified by such observations in at least six independent samples, including genes involved in glutamatergic neurotransmission (mGlur5), NO neurotransmission (PRKG1) and mRNA splicing (A2BP1). We discuss the common and differential features of brain distribution of expression and focal vs multifocal patterns of association within these genes in relation to possible roles in different

aspects of development and maintenance of addictive behaviors. *(Support: NIDA-IRP/NIH/DHHS)*

Disclosures: G.R. Uhl, None; **T. Drgon**, None; **C. Johnson**, None; **D. Walther**, None; **M. Nino**, None.

Nanosymposium

323. Genetic Approaches to Addiction

Location: Room 31C

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 323.2

Topic: C.17. Drugs of Abuse and Addiction

Title: Prefrontal white matter is influenced by catechol-o-methyl transferase (COMT) val158met polymorphism in substance users and healthy controls

Authors: *X. ZHANG[1], D. J. STEIN[2], B. SALMERON[1], M. LEE[1], M. LEE[1], X. GENG[1], C. HODGKINSON[3], P.-H. SHEN[3], Y. YANG[1], D. GOLDMAN[3], E. A. STEIN[1];
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Abstract: The COMT val158met polymorphism is a frequently-studied functional locus that alters both the metabolism of catecholamines and behavior. Healthy controls with the Met^{158} allele have lower enzymatic activity, higher prefrontal dopamine levels and associated differences in frontal cognitive function, and increased limbic activation in response to pain. Significant associations between val158met and susceptibility to cannabis, nicotine, and opiate dependence have been reported. We have reported that smoking-related PFC white matter alterations are related to severity of nicotine dependence, a relatively heritable trait of tobacco smokers. In the current study, we tested the hypothesis that white matter in substance users is influenced by COMT val158met. Diffusion tensor imaging (DTI) data were acquired in 116 controls (17 met/met, 63 met/val, and 36 val/val subjects) and 164 subjects with substance dependence (35 met/met, 80 met/val, and 49 val/val individuals). Data were analyzed using a whole brain DTI method using tract-based spatial statistics with an improved alignment method. A 3 genotype x 2 group ANOVA was used to detect effects on fractional anisotropy (FA). One cluster in the left prefrontal area showed significant an interaction gene by group (threshold: FWE corrected $p<0.05$), another cluster in the left superior longitudinal fasciculus showed a significant gene main effect (The met/met subjects showed the lowest FA data while the FA in val/val individuals was highest.), and three clusters in the right prefrontal area and bilateral

inferior/superior longitudinal fasciculus showed a significant group main effect (Compared to controls, drug abusers showed lower FA.). A secondary ROI analysis including age, gender and ethnicity as co-variables showed a significant interaction and group main effect in both left and right prefrontal white matter. Further, the met/met controls had significantly higher FA in the left PFC than val/val controls, while substance abusers showed the opposite pattern. These results suggest that prefrontal white matter alterations in drug dependent individuals are influenced by the COMT gene and reflect heritable differences related to drug abuse while white matter in some posterior regions is affected by addiction and COMT gene respectively. (Supported by NIDA IRP)

Disclosures: X. zhang, None; **D.J. Stein**, None; **B. Salmeron**, None; **M. Lee**, None; **M. Lee**, None; **X. Geng**, None; **C. Hodgkinson**, None; **P. Shen**, None; **Y. Yang**, None; **D. Goldman**, None; **E.A. Stein**, None.

Nanosymposium

323. Genetic Approaches to Addiction

Location: Room 31C

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 323.3

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant R21-DA024429-01 to Drs. Chua and Strecher

Title: Activation within the self-related processing network to tailored health messages mediates the influence of the STin2 genotype on smoking cessation outcome

Authors: *A. J. JASINSKA[1], H. F. CHUA[2], S.-H. S. HO[3], T. A. POLK[4], L. ROZEK[5], V. J. STRECHER[2];
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Abstract: Each year, a large number of smokers attempt to quit, but on average only 10% remain abstinent for 6 months or more, highlighting a vital need for improved smoking cessation treatments and for better predictors of treatment outcomes. One class of such predictors is genetics. Twin studies suggest that genetic factors account for over 50% of the variance in smoking cessation outcomes but specific genetic variants involved are mostly unknown. Identification of specific genetic variants associated with smoking cessation, and a better

understanding of the neurobiological processes through which these variants influence smoking behavior, could guide the design and selection of the most effective treatment for each smoker. In the current study, we combined genetics, functional MRI, and health communication techniques to examine the role of the STin2 polymorphism in the serotonin transporter gene on the brain processes related to smoking cessation outcome. STin2 is a functional polymorphism, with the 12-repeat allele more efficiently transcribed than the 10-repeat allele. Similar to the S allele of the 5-HTTLPR, the STin2.12 allele is associated with neuroticism, a personality trait considered to be a risk factor for substance abuse and linked to higher rates of failure in quitting smoking.

Because prior neuroimaging work has shown that tailored health messages preferentially activate the self-related processing network, including the medial prefrontal cortex (MPFC) and the precuneus/posterior cingulate regions, we set out to determine whether the influence of the STin2 genotype on smoking cessation behavior is mediated by the response of the self-related processing network to tailored smoking cessation messages.

Ninety-one smokers interested in quitting (44 females and 47 males; mean age 37.5 ± 11.5 years) were recruited for the study. All participants completed a web-based tailored smoking cessation program. Complete data (including genotyping results, fMRI data, and a smoking cessation outcome at 4-month follow-up) were available from eighty-four participants and these results are reported.

Consistent with our hypothesis, we found evidence that the response to tailored health messages in the dorsal MPFC within the self-related processing network mediated the influence of the STin2 genotype on the smoking cessation outcome at the 4-month follow-up ($p < 0.05$).

These findings may be relevant to a broad area of research on the genetics and neurobiology of smoking cessation and may point towards genetically-tailored smoking cessation treatments in the future.

Disclosures: A.J. Jasinska: None. **H.F. Chua:** None. **S.S. Ho:** None. **T.A. Polk:** None. **L. Rozek:** None. **V.J. Strecher:** Employment; Dr. Strecher is the Chief Visionary Officer, Founder, and Shareholder of HealthMedia, a company that develops and licenses computer-tailored health promotion, disease prevention and managment tools.. Consultant/Advisory Board; Dr. Strecher has consulted for pharmaceutical companies that market computer-tailored smoking cessation programs..

Nanosymposium

323. Genetic Approaches to Addiction

Location: Room 31C

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 323.4

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH T32-MH014654-32

NIH P20-DA025995

NIH P60-DA05186

Title: Case-control association analysis for polymorphisms in the mu-opioid receptor interacting protein candidate gene, GPR177, and cocaine dependence

Authors: *L. M. AMBROSE-LANCI, P. J. BLOCH, C. S. SHEEKEY, T. N. FERRARO, K. M. KAMPMAN, C. A. DACKIS, H. M. PETTINATI, C. P. O'BRIEN, F. W. LOHOFF, W. H. BERRETTINI;
Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Addiction susceptibility and treatment responsiveness are greatly influenced by genetic factors. Sequence variations in genes involved in mechanisms of drug action have the potential to influence addiction risk and treatment outcome. The opioid receptor system, specifically the mu-opioid receptor (MOR), is involved in the rewarding effects of many drugs of abuse including cocaine. The MOR interacts with and is regulated by many known MOR interacting proteins (MORIPs) which are predicted to regulate underlying mechanisms in addiction. In light of this, the present study seeks to evaluate the contribution of MORIP candidate gene, *GPR177*, in risk for cocaine dependence (CD). GPR177 is an evolutionarily conserved protein (mammalian ortholog of *Drosophila* Wntless/Evi/Sprinter) which regulates Wnt secretion thereby influencing dendritic morphology. Since cocaine-induced neuroplasticity contributes to CD, we hypothesize that polymorphisms in the *GPR177* gene may be associated with CD. CD individuals (n=309) and unaffected controls (n=256) of African descent were genotyped for 12 single nucleotide polymorphisms (SNPs) in the *GPR177* gene (rs1430746, rs1337406, rs2033349, rs3736934, rs269350, rs2820487, rs983034 (Ile463Val), rs3748705, rs2566758, rs944082, rs1036066, rs2772280) using Taqman® SNP Genotyping technology. Case-control association analysis was conducted for all SNPs (rs2820487 was excluded from further analysis due to low minor allele frequency). No statistically significant differences were observed in allele or genotype frequencies between cases and controls for any single SNP analyzed. For haplotype associations, a sliding window analysis was employed which uncovered a potential protective haplotype. Specifically, the GCT haplotype at SNPs rs269350, rs983034 (Ile463Val), rs3748705 occurred more frequently in the control population (12.9%) compared to the CD population (9.2%; $X^2(4) = 4.039$, p =. 0445). Further analyses are needed to confirm whether this region of *GPR177* is a risk factor for CD. Genotyping is ongoing and final analysis will include 1000 samples each for control and CD populations.
.

Disclosures: L.M. Ambrose-Lanci, None; **P.J. Bloch**, None; **C.S. Sheekey**, None; **T.N. Ferraro**, None; **K.M. Kampman**, None; **C.A. Dackis**, None; **H.M. Pettinati**, None; **C.P. O'Brien**, None; **F.W. Lohoff**, None; **W.H. Berrettini**, None.

Nanosymposium

323. Genetic Approaches to Addiction

Location: Room 31C

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 323.5

Topic: C.17. Drugs of Abuse and Addiction

Support: ICMR, New Delhi, India

CSIR, New Delhi, India

Title: OPRM1 gene: Functional polymorphism in Exon I and correlation with susceptibility to alcohol and opiate dependence

Authors: *S. KAPUR[1], A. PAL[2], S. SHARAD[3], L. DHAKA[4];
[2]Biol. Sci. Group, [1]Birla Inst. of Technol. and Sci. (BITS), Pilani, Rajasthan, India-33303, Pilani, India; [3]Ctr. for Prostate Dis. Research, Uniformed Service Univ., Bethesda, MD; [4]BDK Hosp., Jhunjhunu, India

Abstract: OPRM1 gene encodes the mu-opioid receptor (MOR), which is the primary site of action for opiates, such as opium and heroin, and may also contribute to the effects of non-opioid drugs, such as cocaine and alcohol. MOR has also been implicated in the reward, tolerance and withdrawal effects of alcohol, opiates and other drugs of abuse. This is further substantiated by several findings such as effect of alcohol on beta-endorphin release, effect of MOR agonists and antagonists on alcohol consumption and the observation that both alcohol and opiates activate the dopaminergic reward system. The OPRM 1 gene, contains 2 SNPs C17T (rs 1799972) and A118G (rs 1799971) in the exon1. Variant A118G has been shown to affect the ligand binding to MOR and reported to be associated with drug dependence. The aim of the present study was to delineate the frequency of the C17T and A118G variants in subjects exclusively dependent on either alcohol or opiates. Age and ethnicity matched cases, diagnosed as alcohol-dependent or opiate-dependent, on the basis of DSM IV criteria by a qualified psychiatrist, and controls were recruited after taking informed consent. Subjects using more than one substance of abuse or suffering from other neuropsychiatric ailments were excluded from the study. Genotyping for C17T (Ala6Val) variant and A118G (Asn40Asp) variant was done using DNA isolated from peripheral lymphocytes and PCR-RFLP based method. This pilot study has revealed that a significant difference exists in the distribution of A118G and C17T allele both in alcohol-dependent or opiate-dependent subjects as compared to control subjects. The frequency of 118G allele was much higher in alcohol (0.33) and opiate (0.31) dependent subjects as opposed to the observed frequency (0.18) in controls. The frequency of 17T allele in alcohol and opiate dependent subjects was also found to be higher, 0.30 and 0.17 respectively in comparison to the

frequency observed (0.11) in controls. A 2-fold higher incidence of G allele (OR=2.06, $CI_{95\%}$=1.2704 to 3.3482) and over 3-fold higher incidence of T allele (OR=3.8936, $CI_{95\%}$=2.1709 to 6.9835) was observed in alcohol dependent subjects. Similarly, a 2-fold higher incidence of G allele (OR=2.2002, $CI_{95\%}$=1.3892 to 3.4846) and T allele (OR=1.8005, $CI_{95\%}$=0.9097 to 3.5635). Both the SNPs, C17T and A118G of Exon I of OPRM1 gene were found to be equally associated with alcohol dependence ($p<0.003$) and opiate dependence ($p=0.0007$) respectively.The significant associations observed with the diagnosis of substance dependence, suggest that 118G and/or 17T allele could directly impact an individual's risk factor for both alcohol and opiate dependence in north Indian subjects.

Disclosures: S. Kapur: Research Grant; Indian Council of Medical Research, India. **A. Pal:** Other Research Support; Senior Research Fellowship from Council of Scientific and Industrial Research, India. **S. Sharad:** None. **L. Dhaka:** None.

Nanosymposium

323. Genetic Approaches to Addiction

Location: Room 31C

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 323.6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA-INIA grant U01AA13499

NIAAA-INIA grant U24AA13513

NIDA grant P20-DA 21131

UTHSC Center for Integrative & Translational Genomics

Title: Analysis of the brain transcriptome using RNA sequencing: Genetics of exon use and alternative splicing

Authors: *K. MOZHUI, X. WANG, M. K. MULLIGAN, Z. LI, L. LU, R. W. WILLIAMS; Anat. & Neurobilogy, Univ. Tennessee HSC, MEMPHIS, TN

Abstract: Alternative splicing and quantitative differences in mRNA are important mechanisms by which the mammalian genome produces phenotypic diversity within and between species. Until recently, large-scale studies of the transcriptome have relied on microarrays and these have provided a glimpse of the prodigious complexity of gene expression. For a more in-depth

analysis of transcriptional control we have used high-throughput sequencing to directly measure and characterize mRNA isoforms in the brain and eye of the mouse. We assayed two key strains of mice—C57BL/6J (B6) and DBA/2J (D2)—and a family of over 20 BXD lines derived from B6 and D2. We have DNA sequence data for both parental strains and high-density genotypes for all progeny BXD strains. This enables us to carry out a finer genetic analysis of transcript variance. Genotypes differ significant for a wide variety of neural traits (e.g., neuron number, drug preference, anxiety, seizure susceptibility, adult neurogenesis) and here we focus mainly on genes known to modulate these phenotypes (e.g., Bdnf, GluR2, and GABA-A receptors). Our assays were based on ~35 million 50-nucleotide oriented reads that provided nucleotide level resolution. We report allelic and tissue-specific differences in gene and exon level expression and alternative promoter usage and exons splicing. These define a subset of molecular events underlying the individual variation in behavior and neurophysiology in these strains of mice. These RNA-seq data are also available at www.genenetwork.org.

Disclosures: K. Mozhui, None; **X. Wang**, None; **M.K. Mulligan**, None; **Z. Li**, None; **L. Lu**, None; **R.W. Williams**, None.

Nanosymposium

323. Genetic Approaches to Addiction

Location: Room 31C

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 323.7

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA016194

NIH Grant DA025697

NIH Grant MH077995

NIH Grant AA016828

NIH Grant RR000168

Title: Comparative primate genetics: A new tool for neuroscience connecting function and disease

Authors: *E. J. VALLENDER[1], D. M. PLATT[2], G. M. MILLER[2];
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Abstract: As next generation sequencing technologies vastly increase the amount of genetic and genomic information available, the need to invest this wealth of data with biological meaning presents new challenges and grand opportunities for the neuroscientist. Due to the complexity of the brain and the intimate interrelationships between gene and environment in behavior, neurobiology is simultaneously has the most to benefit by this sudden increase in data and the field for which its application is most difficult. Physiological, behavioral and genomic similarities between humans and other primates provide an unmatched opportunity to identify and elucidate disease-related genes and genetic differences using comparative genetic approaches. In turn these understandings allow for the creation of new, highly translational, models of complex neurogenetic disorders. An explosion of genomic information, including the complete genomes of nearly a dozen primate species, is allowing us to identify functionally relevant differences affecting brain development, structure and activity between humans and non-human primates. Additionally, comparisons of intra-specific patterns of variation between primate species are revealing convergent genetic variation that results in similar consequential effects, both at the level of protein function and expression as well as shared associations with disease-related phenotypes. By comparing evolutionary divergence in regions associated with neuropsychiatric disease in humans, we have observed patterns among the genetic histories of genes and regions associated with specific diseases as well as more broad categories of disease. In this regard, comparative primate genetics reveals a context for understanding the origins of neurological diseases and their underlying causes. Here, we put forth an example of how this approach has allowed us to enhance the nonhuman primate model of alcoholism. We have located genetic variation in rhesus monkeys that parallels, in function and effect, alcoholism-associated variation in humans. Rhesus monkeys that naturally harbor functionally comparable yet evolutionarily distinct OPRM1 variants to those in humans demonstrate enhanced alcohol drinking behavior and a pharmacogenomic response to naltrexone that parallels human alcoholics. This is the first pharmacogenomic model of a human medication response to be developed in non-human primates for a complex polygenetic disorder. Accordingly, using the knowledge gained from comparative primate genetic studies has allowed us to develop a genetically-enhanced non-human primate model for neuropsychiatric disease and behavior.

Disclosures: E.J. Vallender, None; **G.M. Miller**, None; **D.M. Platt**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.1

Topic: D.02. Auditory

Title: Functional reorganization of the auditory-motor integration network following ventral premotor cortex disruption: Evidence from offline rTMS-fMRI

Authors: *K. KORNYSHEVA[1,2], R. I. SCHUBOTZ[1];
[1]Motor Cognition, Max Planck Inst. For Neurolog. Res., Cologne, Germany; [2]Motor Control, Univ. Col. London, Inst. of Cognitive Neurosci., London, United Kingdom

Abstract: In humans, the anatomical position of the ventral premotor cortex (PMv) renders this area a node for auditory-motor integration in the dorsal auditory stream. This function has been corroborated by neuroimaging studies involving tasks that require auditory-motor transformation. Yet, it remains unknown whether the PMv is causally relevant for auditory-motor integration and whether a perturbation of normal brain activity in the PMv can trigger task-dependent compensatory activity increases in interconnected areas. Functional magnetic resonance imaging (fMRI) was used to investigate short-term functional reorganization following 0.9 Hz transcranial magnetic stimulation (rTMS) over the left ventral premotor cortex (PMv) as opposed to no rTMS during auditory-motor integration. A second session comprising fMRI following rTMS over the left angular gyrus (AG) and no rTMS served to control for rTMS unspecific effects. The order of fMRI scans and sessions was counterbalanced across subjects to exclude learning effects. In an event-related design, sixteen healthy subjects had to synchronize cued left or right finger tapping to beat rates (1.7, 2.0, 2.5 Hz) of auditory rhythms (synchronization condition; SC) and produce self-paced tapping during spectrally identical, but temporally scrambled versions of the same rhythms (control condition; CC). As hypothesized, activity in the dorsal auditory stream - superior temporal gyrus and the ventral premotor cortex - was enhanced for SC compared to CC in all scans (Fig. 1A). rTMS over the left PMv caused enhanced activation in the midline of the anterior cerebellum (vermal lobule V, Fig. 1B). To capture an effect that changes over time, a modulation of synchronization accuracy and BOLD signal across four sub-blocks were considered. Preliminary results suggest that a stimulation of the ventral premotor cortex triggers task-dependent compensatory mechanisms in a cerebellar region not directly interconnected with the PMv, but functionally associated with reduced response variability and discrete motor timing.
$$graphic_42475B61-6B6E-417A-BF0C-05069EB97800$$

Disclosures: K. Kornysheva: Employment; Institute of Cognitive Neuroscience UCL. **R.I. Schubotz:** None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.2

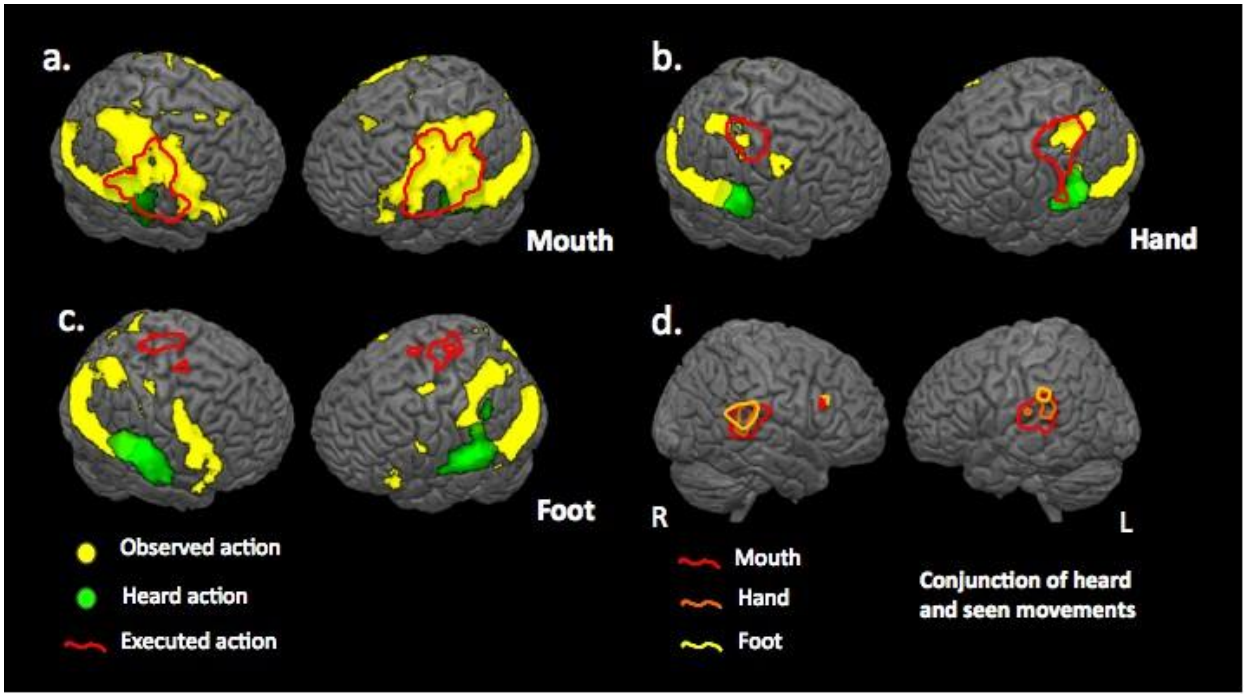
Topic: D.02. Auditory

Support: Wellcome Trust Grant WT074414MA

Title: Multimodal mirror responses for different effectors: Convergence and divergence of sensorimotor processing

Authors: *Z. K. AGNEW, C. MCGETTIGAN, S. K. SCOTT;
UCL Inst. of Cognitive Neurosci., London, United Kingdom

Abstract: It is widely established that perception of action results in activity in motor and/or premotor cortices. This has been demonstrated for both observed and heard actions in response to a range of different object-directed, non-object directed and gestural actions using neuroimaging. Single recordings from non-human primates have revealed the presence of auditory visual mirror neurons in F5 of ventral premotor cortex. We aimed to look at common and divergent encoding of executed, observed and heard simple intransitive actions of the hand, mouth and foot.
We report overlapping activity for observation and execution of both hand and mouth actions in motor cortices (visuomotor mirror responses, Figure 1 a and b). In contrast, the only effector that resulted in common activity during audition and execution of an action (auditor-motor mirror responses) was the mouth. For mouth actions, we report activity common to executing, observed and heard actions in ventral motor regions in both hemispheres. No mirror activity was seen for actions of the foot. In addition to this, we report common encoding of auditory and visual actions for all effectors in supramarginal gyrus in both hemispheres (Figure 1d).
We argue that these differences between modality for mirror responses may reflect the extent to which actions of the different effectors rely on sensory feedback. For example, mouth actions rely on both visual and auditory feedback, whereas hand actions are highly dependant on visual feedback, whereas the footstep movements used in this experiment are rarely performed under visual or auditory feedback. We suggest that this profile is reflected in these patterns of common and divergent encoding of sensory and motor responses to action.



Disclosures: Z.K. Agnew, None; **C. McGettigan**, None; **S.K. Scott**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.3

Topic: D.02. Auditory

Support: AOSR FA9550-10-C-0092

Title: Entrainment to complex rhythms: Tests of a neural model

Authors: *M. J. VELASCO[1,2], L. MAURER[2], E. W. LARGE[2];
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Abstract: Rhythmic entrainment of bodily movements to acoustic patterns is intrinsic to music, observed in every known musical culture. In even very complex musical rhythms people perceive a pulse, and structured patterns of accentuation among pulses, called meter. In general,

rhythmic entrainment involves the coordination of periodic tapping with a complex, multifrequency signal that may have little or no energy at the pulse frequency. We created a set of ten simple and ten complex (syncopated) auditory rhythms. For the simple rhythms, FFT analysis confirmed the presence of energy at the pulse frequency, whereas for the complex rhythms, FFT analysis revealed no energy at the pulse frequency. Next, in a computational simulation, these rhythms produced entrained neural oscillations. The simulation predicted periodic oscillations at the pulse frequency (as well as other frequencies) for both types of rhythms. However, for the complex rhythms, a greater variety of frequencies, a greater number of disruptions, and longer relaxation times were observed. We tested these predictions in a behavioral experiment in which people were instructed to tap periodically with the same rhythms. For the complex rhythms, a greater variety of tapping frequencies, more disruptions and longer relaxation times were found, as predicted by the neural model. These results provide evidence that intrinsic neural oscillations may underlie the perception of pulse and meter in musical rhythms.

Disclosures: M.J. Velasco, None; **L. Maurer**, None; **E.W. Large**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.4

Topic: D.02. Auditory

Support: Technologiestichting STW, The Netherlands

SmartMix Programme, The Netherlands

Title: Rhythm processing decomposed: EEG of perceived and self-imposed rhythmic patterns

Authors: *R. S. SCHAEFER, R. J. VLEK, P. DESAIN;
Donders Ctr. for Cognition, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Rhythmic patterns are at the basis of understanding spoken language, movement coordination and joint action. Perceiving rhythms can be considered a process of attentional chunking over time, often driven by accent patterns. In these patterns, events have varying saliency levels based on their metric position. A rhythmic structure can also be generated internally, by imposing a subjective accent pattern on an isochronous stimulus train. By looking at perceived as well as subjective patterns, we can disentangle low-level perceptual processes

from the cognitive aspects of rhythm processing.
We investigate the event-related potential (ERP) signature of three different metric patterns, and distinguish accented events and two types of unaccented event. The results show differences between accented and all unaccented events, but also show that specific responses to different types of unaccented events can be distinguished, revealing additional structure within the rhythmic pattern. This structure is further investigated by decomposing the ERP using Principal Component Analysis (PCA) over both tasks; perceiving and imagining the patterns. In this way, common activation processes between perceiving a pattern and self-generating it are isolated, and can be visualized for the tasks separately.
The results show the ERP responses to be quite similar between tasks, although the accents in the stimulus cause a larger N1/P2 complex. Two effects appear to distinguish different metric events; an early central effect and a later frontal effect. The PCA results confirm this, and the former is captured in the first two components, explaining 67% of the variance, while the third component explains an additional 7% at frontal locations after about 300 ms.
The main contribution of this study is to show the structure within rhythmic patterns that goes beyond simple series of accented and unaccented events. Additionally, these effects do not greatly differ between tasks. This suggests that top-down processes have a substiantial role in the cerebral mechanisms of rhythm processing, independent of an externally presented stimulus.

Disclosures: R.S. Schaefer, None; **R.J. Vlek**, None; **P. Desain**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.5

Topic: D.02. Auditory

Title: The impact of basal ganglia or cerebellar lesions on attention-dependent temporal processing

Authors: *M. SCHWARTZE, S. A. KOTZ;
Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: The basal ganglia (BG) and the cerebellum (CE) not only engage in motor behavior but also in temporal processing, i.e. mechanisms underlying the encoding, decoding and evaluation of temporal structure (e.g. Ivry and Schlerf, 2008). Whereas the BG and associated cortico-striato-thalamo-cortical circuits are implicated in attention-dependent, interval-based temporal processing, the CE is associated with pre-attentive, event-based temporal processing

(e.g. Buhusi and Meck, 2005). Although they are assumed to work in parallel, these systems have been primarily modeled in isolation. However, recent neuroanatomical and neurofunctional evidence suggests a function of the CE in early stages of sensory processing (Petacchi et al., 2005) and identified connections from non-motor parts of the CE to the supplementary motor area (SMA) which in turn projects to the BG (Akkal et al., 2007). These connections provide a basis for temporal processing in a network of functionally specialized subsystems with the BG involved in the evaluation of temporal structure. In the current study we used the high temporal resolution of the Electroencephalogram (EEG) to investigate attention-dependent temporal processing in patients with focal BG or CE lesions. The EEG was recorded from patients and healthy controls while these participants directed their attention to two-tone auditory oddball sequences (600 Hz standard, 660 Hz deviant) which conveyed either regular or irregular temporal structure. We hypothesize that BG lesions should compromise the evaluation of temporal structure, the recognition of temporal regularity, and the ability to subsequently focus attention on the temporal locus of relevant information. In turn, this difficulty should modulate the amplitude of the event-related potential (ERP) associated with the attentive detection of the deviant element (P300; Linden, 2005; Polich, 2007). Our findings suggest that this manipulation of temporal structure has indeed an impact on P300 amplitude. Moreover, in comparison to controls, both patient groups showed a reduction in P300 amplitude. However, this reduction was stronger for BG than for CE patients. These findings support the notion of dissociable pre-attentive, event-based and attention-dependent temporal processing systems and the supposed role of the BG in the evaluation of temporal structure.

Disclosures: M. Schwartze, None; **S.A. Kotz**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.6

Topic: D.02. Auditory

Title: The basal ganglia in perceptual timing: The effects of Parkinson's disease and deep-brain stimulation

Authors: *M. GRUBE[1], A. MANDAL[1], T. E. COPE[1], F. E. COOPER[1], U. BRECHANY[3], D. J. BURN[2], T. D. GRIFFITHS[1];
[1]Med. Sch., [2]Inst. for Ageing and Hlth., Newcastle Univ., Newcastle-upon-Tyne, United Kingdom; [3]NHS Trust, Newcastle Gen. Hosp., Newcastle-upon-Tyne, United Kingdom

Abstract: This work tests the role of the basal ganglia in duration-based and beat-based timing of auditory events. Both those types of perceptual timing have been shown to activate a common timing network of the brain, including the cerebellum, basal ganglia and prefrontal cortical areas (Penhune et al., 1998; Grahn and Brett, 2007). Recent neuropsychological evidence suggests a functional dissociation between the absolute, duration-based timing and relative, beat-based timing of auditory events: patients with cerebellar degeneration show an impairment in the duration-based timing of single-interval duration but not in the beat-based timing of rhythmic sequences (Grube et al., 2007), whilst patients with Parkinson's Disease show an impairment in the discrimination of rhythms with a metrical beat (Grahn and Brett, 2009). In this work, we seek to dissociate duration- from beat-based timing in patients with Parkinson's Disease as a model of basal ganglia dysfunction and, in addition, test the effect of deep brain stimulation (DBS) on the two types of timing. We report a group of case studies based on four perceptual timing tasks: duration discrimination of single intervals, detection of a regular beat, detection of a deviation from an isochronous beat, and discrimination of sequences with a metrical beat. Each task is performed 3 times, with the stimulator on, off and on. Preliminary data are consistent with a role for the basal ganglia in perceptual timing, a specific function in metrical rhythm perception, with a beneficial effect of DBS.
References:
Grahn JA, Brett M (2007) Rhythm and beat perception in motor areas of the brain. J Cogn Neurosci 19:893-906.
Grahn JA, Brett M (2009) Impairment of beat-based rhythm discrimination in Parkinson's disease. Cortex 45:54-61.
Grube M, Cooper FE, Chinnery PF, Griffiths TD (2007). It's about sub-second time: impaired basic vs. preserved higher-order timing functions in cerebellar degeneration. 37th SfN Annual Meeting.
Penhune VB, Zattore RJ, Evans AC (1998) Cerebellar contributions to motor timing: a PET study of auditory and visual rhythm reproduction. J Cogn Neurosci 10:752-765.

Disclosures: M. Grube, None; **F.E. Cooper**, None; **A. Mandal**, None; **T.E. Cope**, None; **D.J. Burn**, None; **T.D. Griffiths**, None; **U. Brechany**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.7

Topic: D.02. Auditory

Support: CIHR 81135

Title: Neuromagnetic oscillation in musical meter and beat processing

Authors: *T. FUJIOKA, B. R. ZENDEL, B. ROSS;
Baycrest, Univ. of Toronto, Rotman Res. Inst., Toronto, ON, Canada

Abstract: Music, or rhythmic sound, facilitates synchronized body movements even in Parkinson's disease (PD) patients with motor impairment off-medication. Such facilitation, accompanied with timing perception is thought to be related to basal-ganglia-thalamo-cortical functions. Neural oscillations are proposed to be central in the integration of information at local and global levels. Beta-band (13-30Hz) activity is specifically associated with motor system in that beta amplitude decreases prior to and during movement, and increases again after task completion. In our previous magnetoencephalography (MEG) study, beta activity in bilateral auditory cortices showed synchronized modulation with the regular beat stimuli (Fujioka et al. 2009). Subsequently, we showed that when the same beat sequence was interpreted by listeners according to different musical meter context- such as march or waltz - activated the auditory, sensorimotor cortex, basal-ganglia, and hippocampal area differently (Fujioka et al. 2010). The present study is further aimed at investigating whether beta activity is related to musical meter processing in bilateral sensorimotor and auditory cortices.
MEG was recorded from musically trained subjects alternatively listening to the regular beat stimuli (clicks every 390ms) and tapping to the same stimuli in a march or waltz context (every 2nd or 3rd). Beta activity during the listening period in bilateral auditory and sensorimotor cortices was examined using equivalent current dipole estimates and source space projection. The conditions were separated as march-down-beat, march-up-beat, waltz-down-beat, and waltz-up-beat.
Consistent with the previous study with metrically unaccented beat stimuli, the beta modulation was found extended beyond auditory cortices to sensorimotor cortices while in the latter the modulation amplitude was much weaker than the former. Beta desynchronization was found around 160-170 ms after the stimulus onset in all conditions, while the amplitude for the down-beat was larger than that for the up-beat. This distinction, however, was stronger in the march condition than in the waltz condition.
Presentation of regular auditory beats led to beta band modulation synchronized to the tempo of the beats in both auditory areas and motor thalamo-cortical loops, even in the absence of motor activity, explaining why music makes us want to move in time to the beat and the musical meter. Our results suggest that beta-band activity plays an important role for timing perception and predictive motor movements.

Disclosures: T. Fujioka, None; **B.R. Zendel**, None; **B. Ross**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.8

Topic: D.02. Auditory

Support: Neurosciences Research Foundation

Title: Neural dynamics of beat perception

Authors: *J. R. IVERSEN, A. D. PATEL;
The Neurosciences Inst., San Diego, CA

Abstract: Our perceptions are jointly shaped by external stimuli and internal interpretation. The perceptual experience of a simple rhythm, for example, strongly depends upon its metrical interpretation (where one hears the beat). Such interpretation can be altered at will, providing a model of the voluntary cognitive organization of perception. Where in the brain do the bottom-up and top-down influences in rhythm perception converge? Is it purely auditory, or does it involve other systems? To understand the neural mechanisms responsible for beat perception and the metrical interpretation, we measured brain responses as participants listened to a repeating rhythmic phrase, using magnetoencephalography. In separate trials, listeners (n=11) were instructed to mentally impose different metrical organizations on the rhythm by hearing the downbeat at one of three different phases in the rhythm. The imagined beat could coincide with a note, or with a silent position (yielding a syncopated rhythm). Since the stimulus was unchanged, observed differences in brain activity between the conditions should relate to active rhythm interpretation. Two effects related to endogenous processes were observed: First, sound-evoked responses were increased when a note coincided with the imagined beat. This effect was observed in the beta range (20-30 Hz), consistent with earlier studies. Second, and in contrast, induced beta responses were decoupled from the stimulus and instead tracked the time of the imagined beat. The results demonstrate temporally precise rhythmic modulation of beta responses that reflect the active interpretation of a rhythm. Given the suggested roles of beta in motor processing and in long-range intracortical coordination, it is hypothesized that the motor system is involved in the metrical interpretation of sound, even in the absence of overt movement. Preliminary localization analysis supports this view, finding examples of beat-related activity in motor areas.

Disclosures: J.R. Iversen, None; **A.D. Patel**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.9

Topic: D.02. Auditory

Support: AFOSR Grant #FA9550-10-C-0092

Title: 1/f temporal structure and prediction in rhythmic entrainment

Authors: *S. K. RANKIN, E. W. LARGE;
Ctr. Complex Systems &Brain Sci., Univ. Florida Atlantic, BOCA RATON, FL

Abstract: Tempo fluctuations in skilled piano performance exhibit 1/f-type long-range correlations and fractal scaling. In addition, listeners have been shown to predict tempo fluctuations when entraining to natural musical performances. We asked whether people exploit 1/f structure to predict tempo fluctuations in rhythmic sequences. In four related experiments, we found that both natural and synthetic long-range correlations enable temporal prediction by listeners. Temporal subdivision of the tapped period improves prediction. However, additional musical information, i.e. pitches and harmonies, does not improve temporal prediction. Thus, in this series of experiments, 1/f temporal structure was both necessary and sufficient to enable tempo prediction. Fractal temporal structure may enhance interaction between the organism and the environment, such that endogenous processes are better able to perceive structure and adapt to changes.

Disclosures: S.K. Rankin, None; **E.W. Large**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.10

Topic: D.02. Auditory

Support: aivoAALTO program of the Aalto University and the Academy of Finland (National Centers of Excellence Program 2006–2011)

Title: Inter-subject brain synchrony during listening to natural auditory scenes

Authors: *D. AKKAL[1], J. KATSYRI[2], S. MALINEN[1], O. KALLIOINEN[1], S. PAMILO[1], Y. HLUSHCHUK[1], P. TIKKA[3], S. CARLSON[1,4], R. HARI[1];
[1]Brain Res. Unit, Low Tempererature Laboratory, and AMI Ctr., Aalto Univ. Sch. of Sci. and Technol., AALTO, Espoo, Finland; [2]Ctr. for Knowledge and Innovation Res., Aalto Univ. Sch. of Econ., Helsinki, Finland; [3]Dept. of Motion Picture, Television and Production Design, Aalto Univ. Sch. of Art and Design, Helsinki, Finland; [4]Neurosci. Unit, Inst. of Biomedicine/Physiology, Univ. of Helsinki, Helsinki, Finland

Abstract: Background: Despite individual differences, people are able to form mutual understanding of their complex environment. Considerable intersubject synchronization occurs in the human brain during viewing of movies, with high synchrony in the auditory and visual cortices (Hasson et al., 2004). Here we used fMRI to determine to what extent the brain activity elicited by natural auditory scenes, without visual stimulation, would be correlated across individuals.
Methods: Nine healthy adults (18-45 yrs; 3 f, 6 m) were scanned with a 3-T magnet while they were listening to an 8-min track of natural auditory scenes including human voices, musical instruments, nature and objects-related sounds as well as movement-related sounds (composed by O.K.). We used the data- driven intersubject correlation analysis (ISC) to identify across-subject voxel- by- voxel synchronization. After scanning, participants re-listened to the sound track while continuously evaluating its emotional content on the valence and arousal dimensions.
Results: Our preliminary results show statistically significant ISC ($p < 0.001$ unc.; cluster size threshold 20) within a large network encompassing both hemispheres. Specifically, ISC was seen in the superior temporal gyrus, superior frontal gyrus, medial frontal gyrus, parahippocampal gyrus, fusiform gyrus and cerebellum. Emotional ratings were highly consistent ($p < 0.05$) at specific time points, indicating the emotional saliency of several auditory events.
Conclusions: The present findings demonstrate widespread synchrony during natural auditory stimulation including several regions involved in sensory, cognitive and/or emotional processing. These regions may be part of a distributed network supporting mutual understanding of the complex auditory environment. Further analysis will determine the exact nature of the sounds these regions preferentially process.

Disclosures: D. Akkal, None; **J. Katsyri**, None; **S. Malinen**, None; **O. Kallioinen**, None; **S. Pamilo**, None; **Y. Hlushchuk**, None; **P. Tikka**, None; **S. Carlson**, None; **R. Hari**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.11

Topic: D.02. Auditory

Support: Wellcome Trust, UK

Title: Distinct representation of absolute and relative auditory time

Authors: *S. TEKI[1,2], M. GRUBE[2], S. KUMAR[1,2], T. D. GRIFFITHS[1,2];
[1]Wellcome Trust Ctr. For Neuroimaging, London, United Kingdom; [2]Newcastle Auditory Group, Med. School, Newcastle Univ., Newcastle-upon-Tyne, United Kingdom

Abstract: Recent evidence from neuropsychological and neuroimaging studies strongly implicates the cerebellum and the basal ganglia in the perception of time. Here we test the hypothesis that the cerebellum and basal ganglia are implicated in the perception of absolute time intervals and the perception of time intervals relative to a beat, respectively (Grube et al., 2007; Grahn and Brett, 2007). Absolute measurement of time intervals can occur irrespective of the context in which they occur whilst the measurement of time intervals relative to a beat requires such context. We therefore assessed absolute and relative measurement of time intervals by assessing the fMRI BOLD response when subjects assess the difference in duration of two successive time intervals between clicks in the context of 1) a preceding irregular sequence of clicks (where the comparison can only be achieved by absolute mechanisms) and 2) a preceding regular sequence of clicks (when the metrical beat provides an extra cue for relative timing). The contrast between the BOLD responses in condition 1 - 2 provides a measure of absolute timing whilst the contrast between 2 - 1 provides a measure of relative timing.
The contrast 1 - 2 revealed a bilateral olivocerebellar network comprising the inferior olive, vermis, deep cerebellar nuclei including the dentate nuclei. The contrast 2-1 revealed a bilateral striato-thalamo-cortical network consisting of the putamen and caudate nucleus, thalamus, supplementary motor area, pre-motor cortex and the dorsolateral prefrontal cortex.
These data support two timing mechanisms in the brain: 1) an olivocerebellar network that acts as a precision clock to mediate absolute, interval-based timing and 2) a mechanism for relative, beat-based timing including the striatum and neocortex.

References:
1. Grube, M., Cooper, F.E., Chinnery, P.F., Griffiths T.D. (2007). It's about sub-second time: impaired basic vs. preserved higher-order timing functions in cerebellar degeneration. 37th Annual Meeting of the Society for Neuroscience abstract no. 303.5.
2. Grahn, J.A., and Brett, M. (2007) Rhythm and beat perception in motor areas of the brain. Journal of Cognitive Neuroscience 19(5): 893-906.

Disclosures: S. Teki, None; **M. Grube**, None; **S. Kumar**, None; **T.D. Griffiths**, None.

Nanosymposium

324. Sound, Time, Movement, and Rhythm

Location: Room 33C

Time: Monday, November 15, 2010, 8:00 am - 11:00 am

Program Number: 324.12

Topic: D.02. Auditory

Support: Max Planck Society

Title: Population coding in auditory cortex - time scales and intrinsic reference frames

Authors: *C. KAYSER[1], N. K. LOGOTHETIS[1], S. PANZERI[2];
[1]Max Planck Inst. Biol Cybernetics, Tuebingen, Germany; [2]Italian Inst. of Technol., Genua, Italy

Abstract: Natural sounds such as communication signals and music are composed of complex temporal features that cover a wide range of time scales. Our auditory system excels at resolving this temporal structure and temporal features are crucial for identifying sound qualities and understanding speech. Yet, how auditory cortex neurons encode rapid sound features remains a matter of investigation.
To shed light on this issue we recorded the activity of neural populations in caudal auditory cortex of alert macaque monkeys during stimulation with a rapid sequence of synthetic random chords and with naturalistic stimuli. We quantified stimulus discriminability by means of both decoding and information theoretic measures. We used these data to investigate two important questions related to temporal coding in auditory cortex: 1) What is the time scale at which spike patterns of auditory cortex neurons carry sensory information? And 2) is it possible to 'read' such temporal activity patterns without having exact knowledge about external stimulus timing?
With regard to the first question we find that auditory cortex responses are very precise and can encode stimulus information in spike patterns at the millisecond scale. Importantly, we find that 'reading' responses at precisions coarser than 4ms causes a significant loss in the information, which reaches already 10% at an effective precisions of 6ms. This information loss induced by ignoring millisecond precise spike patterns was more prominent during stimulation with random chords, but for a subset of neurons also prevailed during stimulation with natural sounds.
With regard to the second question we find that one can detect the timing of stimulus onset directly from the population of neural responses with a precision of about 8ms. This population derived timing defines an internal reference frame that can be used for temporal response decoding without making reference to an external clock. Quantifying the information lost by using internal rather than external reference frames suggests that the auditory system can well achieve fine temporal stimulus encoding even without precise knowledge about external stimulus timing.

Disclosures: C. Kayser, None; **N.K. Logothetis**, None; **S. Panzeri**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.1

Topic: D.04. Vision

Support: The Gatsby Charitable Foundation

Title: Human monochromatic light discrimination explained by optimal decoding of cone absorptions

Authors: *L. ZHAOPING;
Univ. Col. London, London, United Kingdom

Abstract: The threshold in light wavelength for humans to discriminate two monochromatic inputs, which could differ in both input wavelength and intensity, depends on the wavelength in a particular way. It dips near wavelengths 490 and 590 nanometers (nm) but rises steeply beyond 630 nm (Pokorny and Smith, 1970). Previous works aimed to account for these data by specific neural mechanisms in the retina and cortex. In contrast, this work uses an ideal observer analysis to see if the data can be accounted for as the best color discrimination performance possible from the information available in the cone absorptions. We apply a maximum likelihood decoding of the input wavelength and intensity from the cone absorptions. The wavelength tuning curves of the three cone types, red, green, and blue cones (or long, medium, and short wavelength selective cones), reflect their average absorptions for any monochromatic input. However, due to Poisson noise in the cones, the actual absorptions will deviate stochastically from the respective averages. The brain could decode the best estimates of the input wavelength and intensity responsible for the cone absorptions, and obtain the noise induced uncertainties about these estimates. Computationally (Dayan and Abbott, 2001), these best estimates and their uncertainties correspond to the peak location and the spread, in the input wavelength and intensity, of the conditional probability of the absorptions for the sensory input. Experimentally, peak and spread in this conditional probability should correspond to the perceived monochromatic input and the input discrimination threshold, if the neural processing stages after the cones utilize all the information available in the cone absorptions. This computational decoding scheme is applied to a wavelength discrimination procedure, used by various experiments including the one by Pokorny and Smith, in which observers adjust the input wavelength and intensity of a comparison input field to match a standard monochromatic input field. There is a good agreement between the computationally predicted and experimentally observed wavelength discrimination thresholds as a function of the wavelength. These findings suggest that the post-receptor neural processes for color decoding are optimal. The computational analysis also predicts that the wavelength discrimination threshold should decrease by a quantifiable,

wavelength dependent, amount if the experimental procedure is modified such that observers adjust only the input wavelength but not the input intensity of the comparison input field in the color matching. This prediction can be quantitatively tested experimentally.

Disclosures: L. Zhaoping: None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.2

Topic: D.04. Vision

Support: NIH Grant RO1EY16842

Pew Charitable Trusts

McKnight Endowment Fund for Neuroscience

Sloan Foundation

Title: The adaptive field and predictive object representation in the retina

Authors: *D. B. KASTNER, S. A. BACCUS;
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Abstract: Neurons dynamically change their sensitivity, allowing for a more efficient encoding of their input. Recently, we found that different retinal ganglion cells change their sensitivity in opposite ways following a high contrast stimulus. Some cells adapt by decreasing their sensitivity, while others sensitize, increasing their sensitivity for several seconds after a transition from high to low contrast.
In a natural scene, contrast varies locally depending on the statistics of the image and the motion of objects. We therefore measured the spatial extent over which contrast changed the sensitivity of a cell; a property termed the adaptive field. We measured the adaptive field of a cell by recording from salamander retinal ganglion cells using a multielectrode array. We mapped the sensitivity of each cell using a white noise checkerboard flicker stimulus, and then computed how a small high contrast stimulus, presented at various locations relative to the cell, changed the cell's sensitivity. We found three different adaptive fields in the population of ganglion cells. Two of the adaptive fields showed a simple spatial decay, monotonically decreasing with

distance from the high contrast. One of these cell types always adapted to high contrast, and the other sensitized. The third adaptive field, the most commonly observed, had center-surround behavior. These cells adapted if high contrast was centered over the cell, but sensitized if the local high contrast was adjacent to the cell.
In a natural scene, local image contrast is often generated by the motion of objects. Therefore, instead of high and low contrast, we presented a more naturalistic stimulus composed of a textured object moving in a random walk, alternating between fast and slow speeds. The fast trajectory represented a moving object viewed by the retina, and the slow trajectory represented a fixed object in the presence of fixational eye movements. At the transition from a moving to a fixed object, even though the object identity does not change, the strength of the stimulus on the retina varies greatly.
Cells with center-surround adaptive fields were sensitized by fast motion, displaying elevated sensitivity at the transition from fast to slow motion. Thus, the presence of a moving object was stored as an elevation of sensitivity and firing rate for several seconds after the object stopped. Center-surround antagonism within the adaptive field thus forms a prediction that the object will be near its previous location—a reasonable prediction given that objects in the real world do not disappear.

Disclosures: D.B. Kastner, None; **S.A. Baccus**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.3

Topic: D.04. Vision

Support: NIH Grant EY018718

NIH Grant EY019049

Title: Differential receptive properties of parvalbumin- and somatostatin-containing cortical inhibitory neurons

Authors: W.-P. MA[1], B.-H. LIU[1], L. I. ZHANG[1], Z. J. HUANG[3], *H. TAO[2];
[1]Zilkha Neurogenetic Inst., USC Keck Sch. Med., Los Angeles, CA; [2]USC Keck Sch. Med., LOS ANGELES, CA; [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Cortical inhibitory neurons, the GABAergic non-pyramidal cells, consist of diverse

functional classes that may also be morphologically and/or neurochemically distinct. The precise roles of different subtypes of inhibitory neurons in cortical function have not been well understood, mainly because of the difficulty in studying specific inhibitory neuron subtypes *in vivo*. Here, by two-photon imaging guided cell-attached recordings, we specifically investigated the receptive field (RF) properties of genetically labelled parvalbumin (PV) and somatostatin (SOM) containing inhibitory neurons in layer 2-4 of the mouse visual cortex. We found that SOM neurons exhibit broader spikes, lower levels of spontaneous and evoked firing activity and smaller subfield sizes than PV neurons. Similar as PV neurons, SOM cells mainly exhibit spatially overlapped On and Off subfields when both are detected, although in layer 4 the level of the overlap is lower than PV neurons. Interestingly, while PV neurons usually do not display orientation selectivity, SOM cells do with the level of selectivity comparable to excitatory neurons. SOM cells also display stronger direction selectivity than PV neurons. Furthermore, the onset of evoked spike responses in SOM cells is significantly delayed than PV neurons in the same layer. The differential functional properties of SOM and PV neurons suggest that they may engage in cortical circuits in different manners: PV neurons provide fast inhibition which serves as a general inhibitory gain control, while SOM neurons provide delayed inhibition that modulates excitatory inputs through iso-orientation or cross-orientation inhibition.

Disclosures: W. Ma, None; **B. Liu**, None; **L.I. Zhang**, None; **Z.J. Huang**, None; **H. Tao**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.4

Topic: D.04. Vision

Support: NIH Grant EY01472

NSF Grant 0745253

Robert Leet and Clara Guthrie Patterson Trust Postdoctoral Fellowship

Swartz Foundation

Title: Differences in the 2-D structure of V1 receptive fields mapped with different stimulus ensembles

Authors: *C.-I. YEH, D. XING, R. M. SHAPLEY;
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Abstract: One notion about the V1 simple cell is that its receptive field is a fixed property that can be used to predict all responses of the neuron (i.e. preferred orientation, spatial frequency), a notion that has been challenged by recent findings that receptive fields are stimulus dependent (David et al 2004; Victor et al 2006; Yeh et al 2009). Here we quantified the stimulus dependence by fitting receptive fields with a two-dimensional Gabor function (2DGF, Jones & Palmer 1987). Fits were computed for receptive fields measured with two commonly used stimulus ensembles, sparse noise (SN, Jones & Palmer 1987) and Hartley subspace stimuli (HS, Ringach et al 1997). We calculated spatio-temporal receptive fields by reverse correlation and chose to analyze the map at the time when spatial variance of the map was maximal. Among a total of 334 neurons recorded extracellularly in macaque monkey V1, 158 had mappable receptive fields with both ensembles (signal-to-noise ratio>1.8) and among them 131 were well-fitted with a 2DGF. For the 42/131 simple cells (f1/f0$\geq$1, measured with drifting gratings), the fitted HS and SN maps differed significantly: 1) the aspect ratio of HS maps was larger than that of SN maps (HS: 2.84$\pm$1.49, SN: 1.65$\pm$0.58, $p<0.001$, Wilcoxon signed rank test), 2) the number of subregions was larger for HS than for SN (HS: 2.65$\pm$1.11, SN: 1.33$\pm$0.47, $p<0.001$), 3) the receptive-field size of HS was larger than that of SN (HS: 0.83$\pm$0.28 deg, SN: 0.36$\pm$0.10 deg, $p<0.001$), and 4) black-dominance (stronger OFF-subregion) was more evident for SN than for HS (HS phase: 0.59$\pm$0.30 π, SN phase: 0.71$\pm$0.29 π, $p=0.01$). Furthermore, consistent with previous findings, the discrepancy between HS and SN maps, defined as [|SN-HS|/(SN+HS)], was larger in layer-2/3 than in layer-4: 1) for aspect ratio (L-2/3: 0.31; L-4: 0.17, $p=0.04$, Wilcoxon rank sum test), 2) for number of subregions (L-2/3: 0.38; L-4: 0.26, $p=0.05$), and 3) for receptive-field size (L-2/3: 0.39; L-4: 0.24, $p=0.02$). Overall, receptive-field properties of V1 neurons vary significantly when measured with different stimulus ensembles. Also, the stimulus-dependent discrepancy was significantly larger in the superficial layers, indicating the greater nonlinearity of layer-2/3 simple cells. These results strongly challenge the concept that V1 simple cells have fixed receptive fields.

Disclosures: C. Yeh, None; **D. Xing**, None; **R.M. Shapley**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.5

Topic: D.04. Vision

Support: Feodor Lynen research fellowship of the Alexander von Humboldt-Foundation

Title: Development and specificity of the non-classical receptive field during naturalistic stimulation in mouse primary visual cortex

Authors: *M. PECKA, T. D. MRSIC-FLOGEL;
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Abstract: Neurons in the primary visual cortex (V1) respond with action potentials only to stimuli from a well-localized region of space, defined as the classical receptive field (CRF). However, stimulation of regions outside the CRF can suppress and/or enhance the responses evoked by CRF stimulation. These modulating regions surrounding the CRF are termed the non-classical receptive field (nCRF). Recent studies in V1 have shown that combined stimulation of the CRF and nCRF with naturalistic stimuli increases the sparseness of neural responses compared to CRF stimulation alone. Hence, stimulation of the nCRF using large-field stimuli may be important for highly selective representations of naturalistic stimuli, consistent with the ideas of efficient coding during natural vision.
At present, however, it remains unclear whether the modulating effects of the nCRF are 1) specific to higher-order statistics inherent to naturalistic stimuli; and 2) refined by visual experience after eye opening. To address these questions, we carried out extracellular single-cell recordings from monocular V1 in anaesthetized mice at different postnatal ages to investigate the specificity of nCRF modulation to naturalistic stimuli. We assessed neural stimulus selectivity by analyzing lifetime sparseness, i.e. to what fraction of stimulus frames the neuron responded to during stimulation.
We compared neural responses to naturalistic stimuli confined to the CRF to the responses elicited by large field naturalistic stimulation in adult mice. We observed an overall reduction in firing rate and an enhancement of selectivity by concurrent nCRF stimulation which was qualitatively similar to reports from monkey and cat V1. Furthermore, across our sample of neurons, these effects diminished when the nCRF was stimulated with modified stimuli whose higher order statistics have been altered (white/pink noise). Hence, the enhancement of selectivity by the nCRF was specific to naturalistic stimuli.
To gain insight into the development of this observed specificity, we obtained recordings in V1 of infant animals with limited experience to natural vision (1 to 5 days after eye-opening). We found that, on average, nCRF stimulation still enhanced response selectivity in juvenile mice; however the enhancement was not different for naturalistic and artificial stimuli; hence - in contrast to the adult mice - no specificity to naturalistic stimuli was present in infants.
Together, these findings suggest that the nCRF might be optimized for processing of naturalistic stimuli and that the neural circuits mediating the modulatory influence of the nCRF might be refined by experience after eye-opening.

Disclosures: M. Pecka, None; **T.D. Mrsic-Flogel**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.6

Topic: D.04. Vision

Support: NIH Grant EY016774

Title: Linear summation of feedforward corticocortical inputs

Authors: *A. ZANDVAKILI, A. KOHN;
Dept. of Neurosci., Albert Einstein Col. of Med., BRONX, NY

Abstract: Processing of visual information is accomplished by a feedforward hierarchy of cortical areas, with extensive lateral and feedback projections. While the anatomy of this network has been explored in detail, we know little about the role of patterns of neural activity in relaying signals through this circuitry. It has been suggested that the synchronous discharge of neurons would be especially effective in driving downstream neurons, but this has never been tested directly. To study the sensitivity of downstream neurons to temporal patterns of feedforward corticocortical inputs, we recorded simultaneously from populations of superficial layer neurons in V1 (using a microarray of 100 electrodes) and middle layer cells in V2 (using independently movable tetrodes) in anesthetized macaque monkeys. Area V2 is dependent on feedforward input from V1: visually evoked responses in V2 are abolished by cooling or lesioning V1. This makes the V1-V2 feedforward pathway a useful model system for studying feedforward circuitry.

We calculated crosscorrelograms (CCGs) between all simultaneously recorded pairs of V1-V2 neurons and studied cases where the CCG had a clear peak indicating monosynaptic V1-V2 connection (mean offset of ~2.5 ms). We found that these connections were selective (only cells with well aligned receptive fields were connected) and weak (connection strength was about ten fold lower than the strength reported for thalamocortical connections). To investigate the effect of synchrony, we used cases where two V1 neurons provided input to the same V2 cell. We found that the efficacy of synchronous V1 spikes in driving a target V2 cell was predicted by the sum of the contributions of spikes from the two V1 cells. That is, V2 neurons summed their V1 inputs linearly. We compared the efficacy of near-synchronous events (spikes occurring in two projection neurons with temporal offsets from 1 to 10 ms) and found similar linear summation. Finally, we investigated how the efficacy of input from a single neuron depended on the patterning of its spikes. We found that efficacy was enhanced modestly by brief periods of quiescence (no spikes occurring in the preceding 10 ms), perhaps reflecting an alleviation of weak synaptic depression.

In summary, our data reveal important differences between feedforward corticocortical connections and previously described properties of thamalocortical connections to V1. Specifically, unlike those connections, feedforward inputs from V1 show nearly perfect linear

summation in V2. We conclude that neurons do not act as coincidence detectors of the feedforward input they receive from lower cortical areas.

Disclosures: A. Zandvakili, None; **A. Kohn**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.7

Topic: D.04. Vision

Support: BBSRC Grant BB/G005044/1

Title: The jigsaw puzzle in V1: Influences of surrounding context on non-stimulated early visual regions is independent of memory and basic visual features

Authors: *F. W. SMITH[1,2], L. MUCKLI[2];
[1]Psychology, Univ. of Western Ontario, London, ON, Canada; [2]Psychology & Ctr. for Cognitive Neuroimaging, Univ. of Glasgow, Glasgow, United Kingdom

Abstract: A common theme in several recent theories of cortical function suggests that cortical feedback carries predictive signals relating to forthcoming stimulation (Friston, 2010; Hawkins 2004). We have recently reported less fMRI BOLD activity in primary visual cortex (V1) to spatio-temporally predicted stimuli on the Apparent Motion trace (Alink et al., 2010), implying that cortical feedback to non-stimulated V1 is spatio-temporally precise. In the present work we consider the role of cortical feedback (and lateral interactions) in transmitting contextual information when observers are presented with natural visual scenes.

Under standard conditions of visual stimulation it is difficult to separate out the contributions of feed-forward processing (retina-LGN-V1) from lateral interactions and cortical feedback. In a recent experiment we devised a novel paradigm that was explicitly designed for this purpose: we analyzed the influence of surrounding context on the fMRI BOLD signal in visually non-stimulated parts of V1 and V2 (Smith & Muckli, HBM Abstract 2009). We presented natural visual scenes (lower right) with one quadrant completely occluded by a uniform white field and we mapped the cortical representation of this non-stimulated region in V1/V2. We demonstrated, using multivariate pattern classification techniques, that we could decode the natural scene an observer was viewing in the surround, solely from the signal in non-stimulated V1/V2.
In the present work we have replicated and extended our initial findings with new subjects to

show that previous exposure to the complete visual scenes (where V1/V2 are fully stimulated) is not required to observe this context effect. Moreover, in a further experiment we controlled basic stimulus features (the amplitude spectra) of our visual scenes to ensure that our effects cannot be explained by these basic stimulus features.
Our new results confirm that non-stimulated early visual areas are informed about the surrounding natural visual context, independent of specific memories of the complete scenes, and of low-level stimulus properties. Bayesian models of human vision (e.g. Lee & Mumford, 2003) would suggest that the surrounding visual context biases cortical feedback to the non-stimulated early visual areas.
Alink. A. et al., (2010) J Neurosci. 30(8):2960-2966;
Friston, K et al., (2010). Biol. Cybern. 102(3):227:260.
Hawkins J, Blakeless S (2004) On Intelligence (Times Books).
Lee, T.S., & Mumford, D. (2003). Opt Soc Am A. 20:1434-1448.
Smith, F.W., & Muckli, L. (2009). HBM.

Disclosures: F.W. Smith, None; **L. Muckli**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.8

Topic: D.04. Vision

Support: NIH Grant NEI EY007968

Title: Task-dependent shape selectivity in primary visual cortex

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Abstract: The ability to interpret complex and ambiguous sensory input requires the integration of information over both space and time, as well as cognitive mechanisms to dynamically shape that integration. We have studied these processes in the primary visual cortex (V1), where neurons have been proposed to integrate visual inputs along a geometric pattern known as the association field (AF). The AF describes the rules by which our visual system binds local image segments into a perceptually salient whole, and it may contribute to a host of perceptual phenomena, from contour integration to figure-ground segregation and object recognition. We

recorded extracellularly from single units in macaques to map the shape of the AF in V1, by using a stimulus optimization algorithm to find the global contours that maximally activated individual V1 neurons. We combined the optimization routine with a delayed-match-to-sample behavioral task, in order to examine how the optimal contours for neurons might be molded by the monkeys' expectations for particular cue shapes. We found that neurons in V1 show selectivity for complex shapes, a property previously associated with higher stages in the hierarchy of visual cortical areas. Furthermore, the shape selectivity we observed was sculpted by perceptual task: V1 neurons preferred very different contours, ranging from circles to lines to sinusoidal waves, according to the shapes monkeys were cued to detect. Over the whole network, the optimal modes of geometric selectivity shifted between distinct subsets of the AF, alternately representing different stimulus features known to predominate in natural scenes, as a function of the monkeys' expectations. Moreover, the temporal dynamics of the neural responses were consistent with a theoretical model of intrinsic network interactions in V1. Geometric selectivity first emerged at the peak of the neuronal onset response and gradually matured, together with its task-dependency, over the next forty milliseconds. Our results reveal a sophisticated mode of form processing, whereby the higher-order selectivity of the whole network in V1 is reshaped by cognitive state. Mechanistically, our data support a model in which geometric selectivity emerges from subsets of horizontal connections in V1, which are selectively gated by feedback projections to tailor the network activity to the observer’s expectations.

Disclosures: J.N. McManus, None; **W. Li**, None; **C.D. Gilbert**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.9

Topic: D.04. Vision

Title: Tomographic characterization of population receptive fields in early visual cortex

Authors: C. A. GREENE[1], S. O. DUMOULIN[2], B. M. HARVEY[2], *D. RESS[1];
[1]Neurobiol & Psychol, Univ. Texas Austin, AUSTIN, TX; [2]Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands

Abstract: Purpose: Recently, fMRI measurements were used to estimate properties of visual neuronal populations (Dumoulin & Wandell, Neuroimage **39**:647, 2008). This population receptive field (pRF) approach fits a predefined model shape to the fMRI measurements in order to estimate visual field maps and other neuronal population properties. Here we investigated

whether pRFs could be directly imaged using a tomographic approach that requires no *a priori* shape assumption. **Methods**: We obtained fMRI images across early visual cortex using a GE 3T scanner (2-mm cubic voxels, 2-shot spiral). Stimulation was a thin bar (~1° wide) containing a moving checkerboard pattern that swept slowly (<1°/s) across a 10—20° field-of-view. Bar motion was perpendicular to its long axis. The motion direction and bar orientation of each sweep successively rotated from 0—165° in 15° increments. Between each sweep, blank periods (mean-luminance) were inserted to allow the fMRI response to subside. Assuming a linear response, the fMRI time series corresponded to a projection of the pRF along the long axis of the bar; the multiple sweeps thus formed a sinogram (Fig. 1). This sinogram was blurred by the hemodynamic response function (HRF); blurring was mitigated using a Wiener filter incorporating HRF waveforms measured in each scanning session. The filtered sinograms were then used to reconstruct the pRF using a backprojection algorithm (Fig. 2). Contours were created around the half-maxima and ellipses were fit to these contours to estimate field coordinates and other parameters. **Results**: Reconstruction and analysis by this method requires <20 minutes. Our polar angle and eccentricity maps agree well with the previous model-based approach and with conventional retinotopic mapping. Notably, individual reconstructed pRFs are complex with multiple peaks, non-circular shapes, and significant suppressive surround regions (Fig. 2). **Conclusion**: Tomographic reconstruction of pRFs is a useful approach for estimating visual receptive field properties without *a priori* shape assumptions throughout early visual cortex.$$graphic_{DB2AE77C-E355-47E2-98CE-DAB8FEF5DFB6}$$

Disclosures: C.A. Greene, None; **S.O. Dumoulin**, None; **D. Ress**, None; **B.M. Harvey**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.10

Topic: D.04. Vision

Support: IRG grant 231027

Title: Modeling center-surround configurations in population receptive fields using fMRI

Authors: *W. ZUIDERBAAN[1], D. RESS[2], B. M. HARVEY[1], C. A. GREEN[2], S. O. DUMOULIN[1];
[1]Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands; [2]Univ. of Texas at Austin, Austin, TX

Abstract: Introduction. Antagonistic center-surround configurations are a central organizational

principle of our visual system. Electrophysiological recordings show that stimulation outside a receptive field can lead to decreased neuronal activity that causes negative fMRI responses. Using fMRI we can estimate the region of visual space to which each cortical location responds, i.e. the population receptive field (pRF). Current models of the pRF do not account for center-surround organization or negative fMRI responses. Here we extend the pRF approach in two ways: 1) by adding surround suppression, which gives the model a way to account for negative fMRI responses; 2) by using a tomographic approach that directly reconstructs the spatial distributions of excitatory and suppressive regions.
Methods. We measured fMRI responses to moving bar apertures that reveal a moving checkerboard that sweeps slowly across the stimulus aperture, alternated by periods of mean luminance. In the model-based approach (Dumoulin & Wandell, Neuroimage, 2008), we compare two models of the pRF. The conventional model consisting of a single Gaussian and a new model using differences of two Gaussians (DoG) to incorporate a suppressive surround. In the tomographic approach, the motion direction and bar orientation of each sweep successively rotated from 0-165° in 15° increments Estimates of the pRF were then obtained by backprojection reconstruction. To ensure that differences in the predicted time-series between various models are not caused by a difference in estimation of the baseline activation, the time-series of the mean luminance blocks are used to estimate the parameters of the baseline activity.
Results. Comparing the fits of the models, we found improved variance explained for the DoG model. This improvement was predominantly present in V1/2 and decreased in later visual areas. In cortical locations where the surround contributed significantly, the volumes of the two Gaussians were balanced. This means stimulating the entire pRF yields zero activation. The tomographic pRFs confirm the presence of significant suppression, and show that the suppressive regions are not azimuthally symmetric around the excitatory peak.
Discussion. Our results follow electrophysiological and fMRI findings, which report that increasing stimulus size ultimately will decrease responses. This is explained by our data because stimulating the entire center-surround receptive field does not change the fMRI response. Our results extend the notion of center-surround configuration to the scale of fMRI measurements.

Disclosures: W. Zuiderbaan, None; **D. Ress**, None; **B.M. Harvey**, None; **S.O. Dumoulin**, None; **C.A. Green**, None.

Nanosymposium

325. Vision: Response Properties

Location: Room 1B

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 325.11

Topic: D.04. Vision

Support: NWO (Netherlands) Vidi Grant 452-08-008

Title: Motion integration receptive field effects in human fMRI

Authors: *B. M. HARVEY[1], S. DUMOULIN[2];
[2]Helmholtz Institute, Exptl. Psychology, [1]Utrecht Univ., Utrecht, Netherlands

Abstract: Introduction: Nearby neurons in V1 interact beyond their classical receptive fields, modifying the responses in the receptive field via horizontal and feedback connections (Angelucci & Bullier, J. Physiol., 2003). For behaviorally useful motion integration, the extent of these extra-classical receptive field interactions may be greater for faster motions. Differences in neuronal interactions effectively increase the area of the visual field to which motion sensitive neurons respond when faster motions are presented. Using fMRI we can estimate the region of visual space to which each cortical location responds, i.e. the population receptive field (pRF). The pRF may include neuronal interactions and therefore may reveal the extent of motion integration in the visual field as a change in the estimated pRF size with different motion speeds and directions.
Methods: fMRI images were acquired on a Philips 7T MRI. We recorded fMRI responses to bar apertures moving in 8 different directions. The bars revealed random noise filtered to make black and white patterns with a fundamental spatial frequency range of 0.5-1.0 degrees of visual angle. These patterns moved at speeds between 1 and 8 degrees per second, and in motion directions parallel or perpendicular to the bar orientation. Similar stationary flickering patterns and random dot kinematograms were also examined. In all conditions, the same aperture sizes and positions were used.
Convolution of a pRF model with the stimulus aperture position sequence predicts the fMRI time series. pRF parameters of x and y (visual field position) and σ (size) were estimated for each voxel by minimizing the sum of squared errors between the predicted and observed fMRI time-series (Dumoulin & Wandell, Neuroimage, 2008).
We compared pRF size estimates under different conditions of motion direction relative to the direction of measurement (the direction of bar motion). We also compared different motion speeds. To ensure motion speed effects were specifically related to motion speed, different rates of flicker in static stimuli were also compared.
As eye movements can affect pRF size estimates, we designed stimuli to minimize eye movements. We also measured eye movements during scanning and compared eye movements between different conditions.
Results: Larger pRF size estimates were obtained for faster as compared to slower motion speeds. These changes occurred primarily in V1 but not in extra-striate cortex. The position of pRF centers in the visual field was unchanged.
Conclusion: These pRF sizes increase with increasing motion speed in V1. We propose that these increases reflect the increasing extent of motion-integration mechanisms.

Disclosures: B.M. Harvey, None; **S. Dumoulin**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.1

Topic: D.04. Vision

Support: NSF BCS 04-20794

NSF BCS 05-31177

NSF BCS 06-17699

Title: Voxels in LO can distinguish objects with different arrangements of the same component parts

Authors: *M. D. LESCROART[1], I. BIEDERMAN[2];
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Abstract: There is ample evidence that images of familiar object categories such as faces, body parts, and tools elicit distinguishable patterns of activity in the ventral visual pathway (e.g. Haxby et al., 2001; Kriegeskorte et al., 2008). Which visual features determine the similarity between images in the same categories? This question is unanswerable when experimental stimuli vary in many dimensions, e.g. color, texture, orientation, and curvature, as well as familiarity and behavioral relevance. An influential theory of object recognition (Biederman, 1987) holds that objects can be represented by volumetric parts conjoined in specified relationships. Could differences in the arrangement of objects' parts elicit different patterns of activity in visual cortex in the same way that broadly-defined categorical differences do? In a series of fMRI multi-voxel classification studies, we tested whether any visual areas could distinguish objects that shared the same component parts and only differed in the arrangements of those parts. Stimuli were line drawings of three-part geometrical objects that varied in: a) the arrangement of the three parts and b) the overall orientation of the composite objects. Unlike several prior studies which used diverse sets of colored photos that differed in many attributes, our images were highly similar with no surface variations or familiar interpretations, and thus represent a theoretically clear test of shape selectivity per se. Subjects performed one of two tasks while viewing single presentations of the images by pressing one of three buttons to differentiate either one of three possible arrangements of parts, or one of three different orientations of the objects. In each of nine ROIs throughout visual cortex, a support vector machine classifier was trained to differentiate either groups of objects sharing the same part arrangement, or groups of objects sharing the same global orientation (both classification strategies were possible within the same data set by re-labeling trials for the images). When subjects attended to the arrangement of the parts, the classifier based on voxels in V1 performed

more accurately at separating groups of images of similar global orientation, and more poorly at separating groups of images based on the arrangement of their parts. In LO, this effect was reversed: much greater accuracy was achieved separating different arrangements of parts than different global orientations. LO is therefore more sensitive to the relative positions of an object's parts than to the global orientation of the object.

Disclosures: M.D. Lescroart, None; **I. Biederman**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.2

Topic: D.04. Vision

Support: NSERC post-doctoral fellowship to J.S.C.

NSF grant 0855112 to Y.X.

Title: A link between the processing of object ensembles and texture in the parahippocampal place area

Authors: J. S. CANT, *Y. XU;
Psychology, Harvard Univ., CAMBRIDGE, MA

Abstract: Numerous neuroimaging studies have demonstrated that the lateral occipital complex (LOC) plays a role in perceiving single objects by processing visual cues to outline shape. But objects are not always seen in isolation and are often part of a larger collection, or ensemble, of multiple similar objects (e.g. leaves on a tree). In this sense, object ensembles are similar to visual textures, in that both types of images are homogeneous and contain repeating features that may vary slightly in their size, orientation or color (Portilla & Simoncelli, 2000). Given the similarity between ensembles and textures, and given the finding that a region of the collateral sulcus in the parahippocampal place area (PPA) is sensitive to processing texture (Cant & Goodale, 2007), we conducted a study to examine whether or not the PPA would also be sensitive to processing the ensemble statistics of objects. Moreover, given the role of the LOC in shape perception, we investigated whether or not the LOC would extract shape information out of object ensembles. To address these questions, we used fMR-adaptation and in different trials showed participants a sequence of either three ensemble images or three texture images that were either all identical, all different, or shared object ensemble statistics or texture features. Using an

independent localizer approach, we found that the LOC showed an equivalent release from adaptation (i.e. a rise in activation compared to the 'identical' condition) in trials that depicted a change in the local shape information of object ensembles (i.e. in both the 'shared' and 'different' conditions, which did not differ), but showed no adaptation effects for textures, likely because these images contained no closed contours. In contrast, the PPA showed equivalent levels of repetition attenuation (i.e. a reduction in activation compared to the 'different' condition) in trials where either ensemble or texture features were repeated (i.e. in the 'identical' and 'shared' conditions, which did not differ). These results suggest that in addition to processing the shape of single objects, the LOC also extracts shape information out of more cluttered and ecologically valid visual images. Moreover, in addition to processing visual texture, the PPA is involved in representing ensemble statistics from large collections of objects. Notably, although our stimuli contained minimal amount of 3D scene information, the PPA exhibited adaptation when ensemble and texture features were repeated. This suggests that the PPA may contribute to scene representation by extracting ensemble statistics and texture features in addition to the 3D layout of a scene.

Disclosures: J.S. Cant, None; **Y. Xu**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.3

Topic: D.04. Vision

Support: Taiwan Ministry of Education Five Year Aim for the Top University Plan

NSC-97-2811-B-010-501

NSC-98-2321-B-010-003

98-2923-B-010-001-MY3

Title: Object feature integration within 1 mm^3 of macaque inferior temporal cortex

Authors: *C. P. HUNG, C.-P. LIN, Y.-P. CHEN;
Natl. Yang Ming Univ., Taipei, Taiwan

Abstract: Visual object recognition involves representations and computations across multiple

scales of organization, yet the computational mechanisms acting across these scales is not sufficiently understood to accurately model and predict neuronal responses, due in part to the high dimensionality of the potential feature space and inadequate tools for observation. This problem is especially acute at lower spatial resolutions (e.g. optical imaging and fMRI), where it is difficult to attribute the signals to their neuronal and functional origins. We recorded from spike ensembles of ~64 neurons within 1 mm^3 of macaque inferior temporal (IT) cortex in response to image sets of photos and rendered objects. Linear dimensionality reduction of ensemble responses via principal components analysis reveals multiple spatial components that are consistent across stimulus sets. The first two components explain up to 74% of the variance for single neurons in output layers 2/3 and 5 and consist of a mixture of coalition activity at columnar and mm scales. Integration of opposing features, as defined by joint activation of neighboring opposing coalitions, results in a paradoxical average reduction of activity, whereas the combined absence of features or combination of antifeatures boosts average firing rates. Overall, these results suggest that a substantial portion of the variability of average ensemble responses within 1 mm^3 of IT is associated with local suppression arising at the coalition scale during feature integration. Optical imaging and fMRI of such coalition-defined key feature dimensions are consistent with a common alphabet of such dimensions across animals and across species, and they suggest that such opposing coalitions tend to be neighbors in the cortical map. We suggest that models of IT response variability would benefit from high-resolution maps that capture this coalition-scale representation and its associated mechanisms.

Disclosures: C.P. Hung, None; **C. Lin**, None; **Y. Chen**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.4

Topic: D.04. Vision

Support: NSF CAREER Award (IIS 0546262)

Athinoula A. Martinos Imaging Center at McGovern Institute for Brain Research, MIT

Title: Neural representation of the size of space and the amount of clutter in a scene

Authors: *S. PARK, T. KONKLE, A. OLIVA;
MIT, CAMBRIDGE, MA

Abstract: Estimating the size of a space and the level of clutter within a space is central to our interactions in scenes, for example when deciding whether or not to take a crowded elevator or how to organize furniture and objects in a room. Interestingly, the size of a space is independent from level of clutter: spatial volume is a property defined by the shape of the spatial boundary of a scene while clutter is a property defined by the contents within the spatial boundary. Here, we examined how neural areas respond to scenes that parametrically vary in both volume and the amount of clutter of depicted space. Observers were shown blocks of indoor scene categories and performed a one-back repetition task while undergoing whole brain imaging in a 3T fMRI scanner. 36 scene categories were selected to fully cross the dimensions of scene volume and clutter. The size of depicted space across scene categories varied a 6-point log scale, from small and confined spaces such as closets or showers to expansive areas such as airport terminals or sports arenas. Scene clutter also varied on a 6-point log scale, from very empty spaces such as empty closets or garages, to very cluttered spaces such as full pantries or a full warehouses. Using a regions-of-interest approach, we examined the multivoxel pattern activity across multiple higher-level visual areas. We used a leave-one-category-out method in which a classifier was trained with five categories per ranking and required to predict the volume or clutter of a new scene category, thus requiring generalization across semantic category. We found that while the patterns of activity in the parahippocampal cortex represented both the spatial volume and clutter information within a scene, retrosplenial cortex selectively represented the spatial volume dimension of a scene, and the lateral occipital complex selectively represented the amount of clutter within a scene. There was a significant interaction across region (RSC or LOC) and scene dimension (Volume or Clutter). Furthermore, a whole-brain group random effects analysis with parametric regressors for spatial volume increase showed converging evidence that the retrosplenial cortex and an anterior parahippocampal area had a parametric representation of spatial volume. These data suggest that while scene information is represented in a distributed manner across multiple visual areas, spatial volume information of a scene is coded independently of the amount of clutter within a scene, consistent with previous results showing complementary but distinct neural representations of spatial boundary and scene content information.

Disclosures: S. Park, None; **T. Konkle**, None; **A. Oliva**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.5

Topic: D.04. Vision

Support: LANL 2009006DR

Title: Receptive field properties in primate visual cortical hierarchy from large scale statistics of natural images

Authors: *L. M. BETTENCOURT[1], S. BRUMBY[2], J. GEORGE[3], M. I. HAM[3], G. KENYON[3];
[1]Theoretical Div., [2]ISR Div., [3]Physics Div., Los Alamos Nat Lab., LOS ALAMOS, NM

Abstract: The primate visual cortex is organized hierarchically in terms of neurons with receptive fields that encompass larger and large receptive fields and more complex and abstract features, starting with simple edges in primary visual cortex (V1) to complex objects in inferior temporal cortex (IT). A large body of work has mapped some of the properties of receptive fields at different levels of the hierarchy, thought electrophysiology and other forms of brain imaging. However, no complete systems level understanding of these feature sets has yet emerged.
Over the last few years several computational approaches have started to study the statistics on natural images either directly or via biologically inspired models in order to reveal the nature and statistical properties of these feature sets., especially in V1. However these methods have used small image sets or video, corresponding typically to thousands of frames, but many orders of magnitude smaller than the visual experience of an animal or person. We explore learning of receptive fields in layers V1, V2 and V4 of the dorsal pathway of primate visual cortex over much larger amounts of visual experience, corresponding to a few days of continuous video. We use a model of visual cortex that captures the hierarchical organization of visual cortex and incorporates, in general ways, principles of sparseness and completeness found essential to the learning of the properties of receptive fields.
In this way we construct and characterize statistically receptive fields corresponding to successive layers of visual cortex. We show that lateral competition between cells that share the same receptive field naturally leads to sparseness both in terms of the single cell activation over time and of the activation statistics in a population. We characterize quantitatively the amount of visual information necessary to achieve approximately stationary statistics in these cell populations, which increase progressively up the visual hierarchy, and characterize the resulting feature set in terms of their over-completeness vs. the number of cells involved.
As a result we demonstrate how systems level models of primate visual cortex can be used to test principles of self-organization and learning in the brain, how their results may complement and inform electrophysiological studies and how the resulting feature sets generate more accurate and computationally efficient (sparse) object identification.

Disclosures: L.M. Bettencourt, None; **S. Brumby**, None; **J. George**, None; **M.I. Ham**, None; **G. Kenyon**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.6

Topic: D.04. Vision

Support: NIH Grant R01-EY13455

NIH Grant F32-EY020157

Title: Category-selective visual areas represent retinotopic, but not spatiotopic, location information

Authors: *J. D. GOLOMB, N. KANWISHER;
McGovern Inst., MIT, Cambridge, MA

Abstract: Two important functions of visual processing are identifying objects and determining their location in the environment. Despite the classic view that the ventral visual stream is primarily involved in object identification, these regions also contain considerable information about object location. We applied fMRI multivariate pattern analysis techniques to test whether this location information reflects pure retinotopic position or absolute (spatiotopic) location independent of eye position. We functionally localized several regions in the ventral visual stream, including the lateral occipital complex (LOC), fusiform face area (FFA), parahippocampal place area (PPA), and extrastriate body area (EBA), in addition to occipital and parietal visual regions. We then conducted several experiments to measure category and location information within these areas. Subjects viewed stimuli drawn from different categories (faces, scenes, bodies) and appearing in different locations. The locations varied in both eye position and stimulus position, generating pairs of conditions in which the stimuli occupied different retinotopic (eye-relative) positions but the same spatiotopic (absolute screen) position, the same retinotopic position but different spatiotopic positions, the same in both retinotopic and spatiotopic position, or different in both. In each of the object-selective regions, we found both location-invariant category information and category-invariant location information, replicating previous reports (e.g., Schwarzlose et al, 2008 PNAS). Moreover, the location information was purely retinotopic. That is, the multi-voxel pattern of fMRI response was more similar (i.e., more highly correlated) across conditions that shared the same retinotopic position than across conditions that shared the same spatiotopic position. Furthermore, there was no evidence of any spatiotopic location information in any of the ventral regions. This pattern of results persisted across different experiments in which subjects performed categorization and identification tasks, where location information was irrelevant, as well as tasks explicitly emphasizing spatiotopic (not retinotopic) stimulus position. Retinotopic information also dominated in occipital and parietal visual areas. Interestingly, across all of these areas, information about fixation position could also be decoded, suggesting that while these regions do not appear to contain explicit spatiotopic representations, spatiotopic locations might be implicitly determined by combining information about retinotopic location and eye position.

Disclosures: J.D. Golomb, None; **N. Kanwisher**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.7

Topic: D.04. Vision

Title: Sparse sampling degrades translational invariance for smaller stimuli

Authors: *S. AFRAZ[1], P. CAVANAGH[2];
[1]MIT, CAMBRIDGE, MA; [2]Lab. Psychologie de la Perception, Univ. Paris Descartes, 75006, Paris, France

Abstract: The translation invariance or tolerance of object recognition is frequently attributed to the large receptive fields of cells in the higher brain areas. Here we present evidence challenging this view and we suggest a sampling explanation of translation tolerance. We previously reported significant local biases in object recognition where, for example, the same face would be seen as a female at one location and a male at another. The biases were stable across weeks of testing but the pattern of biases was different for each subject. This position dependence of recognition was greatest for complex image attributes like facial gender, facial age and aspect ratio of objects but smaller for simple image attributes like orientation, spatial frequency and color. We now show that the degree of heterogeneity, the variation of bias across locations, depends on the stimulus size suggesting that the effect is a consequence of sparse neural sampling.
Complete translational invariance could be accomplished if cells at the highest level of the visual system had receptive fields that covered the entire visual field. But this is not the case. We suggest, moreover, that individual units or groups of units that analyze different regions are only coarsely calibrated with each other. As a result, when a stimulus is small relative to the independent analysis areas, it activates only a few cells or groups of cells revealing local biases; the percept can therefore vary from location to location even though the stimulus remains the same. In contrast, a larger stimulus will activate more cells and the variations should average out. We evaluated this sparse sampling hypothesis by varying the size of the visual stimulus, either a face or an oriented Gabor. We measured the perceptual heterogeneity across locations for facial gender and orientation discrimination tasks at 8 different locations, all at 5° eccentricity. The results revealed that heterogeneity is again larger for face stimuli than orientation but that heterogeneity decreases as size increases for both stimulus types, so that at the smallest size, strong heterogeneity is seen even for orientation judgments while at the largest sizes, a fair

degree of translation tolerance is seen even for face stimuli. Based on these results we suggest that perceptual heterogeneity is a general property of visual perception resulting from sparse sampling of stimuli that are small relative to the size of the receptive fields. Translation tolerance emerges when stimuli are large enough, relative to analysis regions, to activate many units, ensuring a categorization dominated by the population mean that will not vary from location to location.

Disclosures: S. Afraz, None; **P. Cavanagh**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.8

Topic: D.04. Vision

Support: NIH grant EY-016464 to RAE

Title: Neural construction of scenes from objects in human occipitotemporal cortex

Authors: *S. P. MACEVOY[1], R. A. EPSTEIN[2];
[1]Dept. of Psychology, Boston Col., Chestnut Hill, MA; [2]Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Human observers have a remarkable capacity to categorize complex visual scenes, such as “kitchen” or “beach”, at a single glance. Although previous work has identified regions of occipitotemporal cortex that respond strongly to scenes (Epstein and Kanwisher, 1998) and to objects within scenes (Malach et al., 1995), specific neural mechanisms underlying scene recognition have not been identified. Here we present evidence for one such mechanism: scene identification through parallel identification of within-scene objects. We recorded subjects’ brain activity with functional magnetic resonance imaging (fMRI) while they viewed scenes drawn from several categories (e.g. kitchen, bathroom) as well as isolated "signature" objects strongly associated with those scenes (e.g. stove, refrigerator, toilet, bathtub). Using multi-voxel pattern analysis (MVPA), we found that activity patterns evoked by scenes in lateral occipital cortex (LO) could be correctly classified by predictor patterns generated by averaging the patterns evoked by their signature objects. This was true even with short stimulus presentation times designed to prevent subjects from directing attention separately to the individual objects within the scenes. Notably, object-based predictor patterns failed to classify scene-evoked patterns in the parahippocampal place area (PPA), even though this region responds strongly to scenes and

is believed to play a critical role in scene recognition (Mendez and Cherrier, 2003; Epstein, 2007). We hypothesize that the PPA and LO represent separate pathways supporting complementary modes of scene recognition, with the PPA supporting recognition based principally upon global scene properties and LO supporting recognition based on parallel analysis of the objects they contain.

Disclosures: S.P. MacEvoy, None; **R.A. Epstein**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.9

Topic: D.04. Vision

Support: NSF BCS 04-20794

NSF BCS 05-31177

NSF BCS 06-17699

NIH BRP EY016093

Title: The coding of object interactions in LOC

Authors: *J. G. KIM[1], I. BIEDERMAN[2];
[1]Psychology, USC, LOS ANGELES, CA; [2]Psychology, Neurosci. Program, USC, Los angeles, CA

Abstract: Our visual experience is generally composed of scenes, where multiple objects are interacting. The coding of relations among objects is fundamental in that it allows the explicit representation of complex structures. A pair of objects can be presented side-by-side, e.g., a bird by a birdhouse, or as interacting, e.g., a bird perched on a birdhouse. Such interactions markedly facilitate cued recall (Epstein et al., 1960), visual search (Green & Hummel, 2004), and target detection (Green & Hummel, 2006) than when the two objects are not interacting. Where are these object interactions encoded in the brain? In a series of fMRI experiments, we show that minimal scenes composed of object pairs presented as interacting elicit greater BOLD responses in LOC, than their side-by-side depictions. This pattern of responses was identical across every experiment. LOC is an area critical for object recognition and where intact shape is first

distinguished from texture (Cant & Goodale, 2009). Novelty of the interactions, e.g., the bird perched on an ear, showed even a greater BOLD response than familiar interactions, but this effect of novelty was absent in the side-by-side depictions. Other regions, such as the PPA, IPS and DLPFC showed a similar but weaker and less consistent pattern of responses as that of LOC, rendering it unlikely that activity in these regions was causal to the effects seen in LOC. In an EEG experiment, we also show that the divergence of the interacting versus side-by-side objects are evident only in the occipito-temporal region (likely LOC), and no other region manifested an effect of the interactions much less as early as LOC. The fMRI results in LOC cannot be explained as a feed-forward effect from early visual areas, foveal magnification, relative size, task difficulty, visual complexity or eye movements. Together, our results provide strong evidence that when we glance at a scene, the relations between objects are processed simultaneously with—rather than after—the perception of the shape of those objects.$$graphic_{21DB544A-D750-4579-8442-8792752F1CB4}$$

Disclosures: J.G. Kim, None; **I. Biederman**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.10

Topic: D.04. Vision

Support: NIMH Intramural Program

Title: High-level scene representations: Its the spaces not the places

Authors: *D. J. KRAVITZ[1], C. S. PENG[2], C. I. BAKER[1];
[1]NIH, BETHESDA, MD; [2]Psychology, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Real-world scenes are complex and heterogeneous, yet we are able to identify and categorize them effortlessly. While the Parahippocampal Place Area (PPA) has been implicated in scene processing, information about scenes is contained in many visual areas, leaving their specific contributions unclear. While, initial reports of PPA focused on the processing of spatial information, a more recent report found that its response could be used to discriminate between semantic categories of scenes. However, similar discrimination was also found in several other regions, including object-selective and early visual cortex (EVC). Ultimately, these studies were limited by the use of only a small number of preselected conditions, making it difficult to compare spatial and non-spatial scene information, or to investigate the fine-grained

representational structure necessary to differentiate between visual regions. Here we use a multivariate ungrouped approach to reconstruct detailed scene representations across visual cortex. We presented 96 diverse, and highly detailed scenes in an ungrouped event-related fMRI paradigm with each scene being a unique condition. 6 exemplars of each of 16 broad semantic scene categories (e.g. beaches, cityscapes) were presented. Scenes were further chosen to equally sample across two spatial factors: expanse (open/closed) and relative distance (near, far), and one non-spatial factor: content (manmade/natural). We used multi-voxel pattern analysis to establish how PPA and other visual areas grouped and discriminated scenes. Importantly, our analyses had no bias for any particular organization to emerge; rather the recovered representational structure is driven by the response of the region. Focusing on PPA and EVC, we found very similar scene discrimination, with a strong correlation between the discriminability of even individual scenes across the two regions. However, the scene representations in PPA and EVC differed strongly in their categorical structure. PPA grouped scenes primarily by expanse, despite large differences in non-spatial information (content, semantic category). In contrast, early visual cortex primarily grouped scenes by relative distance. Neither region evidenced strong grouping by the semantic scene categories and when spatial factors were controlled, there was no discrimination by semantic category evident in either region. These results suggest while many regions can discriminate scenes, each encodes different aspects of complex scenes, providing insight into their relative contributions to scene processing, and the transformation of visual information along the ventral visual pathway.

Disclosures: D.J. Kravitz, None; **C.I. Baker**, None; **C.S. Peng**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.11

Topic: D.04. Vision

Support: NIH Grant EY02966

NIH Grant EY16281

Title: Trans-saccadic memory for border ownership in neurons of the visual cortex

Authors: *P. J. O'HERRON, R. VON DER HEYDT;
Krieger Mind/Brain Inst., Johns Hopkins Univ., BALTIMORE, MD

Abstract: The cortex represents figure-ground organization by assigning border ownership. Border ownership selective neurons respond to a contrast edge with a higher firing rate when the edge belongs to a figure on one side than to the same local edge when it is part of a figure on the other side. This selectivity can be interpreted in two ways. We could assume that the differential response reflects the influence of cues that indicate which side of the edge is foreground. Or, we can speculate that the differential response reflects the influence of an emerging object representation that 'owns' the edge, akin to the 'object file' or 'proto object' hypotheses in psychology. An important property of object files is persistence, because this is what provides continuity of objects across saccades and when objects move. We have previously shown that the response difference ('border ownership signal') persists when the edge in the receptive field is made ambiguous by removing the context (O'Herron & von der Heydt, Neuron, 2009). Here we show that the border ownership assignments persist when a figure moves, or when the eyes make a saccade. We studied single neuron activity in area V2 of awake monkeys during behaviorally induced fixation and saccades. The moving figure test consisted of a three-phase display: (1) A square was presented outside the receptive field. (2) One edge of the square was isolated by occluding the context. (3) The isolated edge was moved into the receptive field. In the first two phases, the neuron was silent, because there was no contrast in the receptive field. In phase 3, the neuron responded to the ambiguous edge. We found that the strength of these responses depended on the side of border ownership in phase 1. Thus, border ownership information had been transferred from neurons that were activated by the figure in phase 1 to neurons that were activated only in phase 3. A similar result was obtained when the edge remained still and the monkey made a saccade that moved the receptive field onto the edge. Because the two groups of neurons are separated in the cortex (as given by the amplitude of movement, or size of saccade), this means that border ownership information is transferred across the cortex. One can explain this transfer of information if one assumes that the presentation of a figure creates a focus of activity in a grouping cell layer at a higher level, which projects back to V2, thereby modulating the neurons representing the features of the object (Craft et al. 2007). The transfer of border ownership signals could then be achieved simply by moving this focus of activity according to the object or eye movement.

Disclosures: P.J. O'Herron, None; **R. von der Heydt**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.12

Topic: D.04. Vision

Support: NSF Award 0546262

Athinoula A. Martinos Imaging Center at McGovern Institute for Brain Research, MIT

Title: Examining how objects of different real-world sizes are represented in ventral visual cortex

Authors: *T. A. KONKLE[1], A. OLIVA[2];
[1]Brain & Cognitive Sci., MIT, CAMBRIDGE, MA; [2]Brain & Cognitive Sci., MIT, Cambridge, MA

Abstract: The size of objects in the world influences how we interact with them, but little is known about how this object property is involved in object processing and representation. Here we examined if the dimension of real-world size is systematically represented in ventral visual cortex. Experiment 1: observers were presented with blocks of small objects (e.g. strawberry, calculator) and blocks of big objects (e.g. car, piano) displayed at the same visual size (8 degrees) while undergoing whole brain imaging in a 3T fMRI scanner. Contrasts of big and small objects revealed that a region in the parahippocampal gyrus was preferentially active to big objects versus small objects, while a subregion along the lateral occipital cortex was preferentially active to small objects versus big objects. Experiment 2: objects with big and small real-world sizes were displayed at two visual sizes on the screen (10 degrees and 4 degrees). The same regions were selective for big or small objects, independent of the visual size presented on the screen, indicating that these regions are tolerant to changes in visual size. Experiment 3: observers were shown blocks of objects grouped by category, with 16 different object categories spanning the range of real-world sizes. We observed parametric modulation of the big and small regions of interest_in the big ROI, activity increased as object size increased ($r=.74$, $p<.01$), whereas in the small ROI the opposite pattern was observed ($r=-.76$, $p<.01$), with no modulation in LOC ($r=-.28$, $p>.1$) or early visual cortex ($r=.05$, $p>.1$). These results show that objects of different real-world sizes are represented in different patches of ventral visual cortex, and suggest that the real-world size of an object is a property that can make predictions about which areas along the ventral visual cortex will be preferentially engaged in that object's processing.

Disclosures: T.A. Konkle, None; **A. Oliva**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.13

Topic: D.04. Vision

Support: DARPA Neo vision 2

NSF-0640097, NSF-0827427

Title: The representation of objects in inferior temporal cortex with and without attention

Authors: *E. M. MEYERS[1], Y. ZHANG[2], N. BICHOT[2], T. SERRE[3], T. POGGIO[2], R. DESIMONE[2];
[1]Brain & Cognitive Sci., MIT, CAMBRIDGE, MA; [2]Dept. of Brain and Cognitive Sciences, McGovern Inst., MIT, Cambridge, MA; [3]Cognitive and Linguistic Sci., Brown Univ., Providence, RI

Abstract: Within a given moment the number of objects that can be recognized is limited, and focused attention is often necessary to select behaviorally relevant objects and ignore distracting information. Despite numerous studies that have described various "enhancing" physiological changes related to attention, it is still unclear whether or how any of these effects impact information content at the population level so as to improve object recognition in cluttered scenes. In this study we used neural population decoding to better quantify how attention related changes influence the information about specific objects in macaque inferior temporal cortex (IT). Results from this work show that before a monkey is cued to attend to an object at a particular location, neural activity contains a mixture of information about the multiple objects that were shown, and that the information about any particular object is greatly reduced relative to when the object is shown in isolation. However, when the monkey is instructed to attend to one of the objects, the population activity reverts to a pattern that is similar to the pattern elicited by the attended object when shown in isolation. Consequently a large amount of information about the attended object is restored. We also find that attention increased information about object position, and that a sudden increase in the salience of a nonattended object can completely override these attentional enhancements. This work not only shows that attention related firing rate changes in IT do indeed impact the information about particular objects embedded in clutter, but it also supports both algorithmic and descriptive computational models of why clutter degrades object recognition performance and how attention restores relevant information at the population level.

Disclosures: E.M. Meyers, None; **Y. Zhang**, None; **N. Bichot**, None; **T. Serre**, None; **T. Poggio**, None; **R. Desimone**, None.

Nanosymposium

326. Encoding of Visually Presented Objects I

Location: Room 24A

Time: Monday, November 15, 2010, 8:00 am - 11:30 am

Program Number: 326.14

Topic: D.04. Vision

Support: the Ministry of Science and Technology of China 2005CB522800

the Ministry of Science and Technology of China 2004CB318101

the National Natural Science Foundation of China 30621004

the National Natural Science Foundation of China 90820307

Title: Hole vs. Whole discriminated by early responses of anterior temporal brain regions: Timing of holistic processing revealed by intracranial recording on human subjects

Authors: *Z. LIU[1], R.-J. WU[2];
[1]Inst. Biophysics, CAS, Beijing, China; [2]Inst. of Biophysics, CAS, Beijing, China

Abstract: A visual object with a hole shows distinct perceptual preference compared with an object without a hole, in which the perception of topological structure stands for the holistic information processing. To investigate the brain regions and the timing of possible neural circuits supporting this perceptual preference, we recorded intracranial event related potentials on human subjects by subdural surface electrodes.
In a discrimination task, a geometrical figure was briefly presented on either side of the center fixation for 10 ms followed by a 100~500 ms ISI. Subjects were required to determine on which side of the fixation the figure had been presented. However, button response was only required for catch trials that take up 1/11 of the total 330 trials. In the catch trial the figure was blurred by Gaussian noise to an extent that it could be easily identified from standard trials. The figure was randomly selected from WHOLE group or HOLE group. There are 5 figures (disc, square, rectangle, parallelogram and trapezium) in WHOLE group. The 5 figures in HOLE group have been carefully designed that they have the same outline and are equal-area to their paired figures in the WHOLE group, but with a hole of the same shape at the center.
Intracranial EEG was recorded from 8 epilepsy patients implanted with subdural electrodes while they were performing the task. Averaged ERPs ranged from -200 ms to 500 ms were calculated for the HOLE and WHOLE group after baseline correction and artifact rejection. A permutation test was applied to identify the effective electrodes that show significant difference between the two conditions. Of total 230 electrodes from all the subjects, 48 electrodes were found to be effectively discriminating HOLE vs WHOLE starting at about 50 ms after stimulus onset, located mainly in anterior temporal areas and frontal areas. Grand average of the ERPs by brain regions revealed that the effective electrodes in anterior temporal areas had the same response pattern that HOLE figures evoked more positive potentials than WHOLE figures ranging from 45 ms ~ 50 ms, while the effective electrodes in frontal lobe had no consistent response pattern for the two groups of figures.
The results demonstrate a crucial role of anterior temporal region regarding of the timing of

holistic information processing, especially considering the well-documented scalp ERPs components in primary visual cortex starting at almost the same latency.

Disclosures: Z. Liu, None; **R. Wu**, None.

Nanosymposium

327. Perception across Movements

Location: Room 5B

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 327.1

Topic: D.05. Visual Sensory-motor Processing

Support: EC-NEST: MEMORY

ERC grant no. 229445 (STANIB)

Title: How transient "remapping" of neuronal receptive fields mediates perceptual stability

Authors: *M. MORRONE[1,2], G. CICCHINI[2], P. BINDA[3], D. BURR[4,5];
[1]Scienze Fisiologiche, Universita' Di Pisa, Pisa, Italy; [2]IRCCS Stella Maris, Pisa, Italy; [3]Facolta' di Psicologia, Univ. Vita-Salute S Raffaele, Milano, Italy; [4]Univ. of Florence, Florence, Italy; [5]CNR - Inst. of Neurosci., Pisa, Italy

Abstract: It is well known that saccades cause profound transient changes to vision, both to the spatial properties of receptive fields of parietal cortex of macaque monkey, and to human perception. It remains unclear, however, how these transient events contribute to stability. A critical, but largely overlooked aspect, is that as neurons update the spatial position of their receptive field before the saccade, responses to the "new receptive field" are delayed in time. This leads to a receptive field that is oriented in space-time (Binda et al., *Journal of Neuroscience*, 2009). We studied in humans how this could lead to stability by measuring perceptual mislocalization of pairs of brief visual stimuli presented successively to the same or different positions. Two bars (6x0.2deg), one white and one black, were briefly flashed above and below screen center at various times relative to the onset of a 20º horizontal saccade, at temporal separations ranging from 20 to 160 ms. Subjects reported both the spatial positions of the two bars, and their apparent temporal order. Single perisaccadic bars were strongly mislocalized, by up to half saccade amplitude. However, when two bars were displayed within 40 - 120 ms of each other, no mislocalization occurred with either, even when the saccades caused them to be separated on the retina by up to 20 deg. This stabilization of spatial localization was also observed for targets separated in space by up to 5 deg, but greatly reduced

when separated by 10 deg. For bars at the same external position, the interaction leading to stability always occurred when the first bar was presented before the saccade and the second within the first 50 ms after saccadic onset. We assume that the interaction between the bars must be mediated by a common neuronal mechanism responding to both stimuli, a mechanism extending over space and time: as the response to the bar displayed to the "future receptive field" is delayed, it arrives simultaneously with the response to stimuli displayed later to the classic receptive field, and are therefore fused. Using data of both single neurons in LIP and psychophysical measurements, we have developed a detailed model of how the spatio-temporal shifts of the receptive fields predict quantitatively these new data and perceptual stability in general.

Disclosures: M. Morrone, None; **G. Cicchini**, None; **P. Binda**, None; **D. Burr**, None.

Nanosymposium

327. Perception across Movements

Location: Room 5B

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 327.2

Topic: D.05. Visual Sensory-motor Processing

Support: NWO/MaGW VICI grant

Title: Temporal uncertainty as the cause of spatial mislocalization

Authors: *J. B. SMEETS, F. MAIJ, R. J. VAN BEERS, E. BRENNER;
Human Movement Sciences, VU Univ., Amsterdam, Netherlands

Abstract: When localizing an object in a visual scene, one has to combine the location of the object's image on the retina with information about the position (orientation) of the eye. In this combination, the brain will take into account the uncertainty of the information, and complement it with prior assumptions. When localizing a flash around the time of a saccade, the relevant uncertainties are not only in the positions, but also in the timing of the retinal position information relative to that of eye position. We propose that around the time of saccades, this temporal uncertainty will be the dominant cause of mislocalization.
We modeled this temporal uncertainty by a normal distribution with a standard deviation of 15 ms. We assumed a tendency to localize in the direction of gaze; this tendency was modeled as a prior with a standard deviation of 5 degrees. Combining a continuous time-varying eye position signal with a brief retinal position signal of which the timing is not known exactly results in a combined position signal with spatial uncertainty around the time of a saccade. During this

period, the spatial prior causes a peri-saccadic spatial compression.
The resulting pattern of mislocalization can be regarded as a combination of a shift and compression towards the saccade target. Interestingly, the pattern shows various asymmetries that are also present in human data; e.g. the moment of maximum mislocalization depends on the flash location. Various parameters of the model, such as saccade amplitude, saccade duration and temporal precision all influence the exact shape of the resulting mislocalization pattern. This can be related to experimental results showing that the mislocalization pattern depends on details of the experimental conditions. The model thus captures many aspects of peri-saccadic mislocalization that have been reported in the literature. We conclude that it is very likely that temporal uncertainty is an important cause of peri-saccadic mislocalization.

Disclosures: J.B. Smeets, None; **F. Maij**, None; **R.J. van Beers**, None; **E. Brenner**, None.

Nanosymposium

327. Perception across Movements

Location: Room 5B

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 327.3

Topic: D.05. Visual Sensory-motor Processing

Title: An explanation of perisaccadic flash mislocalization when the flash occurs together with background stimuli

Authors: *J. R. POLA;
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Abstract: When a person makes a saccade in the everyday visual environment, the perceived location of an object in space remains stable even though the retinal image of the object shifts across the retina as a result of the saccade. The usual account of this phenomenon is that along with the saccade, an extraretinal (exR) signal occurs which serves to cancel perceptually the retinal image shift. One kind of experiment that has been used to explore the exR signal involves asking subjects to report on the location of a perisaccadic flash in the dark. In this type of study, the flash tends to be mislocalized suggesting the existence of an exR signal that changes before, during, and after the saccade. However, Pola (2004; 2007) has offered a retinal-extraretinal signal (R-exR) model suggesting that such mislocalization may not simply come from an exR signal, but from flash retinal (R) signal persistence interacting with the exR signal. In addition to experiments about perisaccadic flash mislocalization in the dark, several studies have investigated such mislocalization in the face of background stimuli. For example, an early experiment by Matin, Matin, Pola & Kowal (1972) was concerned with a perisaccadic flash

occurring either in the dark or along with a small, stationary target present during and after the saccade. In a recent study by Maij, Brenner, Li, Cornelissen & Smeets (2010), a perisaccadic flash was viewed against a saccade goal target in two circumstances: 1) the target jumped, at the time of the saccade, either in the direction or against the direction of the saccade; and 2) the target disappeared at various times before or at the time of the saccade. In general, these experiments show that flash mislocalization is substantially influenced by the spatial and/or temporal characteristics of background stimuli. In the present work, the R-exR model was used to explore what is responsible for flash mislocalization in the studies by Matin et al. (1972) and Maij et al. (2010). In essence, the model suggests that just as R signal persistence interacting with an exR signal is responsible for mislocalization in the dark, the R signal interacting with the particular spatial-temporal features of the background stimuli (as well as the exR signal) gives rise to the magnitude and direction of mislocalization with the background. These findings, along with previous results (Pola, 2004; 2007) indicate that R signal persistence plays a significant role in mislocalization of flashes in a variety of circumstances: in the dark, with multiple stimuli, and with background stimuli.

Disclosures: J.R. Pola, None.

Nanosymposium

327. Perception across Movements

Location: Room 5B

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 327.4

Topic: D.05. Visual Sensory-motor Processing

Support: ANR Chaire d'Excellence (PC)

NIH grant EY018216 (DW)

ED 3CH Paris V (MS)

Title: Motion reveals imperfect spatial constancy for head tilt compared to head translation

Authors: *P. CAVANAGH[1], D. WHITNEY[2], M. SZINTE[1];
[1]Univ. Paris Descartes, Paris, France; [2]Psychology, Univ. of California, Berkeley, Berkeley, CA

Abstract: When we move our head or eyes, stationary objects move over our retina but our visual system discounts this retinal motion so that we see the objects in spatial rather than retinal coordinates. The mechanisms that compensate for head and eye movements can be tested by

moving the target at the time of the saccade or head movement. In the case of eye movements, two dots that presented at different locations, one before and one after a saccade, are seen to move in world coordinates (Cavanagh & Szinte, VSS 2009). In that experiment, we used the deviation from exact spatiotopic motion to estimate errors in the remapping of pre-saccadic locations. We now use the equivalent procedure to test the mechanisms of spatial constancy across two types of head motion: tilt around and translation along the visual axis. In the first case, we asked subjects to make large, rapid head rotations around the visual axis in synchrony with red to green color changes of the fixation spot. Two target dots were presented sequentially, vertically aligned above fixation, one before and one after each head rotation, the vertical displacement between the dots was 1/6 of the eccentricity of their midpoint. Each dot was presented for 200 msec with a 400 msec interval between them. In this condition, the spatial displacement was purely vertical whereas the retinal displacement had a substantial horizontal component. Of 10 subjects, 8 reported that the vertical motion on the screen appeared strongly oblique and 2 reported that it appeared horizontal. This suggests that compensation mechanisms (including efference copy, proprioception, and cyclotorsion) do not correct well for head tilt. In the second case, subjects made large forward and backward translations of the head while fixating and synchronizing their head motions to the red to green color changes of the fixation spot. The near viewing position was approximately half the distance to the screen of the far position. Two target dots were again presented sequentially above the fixation spot, one before and one after each head translation. Now, however, the two target dots were horizontally aligned on the screen, one to left of vertical and the other to the right, separated by 1/6 of their eccentricity. Despite the large vertical displacement of the dot on the retina caused by the head motion, only 3 of 10 subjects reported deviation of the perceived motion from horizontal, while 7 reported that the motion was horizontal, matching its direction in world coordinates. Compensation for head translation therefore appears to be much more effective than that for head tilt, implying a dissociation between the mechanisms that correct for these two types of head movements.

Disclosures: P. Cavanagh, None; **M. Szinte**, None; **D. Whitney**, None.

Nanosymposium

327. Perception across Movements

Location: Room 5B

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 327.5

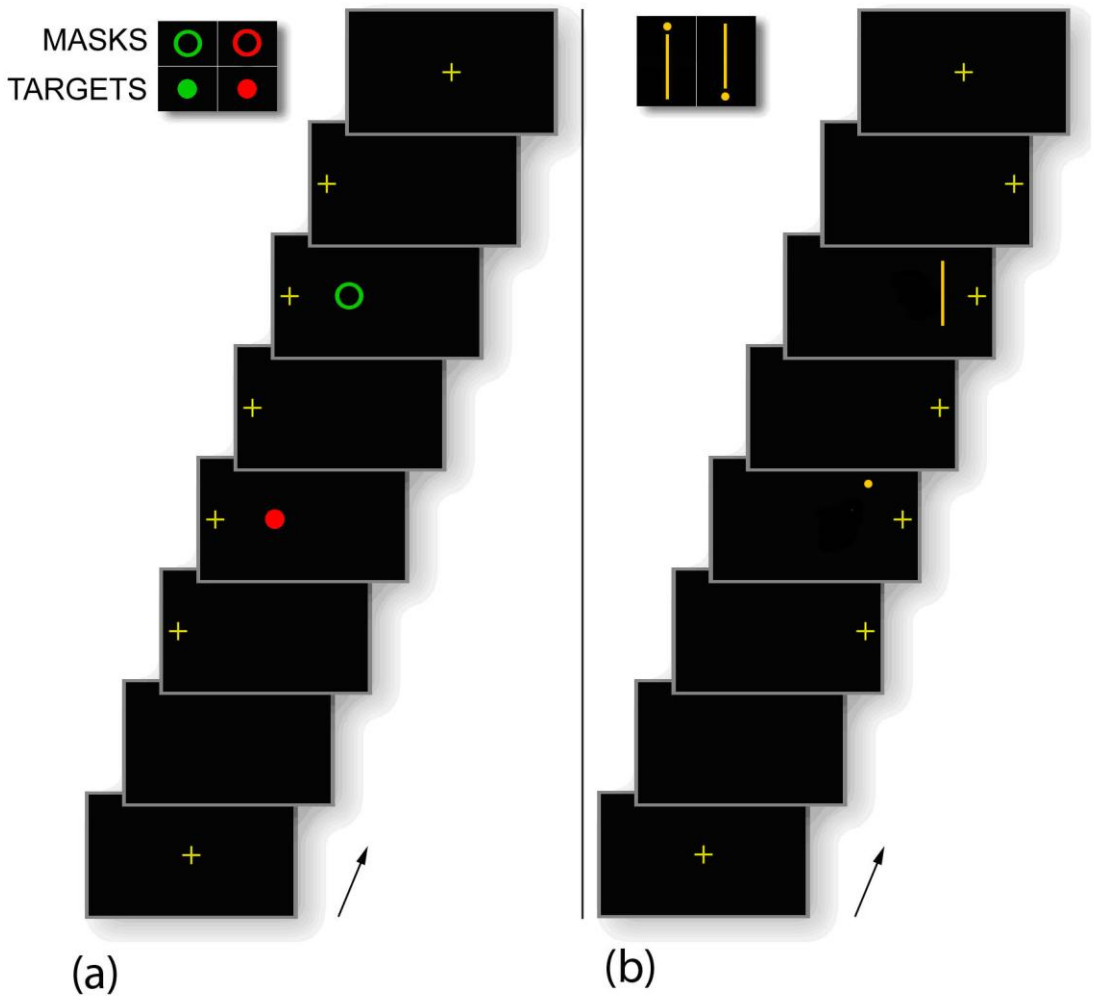
Topic: D.05. Visual Sensory-motor Processing

Support: MIUR PRIN 2007

Title: Perception of spatiotemporal events across saccades

Authors: *D. MELCHER[1], N. DE PISAPIA[2], L. KAUNITZ[2], A. FRACASSO[2];
[1]Dept. of Cognitive Sci., Univ. of Trento, Rovereto, TN, Italy; [2]Ctr. for Mind/Brain Sci., Univ. of Trento, Rovereto, Italy

Abstract: Each saccadic eye movement shifts the retinal coordinates of salient objects in the scene. In order to perceive spatiotemporal events that occur over time, and across saccades, the visual system must successfully match individual objects over separate glances. Specifically, the brain must decide whether to *integrate* the visual information (for the same spatiotemporal object) or to *segregate* the input (into two separate objects). To study this process, we measured spatial localization and perceptual interpretation of two-stimulus events in backward masking and apparent motion paradigms. First, we asked participants to identify the color of a target stimulus (red or green) presented just prior to saccadic onset that was immediately masked by a larger stimulus at the same location. Depending on the timing of the target with respect to the saccade, the target could be mislocalized, causing the two stimuli to be spatiotemporally segregated into two events and thus "unmasking" the target. The improvement in color discrimination when the stimulus was "unmasked" suggests that peri-saccadic mislocalization is related to object remapping rather than spatial uncertainty. In the second experiment, we tested apparent motion and the line-motion illusion across saccades. The strength of the motion percept (based on interpreting two flashes as a single moving object) was determined by the timing of the stimuli with respect to the saccade onset. Overall, the results of both experiments are consistent with the idea that peri-saccadic effects, such as mislocalization and time reversal, are directly related to a spatiotemporal transformation that supports perception of coherent events across saccades.



Disclosures: D. Melcher, None; **N. De Pisapia**, None; **L. Kaunitz**, None; **A. Fracasso**, None.

Nanosymposium

327. Perception across Movements

Location: Room 5B

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 327.6

Topic: D.05. Visual Sensory-motor Processing

Support: NWO, ALW grant 816-02-017

Title: Misjudging where you felt a light switch in a dark room

Authors: *F. MAIJ, D. D. J. DE GRAVE, E. BRENNER, J. B. J. SMEETS;
VU Univ. Amsterdam, Amsterdam, Netherlands

Abstract: When you come home late at night and need to find the light switch in a totally dark room, you will sweep your hand across the wall to feel where the switch is. Once you have felt it you will need to move your hand back to the place at which you felt it to turn the switch. Surprisingly, it is hard to find the switch although you touched it while moving your arm. This is an example of the problem of perception during movement. This problem is well studied in vision: flashes around the time of a saccade are systematically mislocalized. In haptics, similar patterns of mislocalization have been reported when a small vibrator that is attached to the index finger delivers a tap on the finger around the time of an arm movement. However, these studies used short, artificial stimuli that we do not encounter in our everyday environment. In the present study, we tested whether the same mislocalization occurs in a situation in which all objects are continuously present, comparable to the light-switch example.
We asked blindfolded subjects to either move their right index finger to the left or to the right across a table-top from the side of one small cube to the side of another small cube. The cubes were 40 cm apart. We placed a thin aluminum bar on the table on a random position in the path of the movement. The subject was instructed to localize the bar after they had completed the arm movement by indicating the perceived location with their right index finger. We found that subjects systematically mislocalized the bar. The errors depend on the location of the bar during the movement (and thus on the timing) in a similar manner as has been shown with artificial tactile stimuli. This demonstrates that movement related mislocalization is not limited to artificial short-lived stimuli.

Disclosures: F. Maij, None; **D.D.J. de Grave**, None; **E. Brenner**, None; **J.B.J. Smeets**, None.

Nanosymposium

327. Perception across Movements

Location: Room 5B

Time: Monday, November 15, 2010, 8:00 am - 9:45 am

Program Number: 327.7

Topic: D.05. Visual Sensory-motor Processing

Support: CIHR grants to DG and CP

Title: On the illusory perceptual compression of visual space during eye-head gaze saccades

Authors: *D. GUITTON, A. RICHARD, C. PACK;

McGill Univ., Montreal, QC, Canada

Abstract: The spatial location of a stimulus presented briefly near the onset of, and during, an eye saccade is not perceived accurately, a phenomenon known as perisaccadic mislocalization. Two components of mislocalization have been described: a shift of the stimulus's apparent position in the direction of the saccade and a perceived "compression" of its position towards the saccade target. Previous studies of compression have focussed on saccadic eye movements made with the head artificially immobilized. We have proposed a simple equation that expresses the magnitude of compression under such conditions as dependent on the distance, on a retinotopically encoded logarithmic map of the visual field, between two loci of activity: one encoding the location of the visual stimulus on the retina, the other the burst discharge commanding the saccade vector (Richard et al., 2009). Many brain structures involved in saccade generation also encode saccadic gaze shifts, where gaze = eye-in-space = eye-in-head + head-in-space. It is thus important to determine whether compression is towards the end-point of gaze saccades made head-unrestrained (HU). **Methods:** Using a standard compression paradigm (Lappe et al., 2000), we studied mislocalization in HU human subjects who made horizontal saccadic eye-head gaze shifts of 40 to 60 degs.. Subjects were instructed to localize a briefly (12ms) flashed vertical bar presented over a range of horizontal positions between the fixation point and the saccade target and beyond. **Results:** As in previous studies, we found a powerful compression of visual space that depended on the time at which the vertical bar was presented relative to the onset and time-course of the gaze shift. Importantly, compression was toward the intended gaze target, rather than to the spatial location of the initial eye movement. We also found that the duration of compression was nearly constant across gaze-shift amplitudes. **Conclusions**: Our model of compression could be extended to the HU condition and time domain by invoking time-filtered interactions, on a logarithmic map of visual space, between two populations of neurons that encode respectively the gaze-shift vector and visual stimulus position relative to the fovea.

Disclosures: D. Guitton, None; **A. Richard**, None; **C. Pack**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.1

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG028774

Title: The source of the associative deficit in aging: fMRI evidence of the effect of reduced attentional resources for relational processing

Authors: *S.-Y. KIM, K. GIOVANELLO;
Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Episodic memory decline is a well established finding in the field of cognitive aging. The Associative Deficit Hypothesis (ADH: Naveh-Benjamin, 2000) posits that older adults' deficient episodic memory arises from a difficulty in binding/associating contextually-specific information into a cohesive memory unit. According to the ADH, older adults show disproportionate deficits in performance whenever tasks require participants to create and retrieve novel links between arbitrary units of information (e.g., remembering that "dog" and "couch" were shown together at encoding). Such age-related associative/relational memory deficits have been demonstrated with a variety of memory tasks, yet the source of the deficit remains unspecified. One of the most widely investigated factors is an age-related reduction in attentional resources. To investigate the effect of reduced attentional resources on relational memory performance, previous researchers have used a divided attention paradigm to impose attentional loads on young adults during encoding of relational or item information. However, none of the existing studies have found disproportionate relational memory impairments in young adults under divided attention conditions. Using functional magnetic resonance imaging (fMRI), the current study investigated whether a reduction in attentional resources for processing of relational information underlies the memory impairments observed in aging. The behavioral results demonstrated that reduced attentional resources for relational processing in young adults equated their relational memory performance to that of older adults under full attention. Furthermore, the fMRI results demonstrated that aging, as well as reductions in relational attention processing in young adults, significantly reduced activity in the brain areas critical for relational memory formation, namely, the ventrolateral and dorsolateral PFC, superior and inferior parietal regions, and left hippocampus. This converging evidence from behavioral and neuroimaging studies thus documents the first evidence that the reduction in attentional resources for relational processing is the critical factor for the age-related relational memory deficit.

Disclosures: S. Kim: None. **K. Giovanello:** None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.2

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG028774

NIH Grant AG031660

Title: Effects of aging on the neural basis of implicit associative memory

Authors: *I. T. DEW[1], K. S. GIOVANELLO[2];
[1]Psychology, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [2]Psychology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Older adults perform worse than younger adults during intentional, explicit memory retrieval of associative information. However, implicit (unintentional) associative memory retrieval is relatively preserved in aging. Importantly, fMRI studies in young adults have shown that the medial temporal lobe is active during implicit associative retrieval. The present study tested whether MTL function during implicit associative retrieval is spared in aging, or alternatively, whether older adults recruit non-task-related cortical regions to support equivalent behavioral performance. Fourteen young and eleven healthy older adults participated in an event-related BOLD fMRI study. We designed a novel associative version of a speeded classification task in which subjects made speeded judgments about the relationship between objects. We examined differences in neural activity during intact (i.e., repeated) relative to rearranged object pairs. Behaviorally, we observed equivalent implicit associative memory performance between young and older adults (faster reaction time to intact relative to rearranged pairs). Young adults showed activity in regions that have been previously linked with implicit associative memory retrieval, including bilateral hippocampus, left parahippocampal gyrus, and right entorhinal cortex. Relative to the young adults, older adults showed reduced activity in MTL regions, coupled with increased activity in several non-task related regions within prefrontal cortex (PFC), including right dorsolateral PFC, bilateral middle frontal gyrus (MFG), and left inferior frontal gyrus (IFG). Importantly, the magnitude of activity in right DLPFC correlated with the magnitude of behavioral priming in older but not younger adults. The correlation between behavioral priming and activity in right DLPFC is consistent with the hypothesis that older adults recruit prefrontal regions to compensate for MTL dysfunction. This study documents the first evidence of this effect during associative priming, on a task in which no age differences were found behaviorally. Taken together, the findings are consistent with patterns of structure-function reorganization in aging.

Disclosures: I.T. Dew, None; **K.S. Giovanello**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.3

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant K99-NS069788

NIH Grant MH062500

NIH Grant NS19632

Title: Spontaneous relational behaviors: Linking action, memory, and the hippocampus

Authors: *J. L. VOSS[1], D. E. WARREN[2], D. TRANEL[2], N. J. COHEN[1];
[1]Beckman Inst., Urbana, IL; [2]Univ. of Iowa, Iowa City, IA

Abstract: Hippocampal amnesia can be characterized as a deficit of relational memory, compromising performance even at very brief study-test delays. We investigated whether the spontaneous behaviors of amnesic individuals at study time are disorganized as a part of their relational memory deficits. When healthy individuals studied objects arbitrarily positioned on a grid, a prevalent feature of their viewing behavior was to look back-and-forth from an object to the objects studied moments prior, thereby appreciating relations between objects grouped proximally in space and time. This "relational behavior" led to superior subsequent recall of locations for objects studied in this manner vs. studied otherwise, but only when the relational behavior was self-initiated (i.e., not when looking back-and-forth was passive and forced by the computerized display). Furthermore, self-initiated relational behavior was associated with increases in fMRI activity in left anterior hippocampus and frontal cortex, whereas passively looking back-and-forth was not. In addition to standard deficits in relational memory, a group of hippocampal amnesic participants showed markedly impaired relational behavior by rarely ever looking back-and-forth across recently studied objects, even though these objects were studied only seconds before. Furthermore, on the few occasions that relational behaviors did occur in amnesic participants, presumably by chance, they did not benefit subsequent recall as they did in healthy subjects. These findings highlight the crucial role of the hippocampus in guiding study behaviors that boost subsequent memory performance. Critically, hippocampal damage disrupts these behaviors on a timeframe well within the limits of "working memory." In light of these and other recent findings, we consider a view of the linkage between action, memory, and the hippocampus, by which the hippocampus interacts with other structures in the service of using rapidly formed relational representations to direct ongoing behavior.

Disclosures: J.L. Voss, None; **D.E. Warren**, None; **D. Tranel**, None; **N.J. Cohen**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.4

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01 MH076932

Title: The effects of prediction strength on associative novelty signals in human CA1 and medial temporal lobe cortex: A high-resolution fMRI study

Authors: *J. CHEN[1], A. D. WAGNER[2];
[1]Psychology, Stanford Univ., STANFORD, CA; [2]Psychology and Neurosciences Program, Stanford Univ., Stanford, CA

Abstract: Associative novelty occurs when sensory inputs do not match expectations generated from previous experience. The CA1 subfield of hippocampus is hypothesized to act as a comparator that detects mismatches between predictions (retrieved patterns) and reality (current sensory input), a form of prediction error that may signal opportunities for encoding novel events. Here, we investigated whether the magnitude of the putative CA1 prediction error signal is modulated by prediction strength. Specifically, high-resolution fMRI assessed hippocampal subfield responses to different levels of prediction error by manipulating the amount of previous experience prior to event novelty. Twenty-one participants were scanned at 3T while viewing sequences of objects and performing an incidental 1-back task. Each sequence consisted of four objects (a quartet) centrally presented for 1-s each, followed by a second quartet that was either an intact repetition (Intact1) or a rearranged version of the original sequence (Rearr1) in which the third and fourth objects were presented in reverse order. The Rearr1 condition was designed to trigger the retrieval of the initial pattern via repetition of the first two objects and elicit a prediction about the order of upcoming items, which was then violated. To manipulate prediction strength, all Intact1 double-quartets were presented again in the next experimental block, with half appearing in the same order (Intact2) and half in rearranged order (Rearr2). Thus, prediction strength was greater for Intact2 and Rearr2 trials (High Strength) than for Intact1 and Rearr1 trials (Low Strength). fMRI analyses of functionally defined, anatomically restricted ROIs revealed prediction error (mismatch) activity in CA1 ($p<.005$) across both Low and High Strength conditions, as well as an interaction between Intact/Rearr and Strength (pRearr) was larger for High than Low Strength trials. CA1 was the only region to display this interaction. Conversely, parahippocampal cortex simply displayed less activation during High than Low Strength trials ($p<.0001$), suggesting a repetition suppression effect that was insensitive to sequence rearrangement. Finally, perirhinal cortex evidenced both a repetition suppression effect of High<Low Strength ($p=.01$) and a mismatch enhancement across both Low and High Strength trials ($p<.01$), but no interaction. These results show that associative novelty signals can be observed in both CA1 and perirhinal cortex, and that in CA1 the signal is modulated by the

predictive strength of the input.

Disclosures: J. Chen, None; **A.D. Wagner**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.5

Topic: F.01. Human Cognition and Behavior

Support: MH083734

MH059352

NSF Graduate Research Fellowship

Title: Medial temporal lobe contributions to encoding novel cross modal associations: An examination of temporal discontinuity

Authors: *J. D. KOEN[1], A. P. YONELINAS[1], C. M. PARKS[2], M. ALY[1], C. RANGANATH[1]; [1]Dept. of Psychology, Univ. of California, Davis, Davis, CA; [2]Dept. of Psychology, Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: It has been well established that the hippocampus is critical in forming novel associations that can be later recollected, whereas regions in the surrounding medial temporal lobe cortex are involved in encoding item information that supports familiarity-based memory discriminations. However, recent evidence suggests that patients with focal hippocampal damage can exhibit relatively preserved associative recognition under conditions that promote unitization (i.e., encoding the constituents of an association as a single item), which suggests that familiarity may contribute to associative recognition. Evidence in favor of these unitization effects has come from studies of word pairs, and it is unknown if these effects generalize to nonverbal materials. To examine this issue, participants underwent fMRI scanning while encoding pairs of fractals and abstract sounds that were presented simultaneously or sequentially (e.g., temporally discontinuous). Associative recognition was then tested by presenting a mixture of intact and rearranged pairs and requiring subjects to make recognition judgments on a 6-point confidence scale. The ability of participants to unitize the fractal-sound pairs should be disrupted when the constituents of the pairs are presented in a temporally discontinuous manner. Thus, the ability of familiarity and the anterior medial temporal lobes to contribute to associative recognition should

be reduced under such conditions. As predicted, estimates of familiarity were significantly higher for pairs presented simultaneously than pairs presented sequentially. Moreover, preliminary analyses of the fMRI data reveal that activity in multiple MTL subregions was correlated with subsequent associative recognition. Further analyses will assess the extent to which activity in different subregions differentially supported associative recognition in the simultaneous and sequential conditions.

Disclosures: J.D. Koen, None; **A.P. Yonelinas**, None; **C.M. Parks**, None; **C. Ranganath**, None; **M. Aly**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.6

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant K99 MH083945

NIH Grant R01 MH083734

Title: Encoding item, context, and relational information: fMRI adaptation in the medial temporal lobes

Authors: *R. A. DIANA, A. YONELINAS, C. RANGANATH;
Univ. of California, Davis, DAVIS, CA

Abstract: The medial temporal lobes are critical for encoding of episodic long-term memories, however the specific functions of medial temporal lobe subregions remain unclear. The Binding of Item and Context (BIC) model of medial temporal lobe function proposes that the perirhinal cortex processes item information, the parahippocampal cortex processes context information, and the hippocampus processes the conjunction of item and context information in a relational binding. We used an fMRI adaptation paradigm to test the predictions of the BIC model during encoding of episodic memories. Adpatation studies rely on the general finding that repetition of information leads to reduced activation in brain areas that process the repeated information. Thus, repetition of item information (in this case, concrete nouns, e.g. "HAMMER"), context information (unique semantic encoding questions, e.g. "Could you balance this item on your nose?), and relational bindings of item and context information (the joint processing of a noun and an encoding question, e.g. "I can't balance a hammer on my nose.") should lead to reduced

activation in medial temporal lobe subregions that process each type of information. We found fMRI adaptation effects in perirhinal cortex, parahippocampal cortex, and the hippocampus with repeated encoding of item, context, and item-context bindings eliciting different amounts of adaptation in each medial temporal lobe subregion.

Disclosures: R.A. Diana, None; **A. Yonelinas**, None; **C. Ranganath**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.7

Topic: F.01. Human Cognition and Behavior

Support: NIMH Grant 074692

Dart Neuroscience

Title: Persistence of patterns of activity across hippocampal voxels during post-encoding awake rest

Authors: *A. TAMBINI[1], L. DAVACHI[1,2];
[1]Ctr. for Neural Sci., New York Univ., NEW YORK, NY; [2]Psychology, New York Univ., New York, NY

Abstract: Theories of systems memory consolidation posit that consolidation is accomplished by the reactivation of patterns of neural activity corresponding to recent experiences during off-line periods. Supporting these theories, rodent studies have demonstrated that patterns of activity across populations of hippocampal neurons that are representative of an experience are then re-expressed during subsequent off-line periods, such as sleep and awake rest (e.g., Lee & Wilson, 2002; Foster & Wilson, 2006). In humans, the amount of hippocampal activity during sleep has been shown to be correlated with future memory for pre-sleep experiences (Peigneux et al., 2004). Here we asked whether patterns of activity across individual voxels in the human hippocampus that are characteristic of a recent encoding experience persist during rest after encoding (Post-Encoding rest). To this end, we scanned subjects using fMRI during performance of an object-face (OF) and scene-face (SF) encoding task and during pre- and post- task rest scans (Baseline rest, Post-OF rest and Post-SF rest). Hippocampal voxels in each subject were first categorized as being ‘active’ (showing a significant increase in percent signal change from baseline across trials), ‘non-active’ (showing no significant percent signal change from baseline

across trials), ‘de-active’ (showing a significant decrease in percent signal change from baseline across trials), or ‘other’ (those that could not be unambiguously categorized) during both OF and SF encoding, separately. We restricted our analysis of Post-Encoding rest to subjects who showed significantly more ‘active’ voxels than would be expected by chance and found that the mean level of correlation between active voxel pairs (VPs), but not between non-active or de-active VPs, was significantly higher during Post-OF vs. Baseline rest. Second, we found that the correlation between individual active VPs during OF task performance was linearly related to their correlation during Post-OF rest. Critically, VP correlations during OF task performance were significantly more correlated during Post-OF rest compared to Baseline rest. These results provide the first evidence that patterns of activity across the human hippocampus, as reflected in pairwise correlations between individual hippocampal voxels, can persist from encoding to a subsequent off-line rest period.

Disclosures: A. Tambini, None; **L. Davachi**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.8

Topic: F.01. Human Cognition and Behavior

Support: Army Research Office W911NF-09-1-0163

NARSAD UTA09-000453

Title: Multivoxel pattern analysis of cross-episode binding: Reactivation of prior episodic experience during learning supports flexible memory

Authors: *D. ZEITHAMOVA, A. R. PRESTON;
Univ. Texas, Austin, AUSTIN, TX

Abstract: A powerful aspect of episodic memory is the ability to flexibly apply and recombine information from past experience to guide new behavior. One form of mnemonic flexibility is associative inference that requires binding of information across distinct learning episodes to infer new relationships. One process supporting cross-episodic binding—integrative encoding—postulates that overlapping information shared across events may elicit recall of prior experience, enabling integration of new experience into existing representations during learning. However, evidence for such reactivation of prior events during encoding remains speculative. Here, we

used functional MRI and multivoxel pattern analysis (MVPA) to test whether reactivation of content specific representations occurs during encoding of overlapping events in service of generalization. During an associative inference paradigm, object (O) and scene (S) stimuli were organized into triads containing a single stimulus type (3 objects: OOO, 3 scenes: SSS) or cross-domain content (OOS, SSO). During functional scanning, participants encoded overlapping pairs from each triad (e.g., pretzel-frog, frog-beach) across multiple interleaved repetitions and were subsequently tested on inferential relationships between items (e.g., pretzel-beach) after scanning. Using a localizer task, MVPA identified content-specific brain regions involved in scene and object encoding. Comparing encoding activation for pairs of the same content (e.g., SS) where the content of the unseen triad member differed (scenes in SSs vs. objects in SSo), we observed reactivation of content-specific networks reflecting unseen content (e.g., object-network activation during SSo trials) that increased across repetition. Univariate analyses further demonstrated content-general activation in posterior hippocampus whose increase across repetitions of overlapping associations predicted subsequent performance across participants. Together, these results provide direct evidence that reactivation of prior experience during learning enables binding of information across distinct episodes to support the flexible use of memory beyond individually experienced events.

Disclosures: D. Zeithamova, None; **A.R. Preston**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.9

Topic: F.01. Human Cognition and Behavior

Support: NIH grant MH080833

Title: The effects of emotion and encoding strategy on associative memory

Authors: B. D. MURRAY[1], *E. A. KENSINGER[2];
[1]Dept Psychol, Boston Col., Chestnut hill, MA; [2]Dept Psychol, Boston Col., CHESTNUT HILL, MA

Abstract: The ability to bind information together in a meaningful way is a critical aspect of human memory. Imaging studies have shown that memory formation for single items is supported by activation in the rhinal cortices, while activation in the hippocampus supports memory for between-item associations. The present study investigated, using functional MRI,

what neural processes were recruited when participants were instructed to visualize paired associates separately from one another, versus when participants were asked to concatenate two items into a single, holistic representation.
This latter type of associative strategy directs participants to visualize the two items as one single item. For example, given the paired associate "CARD + MOUSE", participants had to either picture a card and a mouse separate from one another, or make a combined mental image of the two (e.g., a mouse playing cards, or a card with a picture of a mouse on it). Some word pairs included an emotional referent ("BOMB + CARTON") while others included neutral referents. Participants visualized pairs while undergoing a functional MRI, and were then given a surprise recognition test (also while in the MRI scanner). At test, participants viewed pairs of words and had to judge them as intact, recombined, or new. During the encoding of pairs that were subsequently correctly recognized as intact, participants recruited dissociable medial temporal lobe processes depending on whether the items were visualized separately, or as a single unit. Visualizing items separately from one another was supported by encoding activity in the left perirhinal and entorhinal cortices, while visualizing the items as a holistic representation was supported by encoding activity in bilateral hippocampus. The emotionality of the referents did not affect the engagement of medial temporal-lobe processes during encoding. These results demonstrate that when participants are directed to envision two novel items as a single object, that associative binding at encoding is mediated by the hippocampus, regardless of the emotionality of the integrated items.

Disclosures: B.D. Murray, None; **E.A. Kensinger**, None.

Nanosymposium

328. Relational Memory

Location: Room 7B

Time: Monday, November 15, 2010, 8:00 am - 10:30 am

Program Number: 328.10

Topic: F.01. Human Cognition and Behavior

Title: Assessing the multidimensional features of memory uncovers selective impairment for relational information in patients with hippocampal damage

Authors: *P. D. WATSON[1], J. L. VOSS[2], D. E. WARREN[3], D. T. TRANEL[3], N. J. COHEN[2]; [1]URBANA, IL; [2]Univ. of Illinois at Urbana-Champaign, URBANA, IL; [3]Univ. of Iowa, Iowa City, IA

Abstract: Although memory representations of experience can be richly multidimensional, memory tests typically sample those dimensions sparsely. Here we attempted to exhaustively

characterize the memory representations formed during a simple object-location paradigm in four neurologically intact participants and in four individuals with hippocampal amnesia. Participants studied sets of 2-5 tangible objects located on a flat surface. Then, after an unfilled 4s delay during which the objects were removed from the surface, memory was tested by requiring participants to reposition the objects back in their original locations. Several different performance metrics characterized the remaining memory representations. The first metric concerned the location of the overall "shape" formed by considering the objects as vertices (e.g., 3 objects create a triangle with specific internal angles and location). Patients showed no significant impairment on this measure irrespective of the number of objects presented. The next measure was the typical measure of distance error in the spatial location of each object. On this measure, both patients and controls showed a graded decrease performance as the set size increased from 2 to 5 objects, but patients showed an overall deficit. More revealing of the specific impairment in hippocampal amnesia was a performance metric focused on the arbitrary relations among objects; that is, the extent to which two or more objects swapped positions relative to their locations at study. Patients showed severe impairment on this metric of relational memory, with a high prevalence of "swapping" errors as the set size increased, whereas comparison participants rarely committed such errors regardless of set size. Even in this simple paradigm with relatively modest memory demands, assessing the richly multidimensional information represented in memory revealed the disproportionate deficit in hippocamal amnesia - and hence the critical role of hippocampus in normal memory - for arbitrary relations among items.

Disclosures: P.D. Watson, None; **J.L. Voss**, None; **D.E. Warren**, None; **D.T. Tranel**, None; **N.J. Cohen**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.1

Topic: F.03. Motivation and Emotion

Support: NIH 085082

Title: A central amygdala neural circuitry controls fear behavior

Authors: *H. CAI, W. HAUBENSAK, P. KUNWAR, D. J. ANDERSON;
Biol., Caltech, PASADENA, CA

Abstract: The central amygdala plays a key role in Pavolovian fear conditioning, however, its cellular complexity makes it difficult to understand in detail how the underlying neural circuits regulate fear responses. Our lab has identified a genetic-marker-labeled subpopulation of CEl (lateral part of central amygdala) neurons, PKC-delta neurons, which inhibit learned fear responses. In order to understand the circuit-level mechanism of how these neurons participate in fear conditioning, we combined optogenetics and electrophysiology to dissect the neuronal circuits that these neurons are involved in. We have mapped both local and long-range (cortical) functional inputs to these neurons, as well as outputs from the neurons. We find that the CEl neurons make inhibitory synapses onto projection neurons in CEm (medial part of central amygdala), which can be back-labeled from the PAG (periaqueductal gray). Our data suggest that these neurons participate in a recurrent inhibitory network that gates CEm output. The structure of the neural circuits characterized here will help us to understand better how the amygdala regulates fear and other emotions.

Disclosures: H. cai, None; **W. Haubensak**, None; **P. Kunwar**, None; **D.J. Anderson**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.2

Topic: F.03. Motivation and Emotion

Support: KKF

DPG

Title: Can we share pain we always feel ? Neural correlates of empathy and pain in patients with pain-predominant multisomatoform disorder

Authors: *M. NOLL-HUSSONG[1], A. OTTI[2], L. LÄER[2], A. WOHLSCHLAEGER[2], C. ZIMMER[2], J. DECETY[3], P. HENNINGSEN[1], R. D. LANE[4], H. GÜNDEL[5];
[1]Klinik fuer Psychosomatische Medizin des Klinikums rechts der Isar, Muenchen, Germany; [2]Abteilung fuer Neuroradiologie des Klinikums rechts der Isar, Technische Univ. Muenchen, Muenchen, Germany; [3]Univ. of Chicago, Chicago, IL; [4]Dept. of Psychiatry, Psychology and Neurosci., Univ. of Arizona, Tuscon, AZ; [5]Klinik fuer Psychosomatische Medizin, Univ. Ulm, Ulm, Germany

Abstract:

Neural responses to painful events can be divided into a sensory-discriminative ("painful") and affective-motivational ("grievous") dimension, and foremost the latter seems to be disturbed in patients with chronic pain disorder. In this naturalistic fMRI-study we compared 19 healthy controls with 21 patients suffering from pain-predominant multisomatoform disorder using an empathetic pain paradigm showing human limbs in graded painful and non-painful situations. Controls attributed by trend significant higher pain-intensity to all (low, medium and high) 'pain' pictures than patients ($p < 0.057$). Significant higher levels were observed for the 'high pain' and 'high and medium pain' condition. To demonstrate the stability of the regions claimed to be part of a network for pain-perception ('pain matrix') , whole group analysis including all 40 participants was performed. In the contrast 'high and medium pain vs. no pain' core regions of the pain-matrix like insula and somatosensory cortex were activated in the whole group. In the control group, 'high & medium pain' pictures elicited activations of left somatosensory cortex, left insula and left dorsal ACC. In contrast to the control group, patients just showed non-significant activations of aforementioned regions in the contrast 'High & Medium Pain vs. No Pain'. Compared to the patients, the control-group showed higher activation of the left perigenual ACC (pACC) in the contrast 'High & Medium Pain vs. No pain'. Moreover, a significant higher activation was found in the left dorsal ACC (BA 24) by ROI-analysis in the control group. Even using the results of the whole-group-analysis as a mask in terms of functional regions-of-interests and providing extremely lenient a priori thresholds did not reveal any other differences in the pain matrix between patients and controls. With increasing a priori pain-intensity (no, low, medium, high), patients showed higher activation in the right SMA, left and right medial prefrontal cortex and right and left somatosensory cortex than controls. Our results suggest altered computational processes in neuronal pain perception in chronic pain patients, especially concerning the empathetic route, and may lead to a deeper understanding of the underlying emotional deficits in patients suffering from pain-predominant multisomatoform disorder.

Disclosures: M. Noll-Hussong, None; **A. Otti**, None; **L. Läer**, None; **A. Wohlschlaeger**, None; **R.D. Lane**, None; **J. Decety**, None; **C. Zimmer**, None; **P. Henningsen**, None; **H. Gündel**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.3

Topic: F.03. Motivation and Emotion

Support: NIH Grant RO1DK079194

NIH Grant DK60685-S2

NIH Grant DK7919481

NIH Grant DK079194-S1

NIH Grant R00163

The Obesity Society New Investigator Grant

Title: Maternal high-fat diet consumption suppresses serotonergic system signaling in juvenile nonhuman primate offspring resulting in increased anxiety and anti-social behavior

Authors: *E. L. SULLIVAN, K. COLEMAN, K. L. GROVE;
Neurosci., Oregon Hlth. and Sci. Univ., Beaverton, OR

Abstract: Maternal nutrition and energy status have long term effects on offspring behavior. Given the prevalence of obesity in developed nations and the comorbidity of metal health disorders and obesity, it is critical to examine the consequences of maternal over nutrition and obesity on offspring behavior and the central pathways that regulate behavior. We previously demonstrated that maternal high-fat diet (HFD) consumption altered the development of the central serotonin system in fetal offspring and increased anxiety-like behavior in female infant offspring. This study used a non-human primate model of diet-induced obesity to examine the long-term consequences of maternal obesity and HFD consumption on the central serotonin system, anxiety like behavior and social behavior of juvenile offspring. Offspring from female Japanese macaques consuming either a low fat control diet (13% of calories from fat) or a HFD (35% calories from fat) were examined. The Human Intruder test and novel object tests were used to assess stress and anxiety responses to a social threat or novel item. These tests were adapted from tests commonly used in children and reliably assess individual differences in primate stress response and anxiety. Behavior in social housing was assessed by frame by frame analysis of videography. In situ hybridization was used to assess the central serotonin system in the dorsal raphe and cerebrospinal fluid serotonin and plasma cortisol and adrenocorticotropic hormone (ACTH) levels were examined using radioimmunoassays. At 11 months of age, offspring from mothers fed a HFD exhibited increased latency to explore novel objects indicating increased anxiety. Moreover, in social housing, offspring from HFD mothers spent more time alone and less time in contact with or engaged in play with peers. At 13 months of age, the serotonin system of HFD offspring was suppressed as indicated by a reduction in tryptophan hydroxylase 2 (THP2; the rate limiting enzyme in serotonin synthesis) in the dorsal raphe and a reduction in cerebrospinal fluid serotonin. Plasma ACTH and cortisol response to stress was similar in HFD and control offspring. This study indicates that maternal HFD consumption causes suppression of the central serotonin system resulting in heightened anxiety and anti-social behavior in juvenile nonhuman primates. As the majority of pregnant women are overweight and consume a HFD, this study has important implications for the metal health status of future generations. Future studies will examine whether transitioning the obese mothers to a

healthy control diet during gestation will reduce the risk of offspring developing anxiety and anti-social behavior.

Disclosures: E.L. Sullivan, None; **K. Coleman**, None; **K.L. Grove**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.4

Topic: F.03. Motivation and Emotion

Support: NIH Grant R01-MH/HD57502

Base Grant to the CNPRC RR00169

Title: Neonatal lesions to the rhesus macaque amygdala result in blunted adult affect

Authors: *E. BLISS-MOREAU[1,2], J. E. TOSCANO[1,2], M. D. BAUMAN[1,2,3], D. G. AMARAL[1,2,3,4];
[1]Psychiatry and Behavioral Sci., Univ. California, Davis, DAVIS, CA; [2]California Natl. Primate Res. Ctr., Davis, CA; [3]The M.I.N.D. Inst., Sacramento, CA; [4]Ctr. for Neurosci., Davis, CA

Abstract: It is widely recognized that the amygdala plays an important role in social and affective processing. Yet, despite extensive research on the amygdala in both humans and non-human animals, few studies have investigated whether the amygdala is critical for developing a normal social and affective behavioral repertoire. To address this question, we have followed the neurological, social and emotional development of a cohort of monkeys that sustained damage to the amygdala at two weeks of age. In the present study, we documented affective behaviors generated by these amygdala-lesioned animals and compared their behavior to that of animals that received hippocampus-lesions or sham operations at two weeks of age. All animals were shown a series of 30-second videos that varied in social content and affective properties. Videos varied across three levels of social content: 1) “non-social” videos included no images of monkeys; 2) “social non-engaging” videos included images of monkeys that were engaged in interactions with each other or by themselves; 3) “social engaging” videos included monkeys engaged in clear expressive behavior directed toward the camera. All videos also varied across three categories of affect: 1) “positive”, 2) “negative”, and 3) “neutral”. Behavior was recorded using a robust behavioral ethogram. Behaviors directed towards the videos (i.e., facial expressions, vocalizations, or other whole-body social signals) were aggregated by video

category. Monkeys in all three lesion conditions differentiated between levels of social content insofar as they were more expressive towards social-engaging videos and least expressive towards non-social videos, across all categories of affective content. Overall, amygdala-lesioned animals were significantly less expressive than control animals. Taken together, these results suggest that neonatal amygdala-lesions result in blunted affective responding even in the presence of provocative social stimuli. The relationship between the amygdala-lesioned animals' blunted affective responding in this task and deficits during naturalistic social interactions will be discussed.

Disclosures: E. Bliss-Moreau, None; **J.E. Toscano**, None; **M.D. Bauman**, None; **D.G. Amaral**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.5

Topic: F.03. Motivation and Emotion

Support: FWO G.0746.09

EF/PF

IUAP

GOA

GSKE

Title: Neural processing of dynamic facial expressions in humans and monkeys: A comparative fMRI study

Authors: *Q. ZHU[1], K. NELISSEN[2], J. VAN DEN STOCK[3], B. DE GELDER[4], H. KOLSTER[1], W. VANDUFFEL[1], M. VANDENBULCKE[3];
[1]Lab. For Neuro- and Psychofysiology, K.U. Leuven, Leuven, Belgium; [2]Dept. of Radiology, MGH, Charlestown, MA; [3]Div. of Psychiatry, K.U.Leuven, Leuven, Belgium; [4]Lab. - Dept. of psychonomics, Tilburg Univ., Tilburg, Netherlands

Abstract: The recognition of facial expressions by conspecifics is fundamental for social

interactions. We hypothesize that a largely common neural system exists for processing faces of humans and monkeys in both species, but that the observation of conspecific-specific emotional expressions would activate mainly higher-order brain areas, e.g. those regions linking facial expressions to their social meaning. We also hypothesize that emotional face processing in humans, but not monkeys, would be lateralized to the right hemisphere in human areas contralateral to language areas.
Two types of dynamic human and monkey facial expressions (fear and chewing) were shown to 23 humans and 3 monkeys in an event-related fMRI design (2s long movie clips, ISI = 4.5-5.5 s, TR = 2 s, 1.25 mm isotropic resolution in monkeys, 2.75 x 2.75 x 3.5 mm in humans). Spatially and temporally scrambled versions of the faces (mosaic-scrambled), with the same motion vectors as in the original movies, were used to control for low-level differences in the dynamic stimuli. A fixation-only condition was also included as ninth stimulus condition within the same run.
Compared to scrambled movies of the faces, monkey and human faces activated the face sensitive regions in temporal and prefrontal cortex, in addition to regions in parietal cortex and the amygdala in both species. The monkey faces evoked larger activations than human faces in all these regions in monkeys, but also in the temporal and parietal human regions. Both the human and monkey faces produced stronger activations in the right compared to the left STS and inferior frontal gyrus in humans. No strong emotion-specific (fear versus chewing) lateralization, however, was observed in the two species. A significant conspecific-specific effect in processing facial expressions (fear vs chewing) was observed in parts of the STS and prefrontal cortex of both species. The amygdala in humans produced a stronger response for human-specific fear faces compared to chewing faces.
Thus, a common neural system for processing faces of monkeys and humans exists in both species. In humans as well as in monkeys, only parts of the STS and PFC are differentially activated by emotional expressions of conspecifics. In humans, facial stimuli activate right STS and inferior frontal gyrus more than their left-sided homotopes.

Disclosures: Q. Zhu, None; **J. Van den Stock**, None; **K. Nelissen**, None; **B. de Gelder**, None; **W. Vanduffel**, None; **M. Vandenbulcke**, None; **H. Kolster**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.6

Topic: F.03. Motivation and Emotion

Title: Anxiety impairs decision-making in rats through differential recruitment of cortico-limbic

circuits

Authors: *L. DE VISSER[1], J. M. BAARS[1], M. LAVRIJSEN[1], C. M. M. VAN DER WEERD[2], L. J. VAN DER KNAAP[2], R. VAN DEN BOS[1];
[1]Neurobio. of Behavior, [2]Utrecht Univ., Utrecht, Netherlands

Abstract: High levels of anxiety have been shown to negatively affect performance in the Iowa Gambling Task (IGT), which measures decision-making performance under conditions of uncertainty. To understand this in more detail, we investigated the relationship between anxiety and decision-making, as well as their neural underpinnings in male rats. In experiment 1, male Wistar rats were screened on a test for anxiety, the elevated plus maze (EPM), before they were subjected to a rat analogue of the Iowa Gambling Task (r-IGT). In this task, male rats gradually increase their choices for the long-term advantageous option as the task progresses. Neural substrates related to performance in the r-IGT were identified by c-fos immunohistochemistry. We observed that high levels of anxiety on the EPM were associated with worse r-IGT performance. Thus, high anxious rats showed a lower number of choices for the long-term advantageous option and collected less rewards than low anxious rats. This was accompanied by differential activation of medial prefrontal areas, i.e. the prelimbic and infralimbic cortex, and of striatal areas, i.e. the caudate putamen as well as the nucleus accumbens shell and core. In experiment 2, inactivation of the prelimbic region of the medial prefrontal cortex (mPFC) using the GABA-receptor agonists muscimol and baclofen resulted in increased anxiety on the EPM. The effects of prelimbic inactivation on r-IGT performance depended upon baseline performance. Rats which showed a low IGT-performance were not affected and showed a progressive increase in IGT-performance after inactivation, whereas rats with a high r-IGT performance did not show any further improvement after inactivation. Collectively, these data show that the effects of anxiety on decision-making in rats may be mediated through a cortico-limbic circuit involving the prelimbic cortex. Moreover, they suggest that this area becomes more strongly involved when rats have learned the contingencies of task. Thus, a lower prelimbic activity may lead to higher levels of anxiety and a lower IGT-performance in male rats.

Disclosures: L. De Visser, None; **J.M. Baars**, None; **M. Lavrijsen**, None; **C.M.M. van der Weerd**, None; **L.J. van der Knaap**, None; **R. van den Bos**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.7

Topic: F.03. Motivation and Emotion

Support: ARC-linkage grant (LP0455104)

Title: Integrative neuroscience: Genes, environment and the emotional brain

Authors: *L. M. WILLIAMS[1,2], S. H. KOSLOW[3], J. M. GATT[1], P. R. SCHOFIELD[4], E. GORDON[5];
[1]Univ. of Sydney Med. Sch., Sydney, Australia; [2]BRAINnet Fndn., San Francisco, CA; [3]BRAINnet Fndn., New York, NY; [4]Prince of Wales Med. Res. Inst., Sydney, Australia; [5]Brain Resource, San Francisco and Sydney, CA

Abstract: BACKGROUND: BRAINnet is a global network of scientists, governed by a foundation, providing access to large multi-modal gene-brain and cognitive behavioral datasets acquired with standardized methods (Fig 1; www.brainnet.net). We present data linking genetic variants and emotional brain function.
METHODS: Data are from 1,000 volunteers screened for depression-anxiety and early life trauma. Functional MRI and autonomic activity were recorded during a facial emotion task. We focused on 5HTT-LPR Short and COMT Met alleles.
RESULTS: Both 5-HTT-LPR Short and COMT Met carriers had heightened activation to fear, in medial prefrontal-anterior cingulate, amygdala and brainstem, and a corresponding increase in heart rate. An opposing pattern was observed for happy (Fig 2). These profiles were exacerbated by early trauma. They correlated with traits of negativity bias implicated in emotional disorder (2,3).
DISCUSSION: Gene-stress interactions modulate neural substrates of negative versus positive emotion, and susceptibility to disorder. Other gene-brain relationships and clinical applications are being examined with BRAINnet data (incl. depression, anxiety, psychoses, ADHD groups).
1. Koslow SH, Williams LM, Gordon E (2010). Mol Psychiatry, 15 (3): 229-30.
2. Williams LM, Gatt JM, Schofield PR et al (2009). NeuroImage 47 (3): 804-14
3. Williams LM, Gatt JM, Grieve SM et al (2010). COMT Val(108/158) Met polymorphism effects on emotional brain function and negativity bias. NeuroImage (in press)

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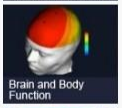

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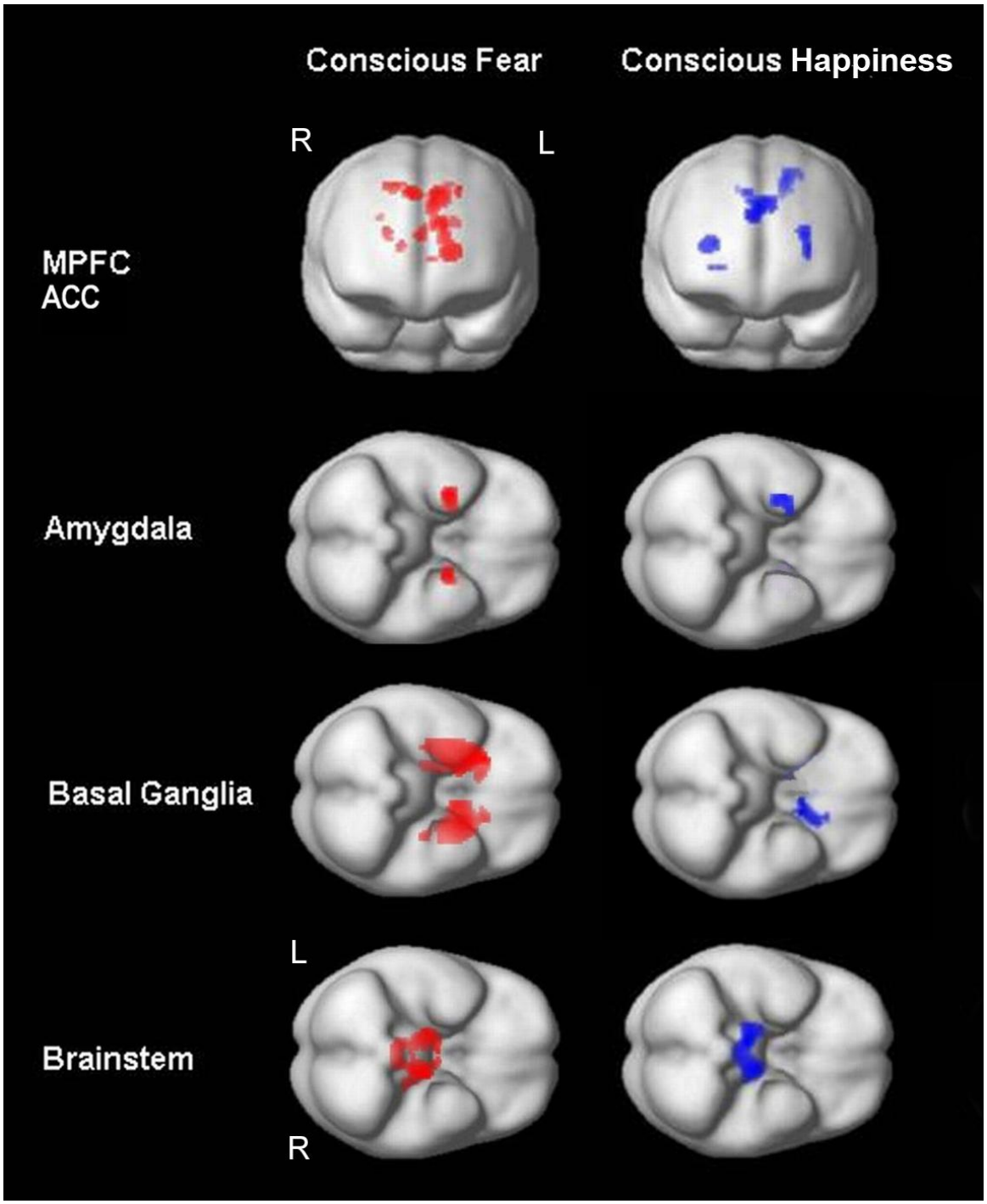

Disclosures: **L.M. Williams:** Research Grant; Australian Research Council, National Health & Medical Research Council. Consultant/Advisory Board; Consultant, Brain Resource. Other; Stockholder, Brain Resource. **S.H. Koslow:** Consultant/Advisory Board; Consultant, Brain Resource. **J.M. Gatt:** Other; Fees, Brain Resource. **P.R. Schofield:** Stock options, Brain Resource. **E. Gordon:** Employment; CEO and Chairman, Brain Resource.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.8

Topic: F.03. Motivation and Emotion

Support: Medical Research Council

Title: Insula and striatum mediate the default bias

Authors: *R. YU[1], D. MOBBS[2], B. SEYMOUR[3], A. J. CALDER[2];
[1]Cambridge, United Kingdom; [2]MRC Cognition and Brain Sci. Unit, Cambridge, United Kingdom; [3]Univ. Col. London, London, United Kingdom

Abstract: Introduction: Whether it be selecting weekly lottery numbers or renewal of an insurance policy, we are repeatedly faced with situations in which we are required to accept the default option, or switch to an alternative. Yet why this occurs is unclear. One explanation is that 'loss aversion' impels the default preference. This predicts that switching is associated with brain areas such as the insula, which are involved in anticipation and receipt of aversive stimuli. Here, we sought to elucidate the neural mechanisms mediating the default bias.
Methods: Eighteen volunteers played a gambling task while undergoing functional MRI scanning. On each trial, they were presented with two cards, one was associated with a win, the other with a loss. During the Decision phase, one card was assigned to the participant and they had the option to either stay with this default card or switch to the other. A following Outcome phase indicated the value of the cards.
Results: Participants' revealed a preference for the default card (57.2% stay vs. 42.8% switch, $P<0.001$). Reaction times for switch and stay were comparable ($t17<1$), suggesting that the difference in mental effort was negligible. Subjective emotion ratings showed that, participants felt stronger frustration for losses after switch (Lswitch) than after stay (Lstay), and expressed more satisfaction for wins after switch (Wswitch) than after stay (Wstay).
For the brain-imaging analysis of decision phase data, stay versus switch decisions elicited ventral striatal activity, whereas the reverse comparison activated the right anterior insula. Furthermore, interpersonal differences in the tendency to switch were associated with decreased activation in an overlapping area of insula cortex together with an increased activation in the dorsal striatum. Our data suggest that the insula underlies the aversive anticipatory affect that may mediate the preference to stay with the default, whereas the dorsal striatum drives the decision to switch.
Analysis of the outcome phase data further emphasized the role of the insula in aversive processes by showing that a comparison of Lswitch with Lstay positively correlated with the

difference in frustration experienced in these conditions in the right anterior insula. Similarly, Wswitch minus Wstay positively correlated with the differential satisfaction in ventral striatum. Thus, switching from the default augments the emotional responses to outcomes in valence specific areas.
Conclusions: Our study highlights the key role of emotion in mediating default bias, casting insights into the power of default preference in both policy-making as well as more routine everyday behaviors.

Disclosures: R. Yu: None. **D. Mobbs:** None. **B. Seymour:** None. **A.J. Calder:** None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.9

Topic: F.03. Motivation and Emotion

Support: NIH Grant MH076137

NIH Grant MH076136

NSF Grant 0631637

Title: Different pathways in medial prefrontal cortex predict effective spontaneous emotion regulation

Authors: *J. SILVERS[1], T. D. WAGER[1,2], J. WEBER[1], K. N. OCHSNER[1];
[1]Psychology, Columbia Univ., New York, NY; [2]Psychology and Neurosci., Univ. of Colorado, Boulder, CO

Abstract: In the past decade there has been a surge in research examining the functional neural underpinnings of emotion regulation. Prior work suggests that lateral, dorsal and medial prefrontal areas are more strongly recruited when individuals are instructed to use cognitive strategies to regulate responses to affective stimuli than when they respond naturally to them. However, little attention has been paid to the neural mechanisms that support spontaneous or uninstructed emotion regulation. To investigate this issue, we used an event-related fMRI paradigm in which 30 healthy adults were instructed to respond naturally to both negative and neutral photographs. After viewing each picture, participants rated their current level of affect. In contrast to the neutral condition, viewing negative pictures elicited more negative affect and

greater activation of the anterior insula, anterior temporal lobe amygdala and periaqueductal gray (PAG). A portion of anterior ventromedial PFC (aVMPFC), which other studies have shown is activated during extinction and processing of positive stimuli and is deactivated by threatening stimuli, showed deactivation in the [negative > neutral] contrast. Correlation analyses identified voxels that showed a significant relationship between [negative>neutral] contrast values and changes in negative affect (i.e., negative-neutral affect rating difference). A conjunction analysis of the correlation and contrast maps revealed that successful spontaneous regulation (smaller increases in negative affect) was associated with greater or comparable aVMPFC activity when viewing aversive stimuli, in comparison to neutral stimuli. Mediation analyses were then performed to identify brain regions that modulated the relationship between activity in aVMPFC and changes in negative affect. These analyses revealed that the relationship between aVMPFC and negative affect was positively mediated by two additional regions of MPFC, one just dorsal to the original aVMPFC ROI, and another in left dorsal MPFC. In contrast, a third and more ventral MPFC area was observed to *negatively* mediate the relationship between the aVMPFC ROI and negative affect. Taken together, these results suggest two things. First, that aVMPFC may support spontaneous emotion regulation or, at least, reduced emotional reactivity to aversive stimuli. Second, that dorsal and ventral portions of MPFC may differentially modulate the strength of the regulatory effect aVMPFC exerts on emotional processing.

Disclosures: J. Silvers, None; **K.N. Ochsner**, None; **J. Weber**, None; **T.D. Wager**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.10

Topic: F.03. Motivation and Emotion

Support: Intramural research

Title: Differential neural and behavioral coupling of fear and happiness

Authors: *M. JABBI[1], T. NASH[2], P. KOHN[2], A. IANNI[3], T. HOLROYD[4], F. CARVER[4], Q. CHEN[5], J. S. KIPPENHAN[6], R. COPPOLA[7], K. F. BERMAN[3];
[1]Natl. Inst. of Mental Health, NIH, BETHESDA, MD; [2]Section on Integrative NeuroImaging, CBDB, [3]Section on Integrative NeuroImaging, [4]MEG core facility, [5]Genes Cognition & Psychosis Program, CBDB, [7]Genes Cognition & Psychosis Program, CBDB, MEG Core Facility, [6]NIMH, Bethesda, MD

Abstract: BACKGROUND
Understanding emotional facial expressions is essential for adequate social functioning. Here, we examined the neural correlates of perception (recognition) of happiness and fear. We tested the hypothesis that the degree to which emotions are recognized will differentially predict BOLD and MEG responses in limbic and frontal regions that vary with dynamics and valence of facial stimuli.
METHODS & RESULTS
Healthy individuals (N=40, 17 females; mean age=30.44) underwent fMRI while exposed to a set of shorter (1s) and longer (3s), dynamic and static emotional (fear/happy) and neutral expressions while being scanned (3T; TR = 2.210s) and rated each facial stimulus for degree of recognized fear/happiness post scanning. Twelve of these individuals were retested with the same paradigm during magnetoencephalography (MEG, a more temporally sensitive measure of neural response).
A random effects analysis in SPM5 after preprocessing (8mm smoothing) and first-level analysis of BOLD response to emotional vs neutral expressions, showed bilateral STS response to dynamic vs static stimuli at $p<0.05$ FDR corrected. Limbic regions (amygdala, insula, ACC and striatum) responded more to 1s than 3s stimuli.
Recognition ratings varied with stimulus dynamics and duration. Static and longer stimuli were better recognized, respectively ($p=0.019$, $F[1,31]=6.14$ and $p=0.005$, $F[1,31]=9.31$). Fear ratings predicted BOLD response in STS, amygdala, and anterior insula/frontal operculum ($p<0.05$ FDR corrected), whilst happiness ratings were coupled with BOLD response in right frontal operculum and OFC ($p<0.005$ uncorrected).
MEG results, analyzed with AFNI, were consistent with BOLD signals in STS and limbic regions which varied with stimulus dynamics and duration respectively ($p<0.05$ FDR corrected) in the gamma frequency band. Gamma frequency band measures are known to reflect higher order cognition and may reflect a robust neurophysiological underpinning of emotional perception in these regions.
CONCLUSION
Here, people's ability to recognize other individuals' emotions predicted their BOLD responses in a valence-specific manner. The activation of salience circuitry, including OFC activations during dynamic emotions may indicate a role for these regions in attributing emotional meaning to arousal and interoceptive responses to emotional facial expressions. These multimodal findings identify a behaviorally relevant OFC/limbic pathway that modulates the way social emotions are understood.

Disclosures: M. Jabbi, None; **P. Kohn**, None; **T. Nash**, None; **J.S. Kippenhan**, None; **K.F. Berman**, None; **A. Ianni**, None; **Q. Chen**, None; **R. Coppola**, None; **T. Holroyd**, None; **F. Carver**, None.

Nanosymposium

329. Neural Bases of Negative Emotional States

Location: Room 2

Time: Monday, November 15, 2010, 8:00 am - 10:45 am

Program Number: 329.11

Topic: F.03. Motivation and Emotion

Support: Hope for Depression Research Foundation

Title: Kappa opioid receptor agonism reduces activity but increases sucrose consumption: A mouse model of the acute role of dynorphin in depression

Authors: *M. R. ZELLNER[1], J. PANKSEPP[3], C. FONTAINE[2], D. W. PFAFF[2];
[1]Rockefeller Univ., New York, NY; [2]Lab. of Neurobio. and Behavior, Rockefeller Univ., NEW YORK, NY; [3]Dept. of VCAPP, Col. of Vet. Med., Washington State Univ., Pullman, WA

Abstract: A prominent feature of depression is loss of motivation, which is associated with anhedonia. Psychoanalytic models of depression attribute impaired motivation to inhibition of drive energy in relation to an ambivalently-cathected object. According to this model, then, separation distress plays a fundamental role in depression. This resonates with a large body of literature linking early stress to HPA axis dysregulation and depression vulnerability in adulthood. Given that kappa opioid receptor (KOR) agonists downregulate activity in the mesolimbic dopamine system, and that dynorphins are released in response to stress, it is important to understand the possible role of the KOR system in depression. Therefore we prepared Swiss-Webster mice with indwelling brain cannulae and delivered microinjections of U-69593, a selective KOR agonist, into the lateral ventricle (130 nmol), the nucleus gigantocellularis and ventral tegmental area (65 nmol each). Mice were then placed into activity chambers and given the opportunity to consume sucrose (10% solution), water, or quinine (60mM). Compared to test sessions with vehicle treatment, U-69593 reduced activity in all groups. Conversely, mice consumed equal or greater amounts of sucrose solution, while consumption of water or quinine was not affected. Our findings suggest that acute KOR agonism may be involved in the suppression of behavioral arousal, while preserving the ability to experience positive sensory affect. This fits in with the model (Watt & Panksepp 2009) that separation distress triggers a SEEKING shut-down mechanism that later becomes a building block of depression; it is possible that dynorphin release triggered by the acute stress of separation serves to downregulate overall activity as a means of preserving a connection with an attachment object, while maintaining the organism's ability to experience gratification upon reunion. Anhedonia may only emerge later after chronic stimulation of these systems.

Disclosures: M.R. Zellner, None; **J. Panksepp**, None; **C. Fontaine**, None; **D.W. Pfaff**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.1

Topic: A.03. Stem Cells

Support: National Science Foundation Graduate Research Fellowship (D.A.L.)

Ruth Kirschstein NRSA Predoctoral Fellowship (D.A.L.)

March of Dimes Research Grant (S.B.)

Klingenstein Foundation Grant (S.B.)

W.M. Keck Foundation Grant (S.B.)

NARSAD Research Grant (S.B.)

Title: The hypothalamic proliferative zone: The identification of a novel neurogenic niche in the postnatal mammalian hypothalamus

Authors: *D. A. LEE[1], T. PAK[1], V. CHARUBHUMI[1], A. MIRANDA-ANGULO[1], H. WANG[1], F. BALORDI[2], G. FISHELL[2], S. BLACKSHAW[1];
[1]Neurosci., Johns Hopkins Sch. of Med., BALTIMORE, MD; [2]Cell Biol., New York Univ. Sch. of Med., New York, NY

Abstract: While new neurons continue to be born postnatally in the constitutively active germinal zones of the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus, other more quiescent germinal zones may exist in the postnatal mammalian brain. Recent studies have demonstrated that constitutively low levels of neurogenesis occur in the mammalian hypothalamus, traditionally considered a non-neurogenic region. The cell of origin of these new neurons is entirely unclear. It is highly contentious whether new hypothalamic neurons are derived from the hypothalamic ventricular zone or from a parenchymal source. In the work presented here, we have observed an enrichment of proliferating cells along the ependymal layer of the base of the third ventricle. We name this proliferative region the hypothalamic proliferative zone (HPZ). We show that the proliferating cells in the HPZ are tanycytes, and we utilize genetic fate mapping tools along with BrdU pulses to independently demonstrate that the HPZ can undergo de novo neurogenesis and gliogenesis in the median eminence of the hypothalamus. Furthermore, these newborn neurons appear to be leptin-responsive, and functionally active. Our findings indicate that this HPZ represents a novel postnatal neurogenic niche with substantial proliferative capacity.

Disclosures: D.A. Lee, None; **T. Pak**, None; **V. Charubhumi**, None; **A. Miranda-Angulo**,

None; **H. Wang**, None; **F. Balordi**, None; **G. Fishell**, None; **S. Blackshaw**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.2

Topic: A.03. Stem Cells

Support: NIH Grant NIA –R01-27505

Title: Neuronal TGF-β signaling regulates survival and maturation of newborn neurons in the adult dentate gyrus

Authors: *Y. HE[1], H. ZHANG[1], P. A. JAEGER[1], O. OLAYIWOLA[2], N. FAINBERG[1], T. WYSS-CORAY[1];
[1]Neurol. and Neurolog. Sci., Stanford Univ. Sch. of Med., Palo Alto, CA; [2]Stanford Univ., Palo Alto, CA

Abstract: Hippocampal memory and learning correlate with neurogenesis in the adult brain. However, molecular signaling that controls adult neurogenesis is poorly understood. Neurogenesis increases after injury, and so does the expression of transforming growth factor (TGF)-β, which has been shown to have neuroprotective effects and has been implicated in developmental neurogenesis. TGF-β acts in a highly contextual manner depending on cell type and environment. Thus we hypothesized that neuronal TGF-β signaling might play a role in regulating adult hippocampal neurogenesis. Here, we developed an inducible transgenic strategy allowing specific activation of TGF-β signaling in neurons of transgenic mice. Confocal microscopy showed that TGF-β signaling was activated in mature neurons but also in the late stage of developing immature neurons in the subgranular zone (SGZ) of the dentate gyrus. Interestingly, activation of neuronal TGF-β signaling led to an increase in the number of immature, doublecortin-positive neurons and accelerated their migration into the granule cell layer. Labeling of dividing cells with Bromodeoxyuridine confirmed the increase in neurogenesis in vivo. Treatment of cultured primary neural progenitor cells with TGF-β promoted their migration in vitro as well. Functionally, mice with activated TGF-β signaling in neurons exhibited enhanced dendritic branching in newly born neurons in the SGZ, increased expression of c-fos, and better performance in spatial memory and learning behavioral tests. Together, these data indicate that neuronal TGF-β signaling is a potent regulator of adult neurogenesis and the maturation of newborn neurons in the hippocampus.

Disclosures: Y. He, None; **H. Zhang**, None; **P.A. Jaeger**, None; **O. Olayiwola**, None; **N. Fainberg**, None; **T. Wyss-Coray**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.3

Topic: A.03. Stem Cells

Support: NIH R01-HL073402

NIH P01- CA096832

Cancer Support Center CA-21765

Title: Prox1 is required for granule cell maturation and intermediate progenitor maintenance during brain neurogenesis

Authors: *A. J. LAVADO, O. LAGUTIN, G. OLIVER;
St Jude Children Resch Hosp, MEMPHIS, TN

Abstract: The dentate gyrus has an important role in learning and memory, and adult neurogenesis in the subgranular zone of the dentate gyrus may play a role in the acquisition of new memories. The homeobox gene *Prox1* is expressed in the dentate gyrus during embryonic development and adult neurogenesis. Here we show that Prox1 activity is necessary for the maturation of granule cells in the dentate gyrus during development and for the maintenance of intermediate progenitors during adult neurogenesis. We also demonstrate that *Prox1*-expressing intermediate progenitors are required for adult neural stem cell self-maintenance in the subgranular zone; thus, we have identified a previously unknown non-cell autonomous regulatory feedback mechanism that controls adult neurogenesis in this region of the mammalian brain. Finally, we show that the ectopic expression of *Prox1* induces premature differentiation of neural stem cells.

Disclosures: A.J. Lavado, None; **O. Lagutin**, None; **G. Oliver**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.4

Topic: A.03. Stem Cells

Support: McDonnell Foundation

Title: Spines of newborn granule cells are regulated by NMDA receptors

Authors: *C. ZHAO[1], Y. MU[2], F. H. GAGE[2];
[1]Salk Inst., LA JOLLA, CA; [2]LOG-G, Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: New neurons are continuously added to the dentate gyrus of the adult hippocampus. We have previously shown that the survival of NR1 deficient newborn granule cells is significantly decreased. To examine whether NMDA receptor-dependent activity plays a role in the integration of newborn granule cells, we examined the morphology of the remaining NR1 KO cells. We found that total spine density is decreased while the mushroom spine density is increased in NR1 KO cells. Electrophysiological recordings of wild-type and NR1 knockout cells confirmed that the knockout cells do not have NMDA receptor dependent activity. In addition, NR1 knockout cells appear to have a higher level of synaptic AMPA receptors. To examine whether this is due to the selective survival of cells with higher AMPA receptors in the absence of the NMDA receptor, we developed a creER-expressing retrovirus so that the deletion of the NR1 gene can be induced after the critical time window of cell survival. Consistently, NMDAR KO cells display decreased spine density and increased mushroom spine density. These observations suggest that NMDA receptor dependent activity plays a critical role in modulating spine number and spine size.

Disclosures: C. Zhao, None; **Y. Mu**, None; **F.H. Gage**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.5

Topic: A.03. Stem Cells

Support: Swedish Research Council

Swedish Cancer Society

Swedish Agency for Innovation Systems

Karolinska Institute

Tobias Stiftelsen and Knut och Alice Wallenbergs Stiftelse

Uehara Memorial Foundation

Wenner-Gren Foundation

Title: EphB signaling controls lineage plasticity of adult neural stem cell niche cells

Authors: *T. NOMURA, C. GORITZ, J. FRISEN;
Karolinska Inst., Stockholm, Sweden

Abstract: Stem cells remain in specialized niches over the lifespan of the organism in many organs to ensure tissue homeostasis and enable regeneration. How the niche is maintained is not understood, but is likely as important as intrinsic stem cell self-renewal capacity for tissue integrity. We here demonstrate a high degree of phenotypic plasticity of the two main niche cell types, ependymal cells and astrocytes, in the neurogenic lateral ventricle walls in the adult mouse brain. In response to a lesion, astrocytes give rise to ependymal cells and ependymal cells give rise to niche astrocytes. We identify EphB2 forward signaling as a key pathway regulating niche cell plasticity. EphB2 acts downstream of Notch and is required for the maintenance of ependymal cell characteristics, thereby inhibiting the transition from ependymal cell to astrocyte. Our results show that niche cell identity is actively maintained and that niche cells retain a high level of plasticity.

Disclosures: T. Nomura, None; **C. Goritz**, None; **J. Frisen**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.6

Topic: A.03. Stem Cells

Support: NIH Grant NS060109

NIH Grant NS054871

Title: Zfp423 is a master transcription factor that regulates the proliferation and differentiation of adult neural stem cells

Authors: L. FLORES-GARCIA[1], J. RAY[4], W. A. ALCARAZ[2], C.-J. HONG[1], F. H. GAGE[4], *B. A. HAMILTON[3];
[1]Med., [2]Biomed. Sci., [3]UCSD Sch. Med., LA JOLLA, CA; [4]Lab. of Genet., Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Retinoic acid (RA) and bone morphogenic protein (BMP) signaling strongly influence stem cell differentiation and renewal by altering transcriptional profiles in responding cells. How cells integrate these and other signaling inputs to form a coherent transcriptional response is not understood. Zfp423, a 30 Kruppel like C_2H_2 zinc finger transcription factor, has been identified independently in complexes with both cell-intrinsic, and RA- or BMP-dependent transcription factors in different cell types. To examine the role of Zfp423 in adult neural stem cells (aNSCs), we derived monolayer cultures of stem cells from the lateral ventricles of *Zfp423*$^{-/-}$ and wild-type littermate controls. Lentiviral-mediated knockdown of *Zfp423* in wild-type cells confirmed the specificity of observed effects within a single cell lineage. Initial growth curve analysis showed a significant increase in proliferation of *Zfp423* mutant or knockdown aNSCs over wild type cells. RA and BMP2 differentiate aNSCs into different proportions of GFAP$^+$, Tuj1$^+$, Olig2$^+$ and DCX$^+$ cells. However, *Zfp423*$^{-/-}$ cells had a significantly reduced differentiation response to either factor, as well as continued proliferation under differentiation treatment conditions compared to wild-type cells. Antibody staining showed that *Zfp423*$^{-/-}$ cells expressing GFAP or Tuj1 have fewer processes and a less mature phenotype than wild-type cells, especially after retinoic acid treatment. Extensive microarray analysis identified gene sets that differ between genotypes under renewal conditions or upon treatment with either differentiation protocol. Double labeling of neural stem/progenitor cell proliferation with IdU/CldU showed a different pattern of proliferation in vivo. Together, our data indicate that *Zfp423* is a key point of integration for extracellular signaling and cell-intrinsic transcriptional control of neural stem and progenitor cells.

Disclosures: L. Flores-Garcia, None; **J. Ray**, None; **B.A. Hamilton**, None; **C. Hong**, None; **W.A. Alcaraz**, None; **F.H. Gage**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.7

Topic: A.03. Stem Cells

Support: NIH Grant 1DP2OD006484-01

Whitehall Foundation

American Heart Association

Title: An orphan nuclear receptor controls adult neural stem cell positioning and activation

Authors: *C.-L. ZHANG, W. NIU, Y. ZOU;
Mol. Biol., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Neural stem cells (NSCs) exist and continually produce functional new neurons in two neurogenic niche of the adult mammalian brain: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus. However, during aging the ability of NSCs to produce neurons rapidly declines. It is not clear how activity of these cells is molecularly regulated. Previously, we demonstrated that TLX, an orphan nuclear receptor, is essential to maintain adult hippocampal neurogenesis and plays a role in hippocampus-dependent learning and memory. Lineage tracing indicates that TLX is expressed in NSCs. Here, we provide further evidence that TLX is required for activation and correct positioning of NSCs in the neurogenic niche. Furthermore, deletion of TLX leads to a precipitous aging process of neural stem cells. Unbiased genome-wide analysis identified several tumor suppressors as downstream targets of TLX. Yet, mutation of several of these suppressors is insufficient to rescue the proliferation deficits of the adult NSCs, suggesting that TLX works as a master regulator in coordinating several pathways to ensure the replication and neurogenic ability of these adult NSCs.

Disclosures: C. Zhang, None; **W. Niu**, None; **Y. Zou**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.8

Topic: A.03. Stem Cells

Support: NIH-NRSA Predoctoral Fellowship 1F31NS066900-01A1

NIH Grant NS042626

Title: Fezf2 regulates the self-renewal and differentiation of neural stem cells in the adult zebrafish telencephalon

Authors: M. A. BERBEROGLU[1], Z. DONG[1], T. MUELLER[1], *S. GUO[2];
[1]Univ. California - San Francisco, San Francisco, CA; [2]Univ. California - San Francisco, SAN FRANCISCO, CA

Abstract: Fezf2 is an evolutionarily conserved forebrain-specific zinc finger transcription factor that is expressed during development and is implicated in patterning as well as neurogenesis in both zebrafish and mice. Despite these findings, the expression of *fezf2* in the adult brain has not been well characterized, and *fezf2* function in the adult brain remains unknown. The zebrafish has recently emerged as a new model system to study adult neurogenesis, given its similarity to mammalian systems and enhanced capability of undergoing adult neurogenesis. Through RNA *in situ* hybridization and using a *fezf2* promoter-driven GFP transgenic line, we show that *fezf2* is expressed in radial glial progenitor cells of the telencephalic ventricular zone in the adult zebrafish brain, which co-express markers of neural stem cells (NSCs) and proliferation. Additionally, we identify the preoptic region and the hypothalamus as *fezf2*-expressing neurogenic regions in the adult zebrafish brain, where *fezf2* labels progenitor cells as well as postmitotic neurons [1].
BrdU/EdU experiments indicate that adult telencephalic *fezf2*-expressing cells can self-renew *in vivo*. To determine whether these *fezf2*-expressing cells give rise to neurons, we are creating a *fezf2*-CreERT2 transgenic line which will allow us to label cells at the adult stage for lineage tracing. Transient experiments at embryonic stages indicate that this approach works to label *fezf2*-expressing cells with precise temporal control. To investigate Fezf2 loss-of-function in NSCs of the adult zebrafish telencephalon, we are using the *too few* (Fezf2) mutant. Analysis of the adult *too few* mutant brain reveals that the mutant telencephalon is substantially smaller in size, and has a significant increase in the number of GFAP-expressing cells in the ventricular zone, as well as an increase in proliferation. No phenotype is observed at the larval stage, supporting an adult role for Fezf2 in the telencephalon. BrdU studies which address self-renewal, cell cycle exit, and the rate of adult neurogenesis in the mutant telencephalon will be presented, as well as the Fezf2 gain-of-function experiments using the available *hsp-GAL4:UAS-fezf2* transgenic line. Our findings establish Fezf2 as a novel marker for NSCs in the adult zebrafish brain, and suggest a critical function of Fezf2 in the maintenance and differentiation of NSCs in the adult vertebrate brain.

Disclosures: M.A. Berberoglu, None; **Z. Dong**, None; **S. Guo**, None; **T. Mueller**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.9

Topic: A.03. Stem Cells

Support: Medical Research Council

Research Council UK

Title: Adult hippocampal neurogenesis and behaviour are altered by diet in mice. What is the role of Klotho?

Authors: *D. STANGL[1], B. MORISSE[1], S. AHMET[1], L. J. AIMONE[2], J. B. AIMONE[2], F. H. GAGE[2], S. THURET[1];
[1]Ctr. for the Cell. Basis of Behaviour & MRC Ctr. for Neurodegeneration Re, King's Col. London, London, United Kingdom; [2]Lab. of Genet., Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: It is now well established that during adulthood, new neurons are generated from adult neural stem cells residing in the dentate gyrus of the hippocampus, a region important for memory and learning function as well as mood in rodents and humans. In the rodent, an increase of neurogenesis in the hippocampus is associated with improved memory and learning abilities, whereas a decreased neurogenesis is associated with symptoms of depression. The level of adult hippocampal neurogenesis can be regulated by factors such as enriched environment, physical activity, aging, and stress but also by diet.
We first present dietary parameters responsible for adult hippocampal neurogenesis and learning/memory as well as mood regulation. We show, in the mouse animal model that meal frequency, independently of calorie intake, affects adult neurogenesis, learning/memory and mood.
We next identified possible molecular mechanisms mediating the effects of diet on adult hippocampal neurogenesis. We show that the gene Klotho is highly expressed in the hippocampus and its expression is up-regulated by 2 fold upon diet-induced increased adult hippocampal neurogenesis. Klotho is a single pass transmembrane protein with an active secreted external domain. Klotho is also known as the ‘ageing suppressor gene’, due to the symptoms of a knockout mouse resembling human aging. In turn, in a mouse model over-expressing Klotho, lifespan is extended up to 30%. Up to date, the role of Klotho in the central nervous system has not been investigated. To further examine the role of Klotho on cellular and molecular mechanisms underlying the influence of food intake on hippocampal neurogenesis, we

used rat adult and human embryonic hippocampal progenitor cell lines. Our data suggest that diet modulates adult hippocampal neurogenesis through Klotho regulation, and underline a central role for Klotho in regulating hippocampal neurogenesis.

Disclosures: D. Stangl, None; **B. Morisse**, None; **S. Ahmet**, None; **L.J. Aimone**, None; **J.B. Aimone**, None; **F.H. Gage**, None; **S. Thuret**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.10

Topic: A.02. Neurogenesis and Gliogenesis

Support: EURYI/DFG (LI 858/6-2)

Marie Curie Excellence Team Grant of EU

Helma

FORNEUROCELL

Title: Role of SoxC transcription factors in adult hippocampal neurogenesis

Authors: L. BERTI[1], M. COVIC[1], L. MU[1], A. HASLINGER[1], V. LEFEBVRE[2], M. WEGNER[3], *E. SOCK[3], D. C. LIE[1];
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Abstract: Neural stem cells in the subgranular zone of the adult dentate gyrus generate new functional neurons throughout life. The genetic programs underlying this process are not fully understood. We have previously shown that the SoxC transcription factor Sox11 is specifically expressed in immature newborn neurons of the hippocampus. We now report that another SoxC family member, i.e., Sox4 is expressed in an overlapping pattern, suggesting an important role for SoxC group proteins in the regulation of adult hippocampal neurogenesis. To study the function of SoxC proteins, we have performed in vivo gain- and loss-of-function experiments. We found that overexpression of SoxC proteins led to prolonged expression of immature neuronal markers, while conditional ablation of Sox4 and Sox11 compromised the neuronal differentiation of newly generated cells. Luciferase-reporter assays and chromatin-

immunoprecipitation studies revealed that SoxC group proteins are involved in the control of expression of immature neuronal proteins. Taken together, these results demonstrate that SoxC transcription factors are essential regulators of neuronal differentiation in adult hippocampal neurogenesis.

Disclosures: L. Berti, None; **M. Covic**, None; **L. Mu**, None; **V. Lefebvre**, None; **A. Haslinger**, None; **M. Wegner**, None; **E. Sock**, None; **D.C. Lie**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.11

Topic: A.03. Stem Cells

Support: NIH R21 DA023701 (AJE)

NIH R01 DA016765 (AJE)

NIH K02 DA023555 (AJE)

NIH T32 DA07290 (to AJE to support NAD, JLA)

NIH F32 NS064632 (to NAD)

CIHR Postdoctoral Fellowship (DCL)

Title: Subpopulations of radial-glia like cells may differentially contribute to adult neurogenesis in the hippocampus in vivo

Authors: *N. A. DECAROLIS[1], M. MECHANIC[1], D. PETRIK[1], S. MALHOTRA[1], J. L. ABLES[1], D. C. LAGACE[1,2], A. J. EISCH[1];
[1]Psychiatry, UT-Southwestern Med., DALLAS, TX; [2]Dept. of Cell. and Mol. Med., Univ. of Ottowa, Ottowa, ON, Canada

Abstract: Radial astrocyte-like cells (RCs, also Type-1 cells, quiescent neural progenitors, or Type-B cells) are the hypothesized source of adult hippocampal neurogenesis, but in vivo experimental evidence is largely correlative. Using inducible Cre-driver transgenic mouse lines to track RCs and progeny, we compare the neurogenic contribution from GLAST- and nestin-

expressing cells over a prolonged time course post-tamoxifen (post-TAM). In the GLAST-CreERT2/R26R-YFP mouse, we find that recombined cells and their progeny contribute to long-term hippocampal neurogenesis and maintain recombined YFP+ progenitor cells even 180d post-TAM. However, in nestin-CreERT2/R26R-YFP mice, YFP+ progenitor cells are depleted by 100d post-TAM. Thus, nestin-expressing RCs do not appear to contribute to long-term hippocampal neurogenesis despite the persistence of YFP+ RCs in nestin-CreERT2/R26R-YFP mice. Experiments are ongoing to assess whether YFP+ RCs from both mouse lines contribute to the recovery of progenitor cells following their pathological depletion with cytotoxic cytosine-β-D-arabinofuranoside (AraC). Current data suggest a heterogeneity of RCs, and may indicate a new model for the lineage progression of adult-generated hippocampal neurons.

Disclosures: N.A. DeCarolis, None; **M. Mechanic**, None; **D. Petrik**, None; **S. Malhotra**, None; **J.L. Ables**, None; **D.C. Lagace**, None; **A.J. Eisch**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.12

Topic: A.02. Neurogenesis and Gliogenesis

Support: 2R01NS014841-31

Title: Maintenance of neural stem cells requires dynamin

Authors: *J. J. BREUNIG, J. I. ARELLANO, K. ANIGHORO, S. FERGUSON, P. DE CAMILLI, P. RAKIC;
Dept Neurobiol, Yale Univ. Sch. Med., NEW HAVEN, CT

Abstract: The Notch pathway has been implicated in cell fate determination in CNS stem cells. Although the details of that process are not totally clear yet in the context of mammalian development, there is growing evidence that endocytosis of Notch ligands and receptors is essential for Notch signaling. We found that Dynamin-mediated endocytosis is essential for Notch signaling and in the maintenance of neural stem cells. Conditional deletion leads to premature loss of neural precursor cells and premature neurogenesis, resembling loss of Notch signaling. In addition, Notch ligands and receptors accumulate on the cellular surface and cleaved NICD cannot be detected in neural stem cells. This phenotype can be reproduced by shRNA knockdown, and can be rescued by overexpression of Dynamin isoforms or an activated form of Notch. Thus, we propose that

Dynamin is essential for neural stem cell maintenance in the embryonic brain.

Disclosures: J.J. Breunig, None; **J.I. Arellano**, None; **K. Anighoro**, None; **P. Rakic**, None; **P. De Camilli**, None; **S. Ferguson**, None.

Nanosymposium

423. Postnatal Neurogenesis III

Location: Room 25A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 423.13

Topic: A.03. Stem Cells

Support: SBO grant (IWT-060838) of the Institute for the Promotion of Innovation through Science and Technology in Flanders

European NoE DiMI LSHB-CT-2005-512146

Fund for Scientific Research grant G.0484.08

Catholic University Leuven IOF-KP/07/001

MoSAIC, the Molecular Small Animal Imaging Center, Catholic University Leuven

Title: Specific labeling and noninvasive imaging of adult endogenous neural stem cells using conditional viral vectors

Authors: *V. REUMERS, R. GIJSBERS, A. IBRAHIMI, S.-A. AELVOET, S. DE SWAEF, I. THIRY, C. VAN DEN HAUTE, Z. DEBYSER, V. BAEKELANDT;
Katholieke Univ. Leuven, Leuven, Belgium

Abstract: Aims: Continuous neurogenesis occurs in the subventricular zone (SVZ) of the adult rodent brain. Neuroblasts, which are the offspring of the endogenous neural stem cells (eNSC) in the SVZ, migrate to the olfactory bulb (OB). We previously showed stable gene transfer in adult eNSC *in vivo* by injection of lentiviral vectors (LV) into the SVZ. Moreover, bioluminescence imaging (BLI) can be used for noninvasive detection and quantification of the migration of neuroblasts by LV marking of the eNSC with firefly luciferase (Fluc). However, since LV transduce both dividing and postmitotic cells, at the site of injection a high BLI signal was observed originating not only from the eNSC but also from mature neurons and astrocytes. We therefore aimed to develop new LV to specifically label the eNSC and improve the *in vivo*

quantification of the number of new neuronal cells in the OB.
Methods: We designed conditional LV based on Cre-mediated recombination. In the LV construct the cDNA of Fluc is present in antisense orientation and flanked by two mutually exclusive loxP sites at the 5' end and two inverted loxP sites at the 3' end. Upon Cre recombination the cDNA between the lox sites will be inverted and thereby expression is induced. Mice were imaged with an optical CCD-camera (IVIS 100).
Results: The conditional LV were tested in cell culture and *in vivo* by cotransduction with a LV encoding Cre recombinase. Successful recombination was detected both in cell culture and *in vivo*. Next, conditional LV were stereotactically injected in the SVZ of Nestin-CreER^{T2} transgenic mice, that express Cre in the eNSC. A long term follow-up study with BLI was initiated. At 8 weeks after injection we detected a specific signal originating from the OB and at later time points this signal increased. Histological analysis showed eNSC specific labeling in the SVZ and labeled cells in the OB, corroborating the *in vivo* BLI data. As a first application we investigated the role of DJ-1, a Parkinson's Disease related gene, in neurogenesis. Long term BLI follow-up showed no significant effect of DJ-1 overexpression on neurogenesis. This was confirmed by histological analyses of the number of labeled cells in the OB.
Conclusion: We developed a new conditional viral vector-based system for specific labeling of eNSC *in vivo*. This approach holds promise to noninvasively follow up modulation of endogenous neurogenesis over time using BLI.

Disclosures: V. Reumers, None; **R. Gijsbers**, None; **A. Ibrahimi**, None; **S. Aelvoet**, None; **S. De Swaef**, None; **I. Thiry**, None; **C. Van den Haute**, None; **Z. Debyser**, None; **V. Baekelandt**, None.

Nanosymposium

424. Dementia Molecular Genetics and Proteome

Location: Room 32B

Time: Monday, November 15, 2010, 1:00 pm - 2:15 pm

Program Number: 424.1

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant AG18379

NIH Grant AG18884

Alzheimer's Association (Zenith Award)

Title: Role of specific microRNAs in regulating neuronal genes implicated in Alzheimer disease

Authors: *J. M. LONG[1], D. K. LAHIRI[2];
[1]Inst. of Psychiatric Res., Indiana Univ. Sch. of Med., INDIANAPOLIS, IN; [2]Inst. of Psychiatric Res., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Alzheimer's disease (AD) is characterized by brain depositions of amyloid plaques and neurofibrillary tangles, synaptic dysfunction, neuron loss, and cognitive decline. These aberrations are believed to result, in part, from the over-production of amyloid-β peptide (Aβ), which is derived from the amyloid precursor protein (APP). Our hypothesis is that overproduction of Aβ may be due to dysregulation of genes involved in AD. Recent studies suggest that in AD, dysregulation of proteins involved in APP trafficking (SORL1), Aβ production (e.g. APP, BACE1) and Aβ clearance (e.g. neprilysin [NEP]) may contribute to excess Aβ deposition. Elucidating how expression of these proteins is regulated will ultimately reveal new drug targets. We have taken the novel approach of studying the regulation of these gene products by microRNAs (miRNAs). These are short, non-coding RNAs that act as post-transcriptional regulators of gene expression through site-specific interactions with the 3'UTRs of target mRNA.
Here we describe the identification of miRNAs that putatively regulate expression of APP, BACE1, NEP and SORL1. Using bioinformatic tools, a set of miRNA predicted to target the human APP, BACE1, NEP and SORL1 mRNA 3'UTR was identified. Chimeric 3'UTR-reporter constructs were then prepared by inserting the APP, BACE1, NEP or SORL1 3'UTR downstream of a reporter *Renilla* luciferase gene. To identify miRNA-target gene interactions, corresponding miRNA mimics were independently co-transfected into HeLa cells along with a specific reporter construct described above. Sensitive reporter assays identified multiple miRNAs that mediate inhibitory (and some stimulatory) effects on luciferase expression for each 3'UTR. Follow-up experiments were limited to APP-directed candidates.
We found that miR-153 and miR-346 inhibited APP 3'UTR reporter expression to a significant degree. To confirm the effects of miR-153 and miR-346 on native protein expression, miRNA mimics were transfected into HeLa cells and changes in APP protein levels were measured by Western blot. Notably, APP levels were significantly decreased by miR-153 but were surprisingly increased by miR-346 relative to either mock-transfected cells or those transfected with a negative control miRNA. Experiments are underway to confirm the site-specific nature of these interactions and to test endogenous regulation of APP by miR-153 and miR-346. Follow-up studies are also underway on candidate miRNA directed against BACE1, NEP and SORL1 3'UTR. Taken together, our results reveal a novel regulatory interaction between important AD-related genes and specific endogenously expressed miRNA species.

Disclosures: J.M. Long, None; **D.K. Lahiri**, None.

Nanosymposium

424. Dementia Molecular Genetics and Proteome

Location: Room 32B

Time: Monday, November 15, 2010, 1:00 pm - 2:15 pm

Program Number: 424.2

Topic: C.02. Alzheimer's disease and other dementias

Support: AHAF grant A2009340

Title: Solution NMR studies of transmembrane domain of APP

Authors: *C. WANG[1], W. CHEN[2], Y. LI[3], E. GAMACHE[1];
[2]Biol., [1]Rensselaer Polytech Inst., TROY, NY; [3]MSKCC, New York, NY

Abstract: While most AD cases are sporadic, a small fraction of patients suffer from familial Alzheimer's disease (FAD) caused by missense mutations in genes encoding γ-secretase and APP. All FAD mutations cause a shift in the cleavage specificity of γ-secretase and result in increased Aβ42/Aβ40 ratios. Twelve FAD mutations have been found within the transmembrane domain of APP (APPTM), but little is known about the structural basis of how these mutations lead to increased Aβ42/Aβ40 ratios. Although intense efforts are being devoted to the structural biology of γ-secretase, the structure-function relationship of APPTM has received much less attention. Solution NMR has been applied to study the structural differences caused by FAD mutations within APPTM. Here we present the structures of APPTM and discuss their relevance to interactions with γ-secretase.

Disclosures: C. Wang, None; **W. Chen**, None; **Y. Li**, None; **E. Gamache**, None.

Nanosymposium

424. Dementia Molecular Genetics and Proteome

Location: Room 32B

Time: Monday, November 15, 2010, 1:00 pm - 2:15 pm

Program Number: 424.3

Topic: C.02. Alzheimer's disease and other dementias

Title: Gene expression profiling of choroid plexus in Alzheimer's disease reveals important implications of csf dynamics

Authors: *E. G. STOPA[1], E. V. NIKONOVA[2], A. A. PODTELEZHNIKOV[2], K. Q. TANIS[2], E. M. FINNEY[2], D. J. STONE[2], L. M. CAMARGO[2], L. PARKER[2], A. VERMA[2], A. BAIRD[3,4], M. C. MILLER[1], J. E. DONAHUE[1], A. GONZALEZ[4], B. ELICEIRI[3], G. D. SILVERBERG[1], P.

M. KLINGE[1], C. E. JOHANSON[1];
[1]Pathol (Neuropathology), Brown Univ., PROVIDENCE, RI; [2]Merck & Co, Inc., West Point, PA; [3]Univ. of California San Diego Med. Ctr., San Diego, CA; [4]Univ. of Birmingham, Edgbaston, United Kingdom

Abstract: In aging, normal pressure hydrocephalus (NPH), and Alzheimer's disease there are striking changes in CSF composition that may be related to altered choroid plexus function. Human Affymetrix 48K gene arrays were used to determine disease-related changes in gene expression within the choroid plexus. Post-mortem tissue samples from healthy controls (mean age/mean PMI: 58 years/22 hours) and patients with advanced (Braak & Braak stage V-VI) Alzheimer's disease (79/18) were snap frozen in liquid nitrogen and stored at -80°C. Samples from diseased control patients with frontotemporal dementia (72/NA) and Huntington's disease (71/19) were also collected. RNA from choroid plexus was extracted using Trizol followed by NuGEN Ovation amplification, and cDNA was hybridized to custom chips at Rosetta/Merck. After RMA normalization, analysis of data was performed using one way ANOVA, and most significant gene sets were further analyzed for biological enrichment using individual (Ingenuity) and combined (Target and Gene Information System) pathway tools.
Clear differences were observed on gene expression level in choroid plexus of advanced AD patients when compared to both the normal and diseased (FTD, HD) control groups. 648 sequences could significantly separate four experimental groups ($p<0.001$, FDR~8%). About half of those sequences were up regulated in neurodegenerative diseases. The up regulated genes represented overall 15 highly enriched biological functions (multiple correction expectation value <0.1). Strikingly, cell adhesion and extracellular matrix re-modeling along with post-translational modification (phosphorylation) were highly enriched in AD patients (expectation <10E04). A significant increase in immune response was evident in AD patients, while oxidative phosphorylation and amyloid processing were both down-regulated. Other observations included decreases in PPARa/RXRa nuclear receptor/retinoic acid, a-adrenergic, glucocorticoid and melatonin signaling, as well as N-glycan, glutathione (antioxidant) and ubiquinone metabolism in AD patients.
This unique resource may be of interest to numerous investigators working on aging CSF dynamics and hydrocephalus. It can be readily shared with investigators wishing to answer specific questions related to their field of investigation.

Disclosures: **E.G. Stopa**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **E.V. Nikonova**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **A.A. Podtelezhnikov**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **K.Q. Tanis**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **E.M. Finney**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **D.J. Stone**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **L.M. Camargo**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **L. Parker**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **A. Verma**, Merck & Co., Inc., Employment; Merck & Co., Inc., Other Research Support; **A. Baird**, None; **M.C. Miller**, None; **J.E. Donahue**, None; **A. Gonzalez**, None; **B. Eliceiri**, None; **G.D. Silverberg**, None; **P.M. Klinge**, None; **C.E. Johanson**, None.

Nanosymposium

424. Dementia Molecular Genetics and Proteome

Location: Room 32B

Time: Monday, November 15, 2010, 1:00 pm - 2:15 pm

Program Number: 424.4

Topic: C.02. Alzheimer's disease and other dementias

Title: The proteostasis network in age-associated neurodegenerative diseases

Authors: *H. GE[1], L. WANG[1], Y. ZHU[1], K. BAILEY[1], F. USECHE[1], W. BALCH[2], A. DILLIN[3], R. MORIMOTO[4], W. NEWMAN[1], P. REINHART[1];
[1]Proteostasis Therapeut. Inc, Cambridge, MA; [2]The Scripps Res. Inst., La Jolla, CA; [3]The Salk Inst. for Biol. Studies, La Jolla, MA; [4]Northwestern Univ., Evanston, MA

Abstract: Age-associated neurodegenerative diseases associated with protein misfolding, such as Huntington's, Parkinson's, and Alzheimer's diseases, generate patterns of neuronal stress responses that allow a systems-based approach for the identification of novel drug targets, disease progression biomarkers, and the effect of putative therapeutics on normalizing network-level alterations. We have developed a novel searchable protein-protein interaction database (Proteostasis Network Explorer) utilizing data obtained across five species from yeast to human. Since aging is the biggest risk factor for many neurodegenerative diseases including the Huntington's, Parkinson's, and Alzheimer's diseases, we have overlaid gene expression datasets of brain aging onto the proteostasis network, and identified sub-networks that are regulated by aging. Furthermore, analyzing transcriptional data from a range of human post-mortem brain tissues, we have identified both positive and negative network responses associated with stress response to neurodegeneration. These data uncover numerous similarities and differences in the proteostasis network in response to aging and age-onset diseases. For instance, we have identified an Hsp70 network, which is up-regulated in Alzheimer's disease, Parkinson's disease, and Huntington's disease. We have also found components of a mitochondrion chaperone network, including the co-chaperone Tom70, to be down-regulated in these three neurodegenerative diseases. Such changes can identify novel pathways and targets for therapeutic intervention, and provide insights into disease-progression biomarkers.

Disclosures: **H. Ge**, Proteostasis Therapeutics Inc, Employment; **L. Wang**, Proteostasis Therapeutics Inc, Employment; **Y. Zhu**, Proteostasis Therapeutics Inc, Employment; **K. Bailey**, Proteostasis Therapeutics Inc, Employment; **F. Useche**, Proteostasis Therapeutics Inc, Employment; **W. Balch**, Proteostasis Therapeutics Inc, Consultant/Advisory Board; **A. Dillin**, Proteostasis Therapeutics Inc, Consultant/Advisory Board; **R. Morimoto**, Proteostasis Therapeutics Inc, Consultant/Advisory Board; **W. Newman**, Proteostasis Therapeutics Inc, Consultant/Advisory Board; **P. Reinhart**, Proteostasis Therapeutics Inc, Employment.

Nanosymposium

424. Dementia Molecular Genetics and Proteome

Location: Room 32B

Time: Monday, November 15, 2010, 1:00 pm - 2:15 pm

Program Number: 424.5

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grants AG18379 and AG18884

Alzheimer's Association (Zenith Award)

Title: Role of the amyloid β-peptide as a transcription factor and Its implication in Alzheimer's disease

Authors: *D. K. LAHIRI, B. MALONEY, Y.-W. GE, J. A. BAILEY;
Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Brain deposition of neuritic plaques that contain the amyloid beta-peptide (Aβ) is one of the major hallmarks of Alzheimer's disease (AD). The current understanding of nonpathological roles for Aβ is incomplete. Our hypothesis is that Aβ interacts with regulatory region(s) of genes involved in AD as a transcription factor. We used a known Aβ-binding decamer from the 5'-flanking region of the p53 apoptosis-associated protein to determine decamers with 80% homology in the 5'-flanking regions of the apolipoprotein E (APOE), Aβ-precursor protein (APP) and β-amyloid cleaving enzyme 1 (BACE1) gene 5'-flanking regions. Oligomers were generated from these homologies and were used for electrophoretic mobility shift assay (EMSA) with Aβ peptides of 42 and 40 amino acids in length. Those oligomers generating positive signal were analyzed by alignment to produce a consensus sequence ("GGATKGGGGT"), frequency matrix, and sequence logo. Fragments of the Aβ peptide of various lengths were used in EMSA vs. the Aβ-binding oligomers. The cytotoxic Aβ25-35 fragment showed greatest DNA-binding affinity. In addition, binding between DNA oligomers and Aβ fragments was concentration-dependent and could be blocked with unlabeled DNA oligomers. Binding of Aβ to APP and BACE1 promoters was confirmed in situ via ChIP in human neuroblastoma cells. Rat neuronal PC12 cells and primary rat cortical neuronal cultures were transiently transfected with luciferase reporter fusions to a 1.2 kb fragment of the APP 5'-flanking region and a 3.3kb fragment of the BACE1 5'-flanking region. Putative or confirmed Aβ binding sites have been identified in both of these 5'-flanking regions. These transfected cultures were treated with three different Aβ peptides. Aβ treatment altered reporter levels for both BACE1 and APP clones in both PC12 and PRCN cultures. Further, Aβ and peroxide

treatment of polymorphic APP promoter fragment-CAT reporter gene fusion clones showed differential response. These results suggest a novel interaction of the Alzheimer's amyloid β-peptide with a specific regulatory motif present in the APOE, APP, and BACE1 gene promoters and that the Aβ peptide is likely to function as a transcription factor or co-factor for more than one gene. In summary, we propose that, whatever other functions it may have, the Aβ peptide acts as a transcription factor that directs normal apoptosis and cell death as well as regulating its own production through feedback on its precursor protein and the necessary β-secretase enzyme (BACE1).

Disclosures: D.K. Lahiri: Research Grant; NIH. **B. Maloney:** None. **Y. Ge:** None. **J.A. Bailey:** None.

Nanosymposium

425. Huntington's Disease: Animal Models I

Location: Room 10

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 425.1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI/High Q Foundation

Title: Dominant negative tumor necrosis factor gene delivery in the yac128 transgenic mouse model of Huntington's disease

Authors: *M. G. TANSEY[1], L. A. TAYLOR[3], X. CHEN[2], I. TREVINO[3], K. RUHN[3];
[2]Physiol., [1]Emory Univ. Sch. of Med., Atlanta, GA; [3]Physiol., UT Southwestern, Dallas, TX

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that has been linked to polyglutamine expansions in the huntingtin gene (htt) resulting in degeneration of GABAergic medium spiny neurons (MSN) in the caudate putamen and cortex leading to uncontrolled movements, emotional disturbances, dementia, and death. While not fully understood, inflammation is also present in HD as brains of mutant htt carriers show increased microglial activation and inflammatory gene expression (e.g. tumor necrosis factor; TNF); an observation mirrored in plasma samples from HD patients. Neuroinflammation is also detectable in mouse models of HD that express mutant htt, such as R6/2, HdhQ150/Q150, and YAC128 transgenic (YAC128) mice. Using the techniques our group has employed to study inflammatory pathogenesis in rodent models of Parkinson's disease and Alzheimer's disease, the objectives of this study were to: i) establish the neuroinflammatory profile of YAC128 Het mice; ii) measure their progressive locomotor deficits using behavioral tests; and iii) investigate the

extent to which TNF-dependent inflammation contributes to their HD-like pathology. These studies demonstrate that at 12 months of age, the inflammatory profile of YAC128 mice was qualitatively different from wild type mice. Specifically, YAC128 mice showed an overall increase in inflammation. Aged YAC128 transgenic animals also exhibited locomotor deficits in the narrow beamwalk and rotarod test. Digital analysis of paw placement during treadmill walking revealed that YAC128 mice displayed significant differences from WT mice in (1) duration of the forelimb propulsion phase of stepping, (2) forelimb stride length variance, (3) forelimb stance factor, and (4) forelimb and (5) hindlimb step angle variance. To investigate the extent to which selective inhibition of soluble TNF could modulate the neuroinflammatory profiles, MSN survival, and locomotor performance in these mice up to 12 months of age, we generated an adeno-associated viral (AAV) vector to deliver dominant negative TNF (DN-TNF) inhibitor (or GFP as a negative control) to the striatum of young (2 mo old) adult YAC128 or WT mice. Although single bi-lateral injections of AAV vectors to mouse striatum resulted in widespread infection of striatal cells and expression of transgenes that was maintained up to 12 months of age, DN-TNF gene delivery did not significantly reduce markers of inflammation or rescue locomotor function deficits observed in YAC128 transgenic mice. Analyses of MSN survival are ongoing. Potential reasons for the limited effects of targeting soluble TNF will be discussed.

Disclosures: M.G. Tansey: Employment; ex-employee of Xencor Inc.. **L.A. Taylor:** None. **X. Chen:** None. **I. Trevino:** None. **K. Ruhn:** None.

Nanosymposium

425. Huntington's Disease: Animal Models I

Location: Room 10

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 425.2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR Clinician Scientist Award

HSC Navigator Award

CHDI TREAT-HD Grant

MSFHR FIND Grant

Title: Huntingtin expression prevents the development of epilepsy in FVB/N mice by reducing seizure-induced neurodegeneration

Authors: *B. LEAVITT[1], J. M. VAN RAAMSDONK[1], L. WAGNER[1], T. W. BREDY[2], J. PEARSON[1], C. SCHWAB[1], Z. MURPHY[1], R. S. DEVON[3], G. LU[1], M. S. KOBOR[1], M. R. HAYDEN[1];
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Abstract: Huntington's disease (HD) is an adult-onset neurodegenerative disorder caused by a trinucleotide CAG expansion in the HTT gene that codes for the protein huntingtin. Huntingtin is a multi-functional, ubiquitously expressed protein that is essential for survival, normal development and adult brain function. The ability of huntingtin to protect neurons against death raises the question as to whether the loss of this function is involved in the pathogenesis of HD, and also whether huntingtin's normal function plays a role in other neurological disorders. Here we show that full-length huntingtin prevents the occurrence of an idiopathic seizure disorder that occurs in FVB/N mice. These mice demonstrate all of the characteristic features of mouse models of epilepsy including astrocytosis, neuronal hypertrophy and up-regulation of brain-derived neurotrophic factor. Interestingly, decreasing huntingtin levels increases the frequency of the seizure disorder in FVB/N mice, while over-expression of huntingtin completely prevents it. Examination of the mechanism underlying huntingtin's ability to modulate the development of this seizure disorder indicates that over-expression of huntingtin acts through promoting the survival of neurons. Our results demonstrate the importance of huntingtin function in a neurological disorder that is independent of HD and suggest that preventing seizure induced neurodegeneration limits the subsequent development of epilepsy.

Disclosures: B. Leavitt, None; **J.M. Van Raamsdonk**, None; **L. Wagner**, None; **J. Pearson**, None; **C. Schwab**, None; **Z. Murphy**, None; **G. Lu**, None; **M.S. Kobor**, None; **M.R. Hayden**, None; **T.W. Bredy**, None; **R.S. Devon**, None.

Nanosymposium

425. Huntington's Disease: Animal Models I

Location: Room 10

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 425.3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS 1R21NS064313-01A1

CHDI Foundation Inc

NIH NS16375

Title: Neuroprotective effects of mycophenol in an inducible cell model and the N171-82Q mouse model of Huntington's disease

Authors: *N. ARBEZ[1], Q. PENG[1], Y. CHENG[1], Q. LI[1], J. FU[1], S. BHAT[2], J. LIU[2], T. ELLIS[5], M. JOYNER[5], R. H. CICHEWICZ[5], W. DUAN[1], C. A. ROSS[1,3,4];
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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterized by an expansion of a polyglutamine repeats in the huntingtin protein. In HD patients, atrophy is detected in the striatum, cortex and other brain regions such as amygdala and thalamus, leading to motor impairments, psychiatric abnormalities and cognitive decline. There is currently no disease-modifying treatment for the disease. In order to develop neuroprotective therapeutic molecules for HD, we previously screened the NINDS 1040 FDA-approved compound library in our inducible PC12 cell model of HD, and found that mycophenolic acid (MPA) significantly protected cells against mutant huntingtin-induced neurotoxicity. However, mycophenolic acid is an immunosuppressant that blocks the de novo synthesis of guanosine monophosphate and does not cross the blood brain barrier (BBB). We synthesized series of MPA derivatives which are predicted to have high BBB penetration, as well reduced immunosuppressive activity, and tested them in the cell model. We found that mycophenol significantly protected mutant huntingtin-induced neurotoxicity concentration-dependently with an EC50 about 3uM in differentiated PC12 cells expressing mutant huntingtin. We then confirmed BBB penetration, and conducted preliminary toxicity study in N171-82Q mouse model. In this study, we have now investigated the effects of mycophenol (50 mg/kg, ip injection) in N171-82Q mice. Mycophenol was administered into mice from 8 weeks of age. We assayed the motor function by accelerating rotarod and monitored general criteria of the mouse health including body weight, grooming and limb clasping. Our preliminary results indicate that mycophenol attenuates the progressive motor deficits assessed with the rotarod testing and improves general health conditions of HD mice. Survival analysis, brain pathology and brain volume changes measured by histology and structural MRI imaging are in progress. The results of this study could lead to further preclinical and possibly clinical trials of mycophenol or derivatives for HD.

Disclosures: N. Arbez, None; **Q. Peng**, None; **Y. Cheng**, None; **Q. Li**, None; **J. Fu**, None; **S. Bhat**, None; **J. Liu**, None; **T. Ellis**, None; **M. Joyner**, None; **R.H. Cichewicz**, None; **W. Duan**, None; **C.A. Ross**, None.

Nanosymposium

425. Huntington's Disease: Animal Models I

Location: Room 10

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 425.4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Transgenic HD mouse model expressing the putative caspase 6 derived huntingin fragment

Authors: *E. A. WALDRON[1], T. RATOVITSKI[1], E. CHIGHLADZE[1], A. T. TEBBENKAMP[2], R. K. GRAHAM[3], M. R. HAYDEN[3], D. R. BORCHELT[2], C. A. ROSS[1]; [1]Psychiatry, Div. of Neurobio., Johns Hopkins Univ., Baltimore, MD; [2]McKnight Brain Inst., Gainesville, FL; [3]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Huntington's disease (HD) is caused by a polyglutamine expansion in the huntingtin (Htt) protein. Proteolytic cleavage of Htt into toxic N-terminal fragments is believed to be a key aspect of pathogenesis Therefore, reducing and/or inhibiting Htt proteolysis is a potential therapeutic target. The best characterized cleavage event is at amino acid 586, and is believed to be mediated by caspase 6. However shorter fragments, potentially generated from the caspase 6 fragment, may be critical mediators. A key correlate of the hypothesis that caspase 6 cleavage is relevant to pathogenesis would be to show that a transgenic mouse expressing this fragment can develop a HD-like phenotype. In addition, the availability of such a mouse model would facilitate studies of Htt cleavage into smaller fragments. We have generated 3 independent lines of transgenic mice expressing the N-terminal 586 aa of Htt with a polyglutamine repeat length of 82 (N586-82Q), under the control of the prion promoter, similar to our previous (N171-82Q) fragment model. Preliminary data suggest they have a progressive HD-like phenotype. The behavioural phenotype was studied using the rotarod test, open field test, and fear conditioning. HD mice show a clear rotarod deficit by four months of age and are hyperactive starting at 5 months. At eight months of age, HD mice display a deficit in learning and memory as evidenced by fear conditioning. Mice also show visible signs of deterioration i.e. loss of body weight and clasping. Histologic studies demonstrate an abundance of Htt aggregates, especially cytoplasmic, throughout the forebrain. Soluble N-terminal fragments appear to accumulate over time, peaking at four months and decreasing by nine months. Efforts to identify the smallest fragment via mass spectrometry are underway. This model provides further validation of N586 mediated toxicity and its contribution to HD pathogenesis, and may provide an excellent means to study the role of post-translational modifications of huntingtin in HD pathogenesis.

Disclosures: E.A. Waldron, None; **T. Ratovitski**, None; **E. Chighladze**, None; **A.T. Tebbenkamp**, None; **R.K. Graham**, None; **M.R. Hayden**, None; **D.R. Borchelt**, None; **C.A. Ross**, None.

Nanosymposium

425. Huntington's Disease: Animal Models I

Location: Room 10

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 425.5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Seelye Trust stipend

NRCGD (NZ) Grant

HRC (NZ) Grant

Title: Increased epigenetic histone acetylation in post-mortem Huntington's disease brain

Authors: *P. J. NARAYAN[1,2], C. A. MCLEAN[3], A. SHEPPARD[3], R. L. M. FAULL[2], M. DRAGUNOW[1,2];
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Abstract: Increasing evidence suggests a pivotal role for epigenetics (eg: histone modifications, DNA methylation) in the expression and interaction of genes with environment, behavior and drug-based therapies for a wide range of neurological disorders (Narayan P, Dragunow M (2009) Pharmacology of epigenetics in brain disorders. Br J Pharmacol 159:285-303). Chemical modifications of histone proteins have been shown to be altered in cell culture and *in vivo* rodent models of Huntington's disease (HD), providing evidence that epigenetics is altered in the disease process. Using immunolabelling and Western blot techniques we investigated the global acetylation levels of histone molecules in the motor cortex of 9 HD and 6 neurologically normal, human post-mortem brains (matched for age/postmortem delay/sex). Cerebellum was labeled as a control region of the brain. Results indicate that histone modifications conducive to gene upregulation are markedly increased in the HD brain compared to normal brain in a region severely affected in the disease process. These findings implicate epigenetic dysregulation in HD. In the future, if these epigenetic changes in the brain are found to correlate with changes in the blood, they may serve as biomarkers which enable presymptomatic diagnosis and allow monitored therapeutic intervention tailored to each individual, before significant and irreversible brain atrophy takes place.

Disclosures: P.J. Narayan, None; **C.A. McLean**, None; **A. Sheppard**, None; **R.L.M. Faull**, None; **M. Dragunow**, None.

Nanosymposium

425. Huntington's Disease: Animal Models I

Location: Room 10

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 425.6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Medical Research Council UK Grant

Title: The search for Huntington's disease modifier genes using random mutagenesis in mice

Authors: *S. CORROCHANO[1], A. ACEVEDO-AROCENA[1], S. CARTER[1], D. C. RUBINSZTEIN[2], S. D. M. BROWN[1];
[1]Med. Res. Council, Harwell, United Kingdom; [2]Addenbrooke's Hosp., Cambridge, United Kingdom

Abstract: Huntington's disease (HD) is an autosomal dominant, progressive, fatal, neurodegenerative disorder caused by an expanded polyglutamine tract (PolyQ) in exon one of the Huntington's gene. Up to 70% of the variance in severity and age at onset is accounted for by the number of glutamine repeats. The rest of the variance is accounted for by environmental and genetic factors (modifiers). Genetic modifiers represent potential targets for the development of new therapeutic approaches to neurodegenerative disease. We have been pursuing a genome-wide genetic screen in order to identify modifier genes that modulate the HD phenotype in the mouse. Transgenic HD females (N171-82Q model) are mated to male mice carrying N-ethyl-N-nitrosourea (ENU) induced random point mutations. We have followed a dominant screen strategy, whereby first generation (F1) HD mice are examined. Initially, individual mice presenting a variation from the standard HD phenotype, presumably carrying an ENU-induced mutation that modifies the HD phenotype, are selected as "phenodeviants". Phenodeviant mice are identified through a variety of simple behavioural analyses including SHIRPA, survival and weight measurements from 8 weeks of age until end-stage (around 22 weeks of age). Once a phenodeviant mouse is identified, it is backcrossed to non-mutagenised C57BL/6J for inheritance testing. Once inheritance is established, a positional cloning approach follows to identify the gene responsible for the new phenotype. Several lines carrying inherited dominant modifiers have been identified, many of which are enhancers. One of the enhancers (Guthrie), which affects onset and severity of tremors, has been mapped to mouse chromosome 15. Other inherited ENU mutant lines are currently being mapped, including two enhancers: Poch and Draggen. We are also currently testing inheritance for two potential suppressor lines. Ultimately, the identification of ENU-induced HD modifying mutations will potentially help to elucidate pathological pathways involved in the disease as well as provide novel therapeutic targets.

Disclosures: S. Corrochano, None; **A. Acevedo-Arocena**, None; **S. Carter**, None; **D.C. Rubinsztein**, None; **S.D.M. Brown**, None.

Nanosymposium

425. Huntington's Disease: Animal Models I

Location: Room 10

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 425.7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS053912

Title: Nicotinamide improves motor deficits and upregulates PGC-1α and BDNF gene expression in a mouse model of Huntington's disease

Authors: T. HATHORN[1], A. SNYDER-KELLER[2,3], *A. MESSER[2,3];
[1]Ctr. for Neuropharm. and Neurosci., Albany Med. Col., Albany, NY; [2]Wadsworth Ctr., New York State Dept Hlth., ALBANY, NY; [3]Biomed. Sci., Univ. at Albany, Albany, NY

Abstract: Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by an expansion of the polyglutamine (polyQ) repeat in exon-1 in the Huntingtin gene (HTT). This results in misfolding and accumulation of the huntingtin (htt) protein, forming nuclear and cytoplasmic inclusions. HD is associated with dysregulation of gene expression as well as mitochondrial dysfunction. We hypothesized that by improving transcriptional regulation of genes necessary for energy metabolism, the HD motor phenotype would also improve. We therefore investigated the protective effects of nicotinamide (NAM), a well-characterized water-soluble vitamin that is an inhibitor of sirtuin1/ class III NAD^+-dependent histone deacetylase (HDAC). In this study, both mini osmotic pump and drinking water deliveries were tested at 250mg NAM/kg/day, using the B6.HDR6/1 transgenic mouse model. Results were similar for both modes of delivery, and there was no evidence of toxicity. We found that NAM treatment normalized mRNA levels of brain-derived neurotrophic factor (BDNF), and increased mRNA levels of PGC-1α, the master regulator of energy metabolism. Critically, NAM treatment was able to improve motor deficits associated with the HD phenotype, tested as time courses of open field, rotarod, and balance beam activities. These improvements were substantial, despite the fact that NAM did not appear to reduce htt aggregation, or to prevent late-stage weight loss. Our study therefore concludes that NAM or similar drugs may be beneficial in clinical treatment of the motor dysfunctions of HD. Combinatorial therapies consisting of small molecules plus approaches such as intrabody gene therapy can then be utilized to also combat the underlying aggregation phenotype and overall physiological decline.

Disclosures: T. Hathorn, None; **A. Messer**, None; **A. Snyder-Keller**, None.

Nanosymposium

425. Huntington's Disease: Animal Models I

Location: Room 10

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 425.8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foudnation Inc

NIH grants

Title: Longitudinal brain volumetric changes measured by structural MRI in preclinical studies of Huntington's disease mouse models

Authors: Y. CHENG[1], Z. HOU[2], Q. PENG[1], J. ZHANG[3], S. MORI[3], C. A. ROSS[1], *W. DUAN[1];
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Abstract: Huntington's disease (HD) is characterized by striatal atrophy that begins long before the onset of motor symptoms. Clinical diagnosis of HD is based on the unequivocal presence of otherwise unexplained extrapyramidal movement disorder. However, structural MRI imaging studies of HD patients found that striatal volumes begin to atrophy at least 11-12 years prior to expected onset, and then continue to shrink. In symptomatic HD, striatal volumes decline predictably with disease course. Previous studies suggest volumetric structural imaging measures could be considered as biomarkers for presymptomatic as well as symptomatic clinical trials of HD. HD mouse models show some behavioral and neuropathological features related to HD, and are widely used in preclinical therapeutic trials. However, there has been relatively little study of *in vivo* longitudinal brain atrophy in relation to other phenotypes and in response to experimental treatments in HD mouse models. We used micro MRI technology T2-weighted images combined with automated morphological analyses to monitor brain volume change longitudinally in mouse models of HD. We found that there are significant and progressive brain atrophy in the striatum, cortex and several other brain regions in fragment mouse models including both R6/2 mice and N171-82Q mice, and more moderate changes in full-length models. The progressive regional brain atrophy is positively correlated with motor behavioral deficits, and responds to experimental treatment with SSRI. This is the first longitudinally study to show structural MRI

measures can detect therapeutic effect in HD mouse models, suggesting that MRI could be considered as a potential biomarker to evaluate therapeutics in HD clinical trials.
Support: CHDI foundation Inc (WD) and NINDS (WD, CAR, JZ, SM).

Disclosures: Y. Cheng, None; **W. Duan**, None; **Z. Hou**, None; **Q. Peng**, None; **J. Zhang**, None; **S. Mori**, None; **C.A. Ross**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.1

Topic: C.07. Epilepsy

Support: NIH R01 NS064154 to RJB and HH

Title: Identification of epilepsy susceptibility loci by convergence of genome wide association and copy number variation data sets

Authors: *R. J. BUONO[1], I. HELBIG[2], H. ZHANG[3], K. WANG[3], F. LOHOFF[4], W. BERRETTINI[4], T. FERRARO[4], H. HAKONARSON[3];
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Abstract: Copy number variation (CNV) is a term used to describe the presence of DNA deletions, duplications and insertions across the human genome and is increasingly recognized as a source of human genetic variation in health and disease. Deletions at chromosome regions 15q11.2, 15q13.3 and 16p13.11 have been implicated as susceptibility factors for idiopathic generalized epilepsy (IGE). As part of an ongoing genome wide association study (GWAS) in a cohort of epilepsy patients, we genotyped 550,000 single nucleotide polymorphism (SNP) markers in patients with IGE (n=412) and patients with cryptogenic focal epilepsy (n=295) all of European descent. SNP genotype frequency and presence of CNVs were compared between epilepsy patients and control individuals (n=4415). We identified deletions at 15q11.2 (n=2), 15q13.3 (n=3) and 16p13.11 (n=2) in 2.4 % of the IGE patients compared to 0.5% in controls ($p = 1.5 \times 10^{-4}$). We also identified an association between duplications at the CNTN4 locus (n=2) and IGE (p=0.03). There were no CNVs found to be in association with focal epilepsy. GWAS data converged with CNV data in that SNPs in the MYH11 (16p13.11) and CNTN4 loci reached genome wide significance levels ($p = 1.3 \times 10^{-9}$ and 3.8×10^{-8} respectively) for IGE. In

conclusion, these data replicate and support previous work that showed deletions in these specific chromosomal regions are associated with a small percentage of IGE cases. In addition, this work demonstrates convergence of data from CNV and GWAS analyses to detect epilepsy susceptibility factors. Indeed, combined CNV and SNP variation in the MYH11 and CNTN4 gene regions identify these as putative epilepsy susceptibility loci. These data support the notion that common forms of epilepsy are caused by a combination of common genetic variation with subtle effects and rare mutations of large effect at specific candidate susceptibility loci.

Disclosures: R.J. Buono, None; **I. Helbig**, None; **H. Zhang**, None; **K. Wang**, None; **F. Lohoff**, None; **W. Berrettini**, None; **T. Ferraro**, None; **H. Hakonarson**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.2

Topic: C.07. Epilepsy

Title: Loss-of-function scn1a mutations associated with gefs+ and sudep

Authors: *Y. LIAO[1], H. KERTI[1], L. SÁEZ-HERNÁNDEZ[2], E. GUTIÉRREZ-DELICADO[2], N. PÉREZ-CASTELLANO[2], M. GARCÍA-TORRENT[2], J. VILLACAST[2], J. MACARRÓN2[2], R. SANZ[2], R. GUERRERO[2], B. GIRALDEZ[2], H. LERCHE[1], J. SERRATOSA[2];
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Abstract: Loss-of-function SCN1A mutations associated with $GEFS^+$ and SUDEP
Sudden unexpected death in epilepsy (SUDEP) is defined as a sudden death with unkown cause in epilepsy patients. Its underlying mechanisms are not kown. In the present study, we identified two de novo misssense mutations in SCN1A (encoding the brain sodium channel $Na_V1.1$) in two families with generalized epilepsy with febrile seizures plus ($GEFS^+$) and and an increased rate of SUDEP. Whole cell patch clamp experiments were performed in transfected tsA201 cells to characterize the functional consequence of these two mutations in human $Na_V1.1$ channels co-expressed with $beta_1$- and $beta_2$-subunits. Both mutant channels showed a clear loss-of-function, one showing no measurable sodium current, the other a significantly reduced sodium current density and a slowed rate of recovery from fast inactivation. The loss-of-function of $Na_V1.1$ predicts a decrease of sodium current amplitudes in GABAergic interneurons and a reduction of their firing rate, which could explain the occurrence of seizures, as has been shown for other

mutations previously. Since expression of $Na_V1.1$ has also been described in the sino-atrial node of the cardiac pacemaking system and also in brain stem nuclei, it would be conceivable that such mutations could alter cardiac rhythm or the central regulation of cardiac rhythm and/or breathing, which may explain the increased rate of SUDEP in those patients.

Disclosures: Y. Liao, None; **H. Kerti**, None; **L. Sáez-Hernández**, None; **E. Gutiérrez-Delicado**, None; **N. Pérez-Castellano**, None; **M. García-Torrent**, None; **J. Villacast**, None; **J. Macarrón2**, None; **R. Sanz**, None; **R. Guerrero**, None; **B. Giraldez**, None; **H. Lerche**, None; **J. Serratosa**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.3

Topic: C.07. Epilepsy

Support: NIH Grant 1RO1NS056314

Title: The role of Lis1 and Ndel1 in dynein-mediated retrograde transport in mature neurons

Authors: *J. P. PANDEY[1], M. T. MESNGON[2], S. HEBBAR[1], D. S. SMITH[1];
[1]Univ. South Carolina, COLUMBIA, SC; [2]Aberdeen Proving Ground, United States Army., Aberdeen,, MD

Abstract: Lissencephaly, which means smooth brain in Greek, is a rare brain formation disorder caused by defective neuronal migration during the early weeks of gestation, resulting in a lack of development of brain folds (gyri) and grooves (sulci). Patients exhibit increasingly severe seizures early in life. Children with lissencephaly are severely neurologically impaired and often die within several months of birth. Classical lissencephaly, characterized by agyria/pachygyria and neuronal mispositioning, is caused by Lis1 haploinsufficiency. Many studies have linked Lis1 to mitosis and migration of neurons during embryonic development. Similar defects in humans may be sufficient to cause the seizures observed in these patients. If developmental defects are solely responsible for the seizures then treatment options are very limited. However, Lis1 expression remains high in the adult brain. Lis1 binds to cytoplasmic dynein, a microtubule motor critical for retrograde axonal transport. It has been shown that LIS1 is required for dynein, but the underlying mechanism is poorly understood. . We showed earlier that Lis1 overexpression alters dynein distribution and microtubule organization in non-neuronal cells, and that Lis1 stimulates dynein's ATPase activity in vitro. If Lis1 reduction causes defects in

organelle transport, neuron function may be compromised, which in turn could cause seizures. Our studies are designed to determine if Lis1 malfunction in mature neurons can contribute to defects in axonal transport, leading to seizures. We have examined the role of Lis1 in dynein-dependent transport in cultured cells both neuronal and non-neuronal cells using steady state distribution and time-lapse analysis of motile vesicles. We find that Lis1 reduction inhibits dynein-dependent retrograde organelle motility in non-neuronal cells, and reduces the pool of the fast-moving organelles in embryonic cortical neurons. Moreover, we find that expression of a Lis1 point mutant unable to bind dynein does not trigger the same changes induced by expression of wild type Lis1. We are now examining how Lis1 reduction, or expression of the dynein-binding mutant, affects retrograde axon transport in adult rat sensory neurons. If Lis1 regulates transport of Lis1 in mature neurons, then knocking it out after brain development occurs may cause seizures or other types of neuronal defects.

Disclosures: **J.P. Pandey:** Employment; University of South Carolina. Research Grant; 1RO1NS056314. **M.T. Mesngon:** None. **S. Hebbar:** None. **D.S. Smith:** None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.4

Topic: C.07. Epilepsy

Support: FRAXA Research Foundation

NIH (HD43428, NS057839, NS035481, NS046769)

NSF (IOS-0725001)

Title: BC1 RNA and FMRP counterbalance the mGluR-MAPK translation pathway in brain

Authors: *J. ZHONG[1,3], S.-C. CHUANG[1,3], R. BIANCHI[1,3], W. ZHAO[1,3], G. PAUL[2], P. THAKKAR[2], A. A. FENTON[1,5], R. K. S. WONG[1,3,4], H. TIEDGE[1,3,4];
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Abstract: Non-protein-coding BC1 RNA is a regulator of mRNA translation. We show that BC1

RNA imposes a tonic brake on group I mGluR-stimulated translation in neurons. In the absence of BC1 RNA, excess protein synthesis leads to heightened neuronal excitability, which manifests as prolonged epileptiform discharges in hippocampal slices and susceptibility to audiogenic seizures. The epileptic tendency in BC1-/- mice is protein synthesis dependent, and can be rescued by the mGluR5 antagonist MPEP and inhibitors of the MAP kinase-signaling pathway, but not by the mTOR inhibitor rapamycin. We observed similar mGluR-MAPK-dependent neuronal hyperexcitability in mice lacking the fragile X mental retardation protein (FMRP), another repressor of mRNA translation. To examine the functional cooperation between BC1 RNA and FMRP, we generated double mutant mice. Mice lacking both repressors have more severe epileptic phenotypes and learning deficits than either of the single mutants, suggesting that BC1 RNA and FMRP functionally converge on the group I mGluR-MAPK translation pathway.

Disclosures: J. Zhong, None; **S. Chuang**, None; **R. Bianchi**, None; **W. Zhao**, None; **G. Paul**, None; **P. Thakkar**, None; **A.A. Fenton**, None; **R.K.S. Wong**, None; **H. Tiedge**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.5

Topic: C.07. Epilepsy

Support: NIH Grant NS048811

Epilepsy Foundation/Milken Family

Title: Epigenetic regulation of the NR2B gene in an experimental epilepsy model

Authors: R. R. PARRISH, *F. D. LUBIN;
Dept. Neurobiol, Univ. Alabama Birmingham, BIRMINGHAM, AL

Abstract: A number of molecular and cellular changes, including N-methyl d-aspartate (NMDA) receptor kinetics and activation, contribute to the development of epilepsy. The NR2B NMDA receptor subunit is highly expressed in the hippocampus and deregulation of NR2B has been implicated in both animal epilepsy models and human epilepsy. The transcriptional mechanisms regulating aberrant expression of NR2B during the development of epilepsy are incompletely understood. In the present study, we investigated whether or not altered epigenetic mechanisms contribute to NR2B gene expression and subsequent protein expression in the

kainate (KA) model of epilepsy. First, we determined the NR2B gene expression profile in hippocampal subregions at three time-points; 1 h following KA-induced status epilepticus (SE), 24 hour post-SE, and 6 weeks post-SE. Real-time PCR revealed that NR2B mRNA levels significantly increased in area CA1 of the hippocampus 1 h after SE. This suggests that KA-induced prolonged seizure activity or SE stimulates the expression of the NR2B gene that may underlie changes in hippocampal organization and contribute to epilepsy in this animal model. Next, we found decreased NR2B mRNA levels in CA1 and CA3 hippocampal subfields at 24 h post-SE. At 6 weeks post-SE when animals were displaying spontaneous reoccurring seizures, NR2B mRNA levels in area CA1 of hippocampus remained significantly decreased, while NR2B mRNA levels returned to baseline levels in CA3 and DG hippocampal subfields when compared to controls. Interestingly, at 6 weeks post-SE hippocampal NR2A mRNA levels remained unchanged compared to control animals. Additionally, we sought to correlate NR2B gene expression changes with NR2B protein levels. Together, these results suggest that alterations in NR2B expression are both spatially and temporally regulated in hippocampus following KA-treatment and epilepsy onset. In conjunction with NR2B gene expression changes, we found that NR2B DNA methylation was also dynamically regulated in a hippocampal subregion-specific manner following KA-induced SE. Pilot DNA methyltransferase (DNMT) inhibitor studies are underway for assessment of the affect of altering hippocampal NR2B DNA methylation on NR2B expression after KA treatment *in vivo* and on seizure frequency in hippocampal slice preparations from KA-treated animals *ex vivo*. Indeed, our studies using DNMT and histone deacetylase inhibitors show promise as therapeutic agents for the recovery of aberrant NR2B in the KA epilepsy model, which might provide novel loci for the treatment of human epilepsy.

Disclosures: R.R. Parrish, None; **F.D. Lubin**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.6

Topic: C.07. Epilepsy

Support: R01 NS-049307 (to HB)

5R01NS055829-04 (to HB)

The Betsy and Jonathan Blattmachr family

Title: Diffusion tensor imaging in absence epilepsy

Authors: *A. M. MISHRA[1], D. COMAN[4], N. DANIELSON[1], C. BASHYAL[1], M. COQUILLETTE[1], M. NEGISHI[4], M. VESTAL[1], B. KILLORY[1], T. VAN RIJN[5], P. M. EDELBROEK[6], R. T. CONSTABLE[4], F. HYDER[4], G. E. J. M. VAN LUIJTELAAR[5], H. BLUMENFELD[1,2,3];
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Abstract: Epilepsy is a disease of abnormal interaction of brain networks. Based on the diffusion properties of water in tissue, diffusion tensor imaging (DTI) provides a noninvasive method for assessing white matter microstructure, allowing detection of differences between healthy and non-healthy brain networks. The purpose of this study is to assess potential morphological changes in childhood absence epilepsy (CAE) in human patients, and in a well-established animal model of CAE. DTI was performed on 9 children with CAE and 9 age and sex matched normal children. We used 10 Wistar albino Glaxo rats of Rijswijk (WAG/Rij), a genetic model of CAE, without treatment (n=9), and 9 WAG/Rij rats after 4 months of treatment started at 30d with the antiabsence medicine ethosuximide (ESX). Children were imaged on a 3T and rats on a 9.4T system. Eigenvalues were derived from the diffusion tensor matrix, and different DTI metrics, including fractional anisotropy (FA), were calculated using Bioimagesuite (www.bioimagesuite.org, Yale University). Registration of the imaging data involved rigid-body and non-linear registration of T2-weighted images via a tensor b-spline algorithm; the composite transformation was then applied to all FA maps and other DTI metrics. After thresholding FA maps, a two sample t-test was performed to assess FA differences. We found that in children with CAE there was significantly decreased FA in the posterior white matter, forceps major region. Decreased FA may imply affected myelin integrity, or loss of axonal density connecting intensely involved brain regions during absence seizures. Of note, the occipital and medial parietal cortex were found to be involved in separate fMRI studies of these subjects. We found that the anterior corpus callosum of treated rats showed increased FA compared to untreated animals, suggesting there is some recovery in morphology of neuronal pathways after ESX treatment. In a previous study, we reported that the tissues integrity of the anterior corpus callosum was compromised in epileptic rats. In conclusion, we have shown that DTI is sensitive for the detection of subtle white matter changes in children with CAE, and can detect improvement in FA abnormalities in a treatment model. These results are important for better understanding the CAE disease process, and suggest that DTI may ultimately serve as a disease biomarker in human therapeutic trials in children with CAE.

Disclosures: A.M. Mishra, None; **D. Coman**, None; **N. Danielson**, None; **C. Bashyal**, None; **M. Coquillette**, None; **M. Negishi**, None; **M. Vestal**, None; **B. Killory**, None; **T. van Rijn**, None; **P.M. Edelbroek**, None; **R.T. Constable**, None; **F. Hyder**, None; **G.E.J.M. van Luijtelaar**, None; **H. Blumenfeld**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.7

Topic: C.07. Epilepsy

Support: NIH R01 NS055829

Betsy and Jonathan Blattmachr family

Epilepsy Foundation Post-Doctoral Research Training Fellowship

Title: Cluster analysis of fMRI data in typical childhood absence seizures

Authors: *X. BAI[1], B. KILLORY[1], J. GUO[1], M. VESTAL[1], R. BERMAN[1], M. NEGISHI[2], E. NOVOTNY[1,3], R. CONSTABLE[2], H. BLUMENFELD[1,4,5];
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Abstract: Typical childhood absence seizures consist of brief 5-10 second episodes of unresponsiveness associated with 3Hz “spike-wave” discharges (SWD) on EEG. Simultaneous EEG and functional magnetic resonance imaging (EEG-fMRI) recordings provide a powerful tool which can be used to investigate brain blood oxygen level-dependent (BOLD) changes relative to seizures. Previously, fMRI data has been analyzed by a model-dependent statistical approach, where the hemodynamic response function (HRF) is explicitly pre-defined. However, there is some important evidence that the actual hemodynamic response may vary from one brain area to another. In the present work, we used a model-free clustering approach to investigate fMRI changes before and after childhood absence seizures. A total of 51 seizures in 8 pediatric patients with typical childhood absence epilepsy were acquired during EEG-fMRI. Our analysis procedure involved three steps: (1) We averaged the time courses of single voxels during the time period from -20s to +40 s relative to seizure onset across patients. (2) Next, 116 gray matter anatomic volumes of interest (VOI) were segmented from the SPM MRI template and a mean fMRI time course was computed within each VOI. The correlations between each pair of mean time courses of VOIs were computed and analyzed to determine the number of expected clusters by a hierarchical clustering method. (3) Finally, correlations between pairs of time courses of voxels were computed. A k-mean method was performed to create partitions of voxels exhibiting similar time courses, using the number of expected clusters determined in step 2. Our analysis showed that 116 anatomic VOIs can be separated into four clusters by using hierarchical clustering method. By using the k-mean method, we observed the partition of areas into clusters as follows: 1) thalamus and occipital cortex; 2) lateral and part of medial parietal cortex, medial temporal, basal ganglia, and cerebellum; 3) part of medial frontal, lateral frontal, orbital frontal,

and rolandic cortices; 4) part of medial frontal, medial parietal, medial temporal cortices and insula. Except for in the first cluster of thalamus and occipital cortex, fMRI changes of the other three clusters were significantly different from the conventional HRF. In summary, our results demonstrate a complex sequence of changes in absence seizures not detectible by conventional HRF modeling. The clustering method can effectively identify regions of similar activation. Finally, these results suggest that the clustering method might be a very helpful tool for analysis of activation patterns in fMRI for other types of generalized seizures.

Disclosures: X. Bai, None; **B. Killory**, None; **J. Guo**, None; **M. Vestal**, None; **R. Berman**, None; **M. Negishi**, None; **E. Novotny**, None; **R. Constable**, None; **H. Blumenfeld**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.8

Topic: C.07. Epilepsy

Support: NIH R01 NS055829

Betsy and Jonathan Blattmachr Family

NIH MSTP TG 2T32GM07205

Title: Variability in attention performance and fMRI signal changes during typical childhood absence seizures

Authors: *J. N. GUO[1], N. B. DANIELSON[2], B. D. KILLORY[3], X. BAI[2], M. NEGISHI[4], M. VESTAL[2], R. BERMAN[2], C. VEGA[5], M. SPANN[5], E. J. NOVOTNY[2], R. T. CONSTABLE[4], H. BLUMENFELD[6];
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Abstract: Childhood absence epilepsy (CAE) is characterized by brief 3-4 Hz spike-and-wave discharges on electroencephalography (EEG), often accompanied by lapses in attention. Interestingly, the degree of behavioral impairment is highly variable across patients and seizures. Previous studies have shown that although absence seizures are considered generalized events, they commonly exhibit early focal changes in areas associated with the attention network such as

the orbital/medial frontal cortex, medial/lateral parietal cortex and thalamus. Our main hypothesis is that these focal changes may be related to the variability in behavior observed during absence seizures. In the present study, we analyzed 175 seizures in 13 pediatric patients with CAE. Simultaneous EEG-fMRI at 3T was performed on all patients as they performed either a continuous performance task (CPT) or a repetitive tapping task (RTT) to measure attention. The time course and amplitude of blood oxygen level-dependent (BOLD) fMRI signal changes were analyzed using a combination of statistical parametric mapping (SPM) and in-house software. We found that seizures caused impaired performance during tasks. On average, the impairment was more severe on CPT than on RTT testing. However, omission error rates were variable on both tasks from patient to patient and even from seizure to seizure within the same patient. Analysis of EEG revealed a possible association between seizure duration and degree of impairment. However, in some cases omission errors occurred even with very brief spike-wave seizures. During seizures, a dynamic sequence of fMRI changes was observed in orbital frontal, parietal, and other cortical areas as well as the thalamus. However, the fMRI changes were also highly variable and not clearly related to features of the EEG recordings. Ongoing analyses are investigating possible relationships between variable fMRI timecourses in cortical and subcortical attention networks and variable task performance. By establishing the relationship between behavior and BOLD signals, we hope to provide insight into the mechanisms by which attention and consciousness are disrupted during absence seizures.

Disclosures: J.N. Guo, None; **N.B. Danielson**, None; **B.D. Killory**, None; **X. Bai**, None; **M. Negishi**, None; **M. Vestal**, None; **R. Berman**, None; **C. Vega**, None; **M. Spann**, None; **E.J. Novotny**, None; **R.T. Constable**, None; **H. Blumenfeld**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.9

Topic: C.07. Epilepsy

Support: NIH R01 NS055829

Betsy and Jonathan Blattmachr family

Title: Impaired attention and network connectivity in childhood absence epilepsy

Authors: B. D. KILLORY[1], X. BAI[2], M. NEGISHI[2], C. VEGA[2], M. SPANN[2], M. VESTAL[2], R. BERMAN[2], N. DANIELSON[2], J. GUO[2], S. FOOTE[2], C. MCAULIFFE[2], E. J. NOVOTNY,

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[1]Neurosurg., Barrow Neurolog. Institute, St. Joseph's Hosp., Phoenix, AZ; [2]Yale Univ. Sch. Med., New Haven, CT

Abstract: Patients with childhood absence epilepsy (CAE) experience 3-10 second seizures characterized by 3-4 Hz spike and wave discharges on electroencephalogram (EEG) that cause impaired consciousness. CAE represents approximately 10% of all diagnosed childhood epilepsies, and causes impaired attention and social dysfunction in school-age children, even with medical control of seizures. The mechanism of impaired interictal attention is currently unknown, and in this study we sought to investigate disruption in attention networks using blood oxygen level dependent functional magnetic resonance imaging (BOLD fMRI).
We used the Continuous Performance Task (CPT) of attentional vigilance and a control motor task - the Repetitive Tapping Task (RTT) - to examine interictal attention in children with CAE. Each subject underwent simultaneous 3 T fMRI-EEG and CPT/RTT testing. Using Statistical Parametric mapping software, CPT and RTT activations above fixation were modeled after regressing out both head motion and seizures. Attention regions of interest (ROI) were defined as CPT>RTT regions that survived direct comparison in a two-sample t-test. Correlation analyses were performed between fMRI signal increase (beta value) within each ROI and subjects' performance on CPT. A smaller cohort of 16 patients and 16 controls, matched for age and gender, underwent fixation-only fMRI-EEG testing. Resting functional connectivity was calculated using the attention ROIs as seed areas in this cohort.
As expected, all behavioral measures reflecting inattention were significantly higher in patients. Attention ROI's included the bilateral insula/frontal operculum (In/FO) and medial frontal lobe. Correlation analysis revealed that for all measures of inattention on the CPT task, impaired attention was associated with decreased medial frontal activation during CPT. Analysis of resting functional connectivity between attention ROI's revealed an overall decrease in this 'attention network' in patients over controls. Patients demonstrated significantly impaired connectivity between the right In/FO and medial frontal lobes, and the degree of this impairment correlated with an index of seizure severity.
CAE patients perform more poorly on a task of attentional vigilance, and our fMRI results demonstrate impaired function in an attention network comprising anterior insula and medial frontal cortex, related to disease severity. These findings provide an anatomical and functional basis for interictal impaired attention in CAE, which may allow improved treatments to be developed targeted at these networks.

Disclosures: B.D. Killory, None; **X. Bai**, None; **M. Negishi**, None; **M. Vestal**, None; **S. Foote**, None; **R. Berman**, None; **C. Vega**, None; **M. Spann**, None; **N. Danielson**, None; **J. Guo**, None; **E.J. Novotny**, None; **R.T. Constable**, None; **H. Blumenfeld**, None; **C. McAuliffe**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.10

Topic: C.07. Epilepsy

Support: NIH Grant 4044010091

NIH Grant 4044010051

Epilepsy Foundation Pre-Doctoral Research Training Fellowship

Title: Effects of a mutation in the GABAA receptor β3 subunit associated with childhood absence epilepsy

Authors: *K. N. GURBA[1], C. C. HERNANDEZ[2], N. HU[2], W.-Y. LO[2], R. L. MACDONALD[2]; [1]Neurosci., [2]Neurol., Vanderbilt Univ., NASHVILLE, TN

Abstract: Reduced inhibition has been implicated in many epilepsies and epilepsy syndromes, including childhood absence epilepsy (CAE). The major inhibitory neurotransmitter in the brain is γ-aminobutyric acid (GABA), which produces fast and tonic inhibition by acting at GABAA receptors (GABARs). Recently, a point mutation in the N-terminus of the GABAA receptor β3 subunit (β3(G32R)) was identified in a family affected with CAE. To characterize the assembly, trafficking, and function of receptors containing mutant subunits, we co-expressed wild-type and/or mutant β3 subunits together with α1 and γ2L subunits in HEK293T cells. When we assessed surface expression levels with flow cytometry, surface biotinylation, and Western blotting, we found that co-expression of the β3(G32R) subunit was associated with unchanged α1 subunit levels, increased β3 subunit levels, and slightly decreased γ2L subunit levels; conversely, total cellular expression of all subunits remained unchanged. These observations are consistent with a stoichiometric change in receptor subunit composition. To evaluate the impact of the β3(G32R) mutation on GABAR function we used whole-cell concentration-jump and single-channel recording. Receptors containing the β3(G32R) subunit had reduced current amplitude and single-channel opening duration, accelerated deactivation, and multiple changes in kinetics of desensitization. Some of these functional effects may be due to altered receptor stoichiometry, as the G32R mutation seems to favor incorporation of a β3 subunit rather than a γ2L subunit in the fifth receptor subunit position. The full effect of the β3(G32R) mutation, however, is likely more complex, because a change in stoichiometry cannot fully explain the observed changes in receptor kinetics. Interestingly, we also observed that β3(G32R) subunits were more extensively glycosylated than wild-type β3 subunits. β3 subunits contain three potential N-glycosylation sites, including one at residue N33. To determine if the G32R mutation increased glycosylation at N33 and, furthermore, if altered glycosylation was responsible for the changes in subunit expression, we inactivated N33 in both β3 and β3(G32R) subunits by mutating the glycosylated asparagine to glutamine (N33Q). Upon further investigation, we found that the G32R mutation increased glycosylation at N33 and that glycan occupancy at that site affected receptor function; however, the mutation also affected the function of β3 subunit-

containing GABARs independent of glycosylation. We conclude that the β3(G32R) mutation may contribute to CAE by reducing GABAergic inhibition through multiple mechanisms.

Disclosures: K.N. Gurba, None; **C.C. Hernandez**, None; **N. Hu**, None; **W. Lo**, None; **R.L. Macdonald**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.11

Topic: B.02. Ligand Gated Ion Channels

Support: NIH Grant 5R01NS051590

Title: The nonsense mutation GABRG2(Q40X) decreased mRNA and altered $GABA_A$ receptor assembly and trafficking and was rescued by gentamicin-induced stop-codon readthrough

Authors: *X. HUANG[1], J. TOPLON[2], N. HU[2], R. MACDONALD[3], M. TIAN[4];
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Abstract: The GABRG2 nonsense mutation, Q40X, was identified in twin sisters with severe myoclonic epilepsy of infancy who were heterozygous for the mutation (Hirose S et al., Epilepsy Res, S1: S206, 2006). We introduced the mutation into a bacterial artificial chromosome (BAC) construct containing the human GABRG2 gene. When expressed in HEK 293T cells, both wild-type and mutant BACs generated several truncated mRNAs. Truncated mRNAs transcribed from wild-type and mutant BACs and normal human brain total RNAs had the same nucleotide sequences, and the Q40X mutation did not enhance magnitude or relative distribution of their expression. Compared to wild-type full length mRNA transcripts, mutant full length transcripts were decreased in amount by 40%, suggesting that the mutation induced nonsense mediated mRNA decay (NMD). If all mutant full length transcripts were degraded by NMD, patients would have epilepsy due to GABRG2 haploinsufficiency. However, haploinsufficient $\gamma2^{+/-}$ knockout mice do not have epilepsy, suggesting that there may be additional cellular abnormalities associated with the mutant gene product. NMD efficiency varies among cell types, and more mutant mRNA would be expressed in cells with low NMD efficiency. If not degraded by NMD, mature mutant mRNA would be translated to an immature γ2 subunit truncated at the end of the signal peptide. An HA-epitope tag was added to the signal peptide of $\gamma2S^{FLAG}$ subunit cDNA construct containing a FLAG-tag in the mature peptide. When expressed in HEK293T

cells, the wild-type $\gamma2S^{HA/FLAG}$ subunit showed both HA- and FLAG-bands, but the $\gamma2S(Q40X)^{HA/FLAG}$ subunit had only HA-bands. The total amount of HA-bands found with $\gamma2S(Q40X)^{HA/FLAG}$ subunits was also increased compared to wild-type subunits. We coexpressed α1 and β2 subunits with mutant γ2S(Q40X) subunits (1:1:1 cDNA ratio) (homozygous expression) or with wild-type γ2 and mutant γ2S(Q40X) subunits (1:1:0.5:0.5 cDNA ratio) (heterozygous expression). We determined total and surface subunit expression patterns using flow cytometry and patch clamp recording. Results were compared to coexpressed α1β2γ2S and and to α1β2 subunits coexpressed with an empty pcDNA vector (null allele condition). The Q40X mutation significantly altered wild-type α1β2γ2S receptor surface and total expression pattern, as well as surface receptor function. Aminoglycosides have been reported to suppress PTCs, resulting in translation of full length protein. The aminoglycoside gentamicin significantly improved γ2S(Q40X) mutant subunit expression and receptor trafficking and function.

Disclosures: X. Huang, None; **J. Toplon**, None; **N. Hu**, None; **R. Macdonald**, None; **M. Tian**, None.

Nanosymposium

426. Genetic Epilepsies

Location: Room 24A

Time: Monday, November 15, 2010, 1:00 pm - 4:00 pm

Program Number: 426.12

Topic: B.02. Ligand Gated Ion Channels

Support: NIH 5R01NS051590

Title: The intronic mutation, ivs6+2t->g, associated with cae altered gabrg2 mrna intron splicing and generated stable mutant gabaa receptor γ2 subunits that altered assembly and trafficking and induced cellular stress

Authors: *M. TIAN[1], R. MACDONALD[2];
[1]Pharmacology, Neurol., Vanderbilt Univ. Med. Ctr., NASHVILLE, TN; [2]Neurology, Pharmacol. and Mol. Physiol. and Biophysics, Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: An autosomal dominant intron 6 splice donor site mutation, IVS6+2TàG, in the $GABA_A$ receptor γ2 subunit GABRG2 gene is associated with childhood absence epilepsy and febrile seizures (Kananura et al., Arch Neurol., 59:1137, 2002). We used minigenes and bacterial artificial chromosomes to study splicing of wild-type and mutant GABRG2 mRNAs in HEK 293T cells and demonstrated that the mutant intron 6 was spliced out between the wild-type splice acceptor site and a cryptic site downstream of the wild-type donor site. Mature mutant

mRNA was subject to nonsense mediated decay (NMD), but undegraded mRNAs were expressed as stable proteins (γ2-PTC subunits). Because NMD is not 100% efficient and its efficiency may vary among cell types, γ2-PTC subunit levels would be higher in neurons with low NMD efficiency. The γ2-PTC subunits had no predicted transmembrane domains but were not secreted and were trapped in the ER. When expressed alone, γ2-PTC subunit total levels were higher than γ2L or γ2S subunit total levels. The γ2-PTC subunits oligomerized with low efficiently to α1 and β2 subunits, but co-expression with α1 and β2 subunits did not result in trafficking of γ2-PTC subunits to the cell membrane. Colocalization of α1 and γ2-PTC subunits with α1β2γ2-PTC subunit coexpression was significantly decreased in permeablized cells and to background levels in unpermeablized cells. When α1 and β2 subunits were coexpressed with mutant γ2-PTC subunits (1:1:1 cDNA ratio) (homozygous expression) or with wild-type and mutant γ2 subunits (1:1:0.5:0.5 cDNA ratio) (heterozygous expression), surface and total levels of α1, β2, and wild-type γ2 subunits were similar to co-expression with an empty pcDNA vector. GABAergic currents recorded from cells with α1β2γ2-PTC subunit coexpression had peak currents close to α1β2 receptors and were inhibited by 10 μM Zn^{2+}. These data suggested that the GABRG2(IVS6+2TàG) mutation generated a stable, but nonfunctional, protein, thus producing disinhibition due to GABRG2 haploinsufficiency. However, $\gamma 2^{+/-}$ knockout mice do not have epilepsy, suggesting that there may be additional cellular abnormalities associated with the mutant subunit. When the γ2-PTC subunits were overexpressed in HEK293T cells, the ER stress marker protein BIP was increasesd, suggesting that γ2-PTC subunits could induce ER stress. In summary, the GABRG2(IVS6+2TàG) mutation generated a stable mutant protein that was trapped in the ER, and although γ2-PTC subunits oligomerized with α1β2 subunits, they were not assembled into $GABA_A$ receptors and did not alter expression and trafficking of other subunits. When expressed in cells at high levels, they induced ER stress.

Disclosures: M. Tian, None; **R. Macdonald**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR

Title: Involvement of the IL-1 system in neutrophil and proinflammatory monocyte recruitment after SCI

Authors: D. BASTIEN, N. VALLIÈRES, N. FORTIN, *S. LACROIX;
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Abstract: CNS injury stimulates the expression of several proinflammatory cytokines and chemokines, some of which including MCP-1 (also known as CCL2), KC (CXCL1), and MIP-2 (CXCL2) act to recruit Gr-1$^+$ leukocytes at lesion sites. While earlier studies have reported that neutrophils and monocytes/macrophages contribute to secondary tissue loss after spinal cord injury (SCI), recent work has shown that depletion of Gr-1$^+$ leukocytes compromised tissue healing and worsened functional recovery. Here, we demonstrate that astrocytes distributed throughout the spinal cord initially contribute to early neuroinflammation by rapidly synthesizing MCP-1, KC, and MIP-2, from 3 up to 12 hours post-SCI. Chemokine expression by astrocytes was followed by the infiltration of blood-derived immune cells, such as type I proinflammatory monocytes (M1) and neutrophils, into the lesion site and nearby damaged areas. Astrocytes from mice deficient in MyD88 signaling produced significantly less MCP-1 and MIP-2 and were unable to synthesize KC. Analysis of the contribution of MyD88-dependent receptors revealed that the astrocytic expression of MCP-1, KC, and MIP-2 was mediated by the IL-1 receptor (IL-1R1), and not by TLR2 or TLR4. Flow cytometry analysis of cells recovered from the spinal cord of MyD88- and IL-1R1-knockout mice confirmed the presence of significantly fewer M1 monocytes and the almost complete absence of neutrophils at 12 hrs and 4 days post-SCI. Importantly, both IL-1α and IL-1β were found to contribute to the recruitment of these immune cells. Together, these results indicate that IL-1α and IL-1β are rapidly released at sites of SCI where they regulate the entry of neutrophils and, to a lesser extent, M1 monocytes through activation of the IL-1R1/MyD88 pathway. *Work supported by the CIHR.*

Disclosures: D. Bastien, None; **N. Vallières**, None; **S. Lacroix**, None; **N. Fortin**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: MDA

Title: CD4+ T cells induce alternatively activated microglia in the mSOD1 mouse model of ALS

Authors: *J. S. HENKEL, D. R. BEERS, B. LIAO, W. ZHAO, S. H. APPEL;
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Abstract: The innate and adaptive immune systems play pivotal and interdependent roles regulating the rate of disease progression in amyotrophic lateral sclerosis (ALS). In a transgenic mouse model of ALS overexpressing mutant superoxide dismutase (mSOD1), CD4+ T cells have been shown to slow disease progression by inducing a stable phase and influencing microglial activation, indicating a dialogue between T cells and microglia. These data suggest that T cells may be slowing neurodegeneration by directing microglia toward a neuroprotective alternatively activated M2 phenotype during the stable phase. To determine whether disease-stage dependent T cells modulate microglial M1/M2 phenotypes in mSOD1 mice, T cells in blood and lymph nodes were analyzed by FACS at critical time points during disease. CD4+CD25+ and CD25+FoxP3+ T cells were increased in blood and lymph nodes during the stable phase of disease but declined as the rate of progression accelerated. Specific microglial markers in lumbar spinal cord of mSOD1 mice were analyzed during the same critical time points using quantitative RT-PCR and compared with their wild-type (WT) littermates. During the stable phase, there was an increase in the expression of protective microglial M2 phenotypic marker such as BDNF, YM1, and CX3CR1. When isolated *ex vivo*, microglia from stable phase mSOD1 mice also expressed enhanced M2 markers, attenuated M1 markers, and more importantly, enhanced the survival of co-cultured motoneurons compared to WT microglia. Following the stable phase and during the rapid progression phase, the balance shifted to an M1 phenotype, with increased expressions of NOX2 and IL-1β mRNA in lumbar spinal cord of mSOD1 mice compared to their WT littermates. *Ex vivo* isolated mSOD1 microglia harvested from mSOD1 mice during the rapidly progressing phase expressed enhanced M1 markers, decreased M2, and were neurotoxic compared to WT microglia. To verify that the T cells present during the stable phase were neuroprotective CD4+ or CD4+CD25+ T cells from stable or rapidly progressing phases were passively transferred, without *ex vivo* activation, into mSOD1/RAG2$^{-/-}$. mSOD1 stable phase CD4+ T cells prolonged survival compared with WT CD4+ T cells or with rapid progression phase mSOD1 CD4+ T cells. In summary, CD4+CD25+ T cells present during the stable phase slow the rate of progression at least partially by inducing an M2 neuroprotective microglial phenotype. However, later in disease, this protective phenotype is lost and the injurious M1 phenotype prevails. These glial/T cell interactions establish novel targets for therapeutic intervention and validate immunomodulatory therapies in ALS.

Disclosures: J.S. Henkel, None; **D.R. Beers**, None; **B. Liao**, None; **W. Zhao**, None; **S.H. Appel**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Lois Pope LIFE Fellowship

The Miami Project to Cure Paralysis

University of Miami Fellowship

Title: The pro-inflammatory role of eph receptors following spinal cord injury

Authors: *S. J. GLASS, Z. ZHUANG, M. H. THEUS, D. J. LIEBL;
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Abstract: Activation of the inflammatory response following spinal cord injury (SCI) is thought to exacerbate cellular damage; however, the mechanisms initiating this process are poorly understood. Microglia and astrocytes, the resident cells of the central nervous system (CNS), are recruited as part of the innate immune response following traumatic insult. Once triggered, these cells can initiate an inflammatory response by secreting pro-inflammatory cytokines and chemokines. Here, we examined the role of EphB3/A4 receptors in the immune response following SCI. We show that EphB3 and EphA4 are present on microglia and astrocytes in the adult spinal cord and that stimulation with ephrinB3 ligand in culture mediates the release of several pro-inflammatory cytokines. Eph receptor stimulation in astrocytes also increases mRNA transcript levels of multiple cytokines known to play a role in the immune response, which is significantly attenuated in the absence of EphB3 and EphA4. Cytokine protein release, as measured by ELISA, is also increased in both wild type (WT) astrocytes and microglia following stimulation. Interestingly, this affect is attenuated in astrocytes but not microglia derived from EphB3/A4 double knockout (EphB3/A4$^{-/-}$) mice. To test whether inflammatory cytokine production following SCI is mediated by EphB3/A4, tissue was collected from sham or SCI WT and EphB3/A4$^{-/-}$ mice at 4 and 24 hours and analyzed using cytometric bead array. We show that the expression of interleukin-6 (IL-6) and chemokine ligand 2 (CCL-2) are significantly increased at 4 and 24 hour post-SCI in WT mice, which is significantly attenuated in the EphB3/A4$^{-/-}$ mice. These data suggest that EphB3 and/or EphA4 play a novel role in initiating the acute pro-inflammatory response following SCI.

Disclosures: S.J. Glass, None; **Z. Zhuang**, None; **M.H. Theus**, None; **D.J. Liebl**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH

Craig Neilsen Foundation

Title: P2X4 receptor regulates inflammasome activation following injury to the spinal cord

Authors: *J. P. DE RIVERO VACCARI[1], G. YURCISIN[1], W. DIETRICH[1], Y. DE KONINCK[2], D. BASTIEN[3], S. LACROIX[3], R. KEANE[4];
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Abstract: P2X4 and P2X7 are the predominant P2X receptor subtypes expressed on immune cells. Recent studies have revealed that these receptor subtypes traffic between intracellular compartments and the plasma membrane and form protein interactions with each other to regulate ATP-dependent signaling. Our recent studies have shown that P2X7 receptors form protein interactions with the NLRP1 inflammasome in neurons, but whether P2X4 receptors regulate inflammasome signaling is largely unknown. Here, P2X4-/- and C57BL/6 wild type (WT) control mice were subjected to moderate contusion injury (70 kdyn) using the Infinite Horizon SCI device. Animals were sacrificed and cords were removed at 30 minutes, 6 hours and 3 days after trauma. Immunoblot analysis of spinal cord lysates showed that cleaved casapase-1 and IL-1β were elevated in the P2X4-/- mice when compared to WT mice. In contrast, the levels of IL-18 and P2X7 were not significantly different between the WT and KO micegroups at any of the time points tested. In situ hybridization studies indicated that neurons in the spinal cord express P2X4 receptors, consistent with our previous studies demonstrating NLRP1 inflammasome activation in neurons. Lesion volume analysis revealed that the P2X4-/- animals had significantly less damage and more tissue sparing in the cord at 45 days after injury when compared to WT mice. TThese results show that P2X4 receptors regulate inflammasome signaling involving caspase-1 activation and IL-1β processing in neurons and suggest that .the lack of P2X4 receptor expression influences tissue preservation following SCI.

Disclosures: J.P. De Rivero Vaccari, None; **G. Yurcisin**, None; **W. Dietrich**, None; **Y. De Koninck**, None; **D. Bastien**, None; **S. Lacroix**, None; **R. Keane**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS NS037846

The Craig H. Neilsen Foundation

Title: Activating toll-like receptor 2 promotes macrophage-mediated regeneration without concurrent neurotoxicity

Authors: *J. C. GENSEL[1], K. A. BECKWITH[2], P. G. POPOVICH[1];
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Abstract: Zymosan-activated macrophages (ZAMs) promote regeneration of injured peripheral and central nervous system axons but also cause tissue pathology and cell death (Gensel et al., 2009). Here, we test the hypothesis that the divergent effects of ZAMs are induced by the concurrent activation of toll-like receptor 2 (TLR2) and dectin-1 receptors. With the goal of uncoupling the beneficial and detrimental effects of zymosan activation to promote regeneration without toxicity, we compared the effects of activating macrophages in vivo or in vitro with selective agonists of TLR2 or dectin-1 receptors. All responses were compared to those achieved with zymosan activation alone. Intraspinal microinjection of dectin-1 agonists mimicked the effects of zymosan, i.e., activated macrophages co-localized with zone of focal necrosis, overt axon pathology and demyelination. In contrast, intraspinal injection of Pam2CSK4, a synthetic TLR2 agonist, elicited a robust macrophage response but with little axon or myelin pathology. In vitro, zymosan or Pam2CSK4-stimulation of macrophages increased axon growth from adult DRG neurons, even in the presence of proteoglycans, i.e., potent inhibitory substrates that dominate sites of CNS injury. Importantly, Pam2CSK4-activated macrophages promoted axon growth without also causing toxicity. In contrast, activation of macrophages via dectin-1 caused neurotoxicity. Collectively these data suggest that selective activation of macrophage TLR2 can promote axonal regeneration without simultaneously eliciting neurotoxic effector functions.

Disclosures: J.C. Gensel, None; **P.G. Popovich**, None; **K.A. Beckwith**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Institute of Neurological Disorders and Stroke (R01NS061973)

Title: Thrombospondin-1 is detrimental to neuronal survival in acute CNS injury: The immune link

Authors: *J. WALSH, J. KIPNIS;
Univ. of Virginia, Charlottesville, VA

Abstract: Thrombospondin 1 (TSP1) is an extracellular matrix protein secreted by glial cells and is known for both its major role in cell migration, angiogenesis, and immuno-modulatory functions. Recently, TSP1 has gained even more of attention from neuroscientists, since beyond its well-studied properties it has been shown to control synaptogenesis in developing brain, to play a role in stroke, and to be important in adult neurogenesis. However, despite the fact that it has been shown that TSP1 protein levels increase after spinal cord injury, very little has been done to determine what role it is playing at the site of injury. With this in mind, we decided to explore the effects that TSP1 is having on traumatic CNS injury using an optic nerve injury as our model. We have found that TSP1, but not its closely related family member TSP2, is upregulated at the site of injury, but is downregulated distally on the same nerve. This TSP1 response to injury appears to be maladaptive, as $TSP1^{-/-}$ mice show decreased secondary degeneration of retinal ganglion cells (RGCs) compared to wild type mice. The effect of TSP1 in CNS injury is not mediated through TSP-induced synaptogenesis, as neuroprotection is not affected by treatment with the alpha2delta1 receptor antagonist, gabapentin, which was previously shown to block TSP1-induced synaptogenesis. One strong candidate pathway for this neuroprotective response is an increased protective T cell response to the injury. Indeed, $TSP1^{-/-}$ mice exhibit an increased number of activated CD4+ splenocytes within hours of CNS injury. Correlating with this heightened immune activation in $TSP1^{-/-}$ mice are their decreased levels of active TGFβ, a molecule known to be important in differentiation of peripherally-induced regulatory T cells. Therefore, we show that TSP1 is an important modulator of the adaptive immune system whose presence attenuates neuroprotection after traumatic CNS injury.

Disclosures: J. Walsh, None; **J. Kipnis**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: PVA

AHFMR

CIHR

CRC

Title: Elucidating the role of microglia in secondary degeneration following spinal cord injury in real-time using two-photon microscopy

Authors: *D. P. STIRLING, K. A. CUMMINS, P. K. STYS;
Clin. Neurosciences, Hotchkiss Brain Institute/ Univ. of Calgary, Calgary, AB, Canada

Abstract: Spinal cord injury (SCI) induces a robust inflammatory response mediated in part by the rapid activation of microglia, however, whether they play a destructive or protective role after SCI remains unclear. To better understand the role of microglia following SCI we utilized two-photon microscopy to ablate cervical dorsal column axons (primary injury) from live murine spinal cord preparations and documented the subsequent changes in axon, myelin and microglia as the lesion evolved (secondary injury) over time. Towards this goal we generated double transgenic mice that express EGFP (enhanced green fluorescent protein) in microglia (CX3CR1-GFP+/-), YFP (yellow fluorescent protein) in axons (Thy1-YFP+), and applied lipophilic fluorescent dyes to visualize myelin. In control conditions, time-lapse recordings of live spinal cord white matter revealed parallel-aligned YFP+ axon cylinders ensheathed in myelin with largely constant diameters along the length of the axon. Few spheroids or other morphological signs of axonal degeneration were present. In support, microglia were highly ramified and frequently extended/retracted processes. In contrast to baseline conditions, transected axons formed end bulbs that began to swell both rostral and caudal to injury within 5 minutes of ablation. Microglia responded immediately to laser ablation by extending processes several microns to wall of the ablation site. As microglia density increased over time, transected axons formed end bulbs that “died back” from the ablation site both rostral and caudal to injury. Swelling of axonal end bulbs partially detached from their myelin sheath were a common finding remote to the ablation site along with axonal spheroids tightly encased in myelin. In distinction, large swollen empty tubes of myelin remained at the border of the ablation site. Fiber loss adjacent to the ablation site, and in close apposition with microglia, was a prominent feature; Lesion width at 4 hours post-ablation was increased by ~70% versus ~ 26% at 5 minutes after injury. These data suggest that significant numbers of axons that were spared by the original laser ablation succumbed to secondary degeneration in a delayed fashion. We conclude that laser ablation is a useful model to examine spinal microglial activation and changes in myelin and axons in real-time using two-photon microscopy. Studies are currently underway to examine the

effects of microglial modulators on axon and myelin sparing following axonal injury.

Disclosures: D.P. Stirling, None; **K.A. Cummins**, None; **P.K. Stys**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY05690

The Sheldon and Miriam Adelson Medical Research Foundation

Title: Role of inflammatory mediators in stimulating optic nerve regeneration

Authors: *L. I. BENOWITZ[1], T. KURIMOTO[1], S. NAKAO[2], G. HABBOUB[1], H.-Y. GILBERT[1], K. OMURA[1], Y. YIN[1], A. HAFEZI-MOGHADAM[2];
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[2]Angiogenesis lab, Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Med, Sch., Boston, MA

Abstract: Although inflammation often has destructive effects in the nervous system, the induction of a controlled inflammatory response in the eye transforms retinal ganglion cells (RGCs) into an active growth state and improves cell survival after optic nerve injury. Inflammation stimulated by lens injury, intraocular peripheral nerve implants, or zymosan injections enables RGCs to regenerate axons for considerable distances beyond the site of optic nerve damage (1,2). Prior work from this lab indicates that the primary mediator of this phenomenon is oncomodulin (Ocm), a small, Ca2+-binding protein (3). However, the role of various inflammatory cells in producing Ocm and promoting axon regeneration is not fully understood, and others have maintained that macrophages are unimportant, and that CNTF derived from astrocytes mediates the effects of inflammation on optic nerve regeneration. Here, we examined the role of different inflammatory mediators and Ocm on axon regeneration in mice. In mice lacking the gene for either CD18, a part of the complement receptor, or PSGL-1 (P-selectin glycoprotein ligand 1), a protein involved in leukocyte recruitment, regeneration was strongly attenuated following intraocular injection of Zymosan. In addition, in wild-type mice, regeneration induced by Zymosan injections was fully blocked with a peptide that prevents Ocm from binding to its receptor. Inflammation-induced regeneration can be augmented by elevation

of cAMP. This enhancement is associated with increased binding of Ocm to its receptor, and is likewise eliminated with the blocking peptide. These results support a central role for Ocm and macrophages in mediating the pro-regenerative effects of intraocular inflammation. Deletion of the gene for PTEN, a phosphatase that suppresses signaling through the PI3 kinase-Akt pathway, acts synergistically with intraocular inflammation and cAMP elevation, and enables RGCs to regenerate axons through the entire length of the optic nerve, across the optic chiasm, and into the thalamus. Thus, inflammatory mediators can profoundly affect CNS axon regeneration.
(1) Leon, S., Yin, Y. et al., J Neurosci 20, 4615-26 (2000); (2) Berry M, et al., J Neurocytol 25:147-170(1996); (3) Yin, Y. et al. Nat Neurosci 9, 843-52 (2006); (4)Park, K. et al., Science 322, 963-966 (2008)

Disclosures: L.I. Benowitz, None; **T. Kurimoto**, None; **S. Nakao**, None; **G. Habboub**, None; **H. Gilbert**, None; **K. Omura**, None; **Y. Yin**, None; **A. Hafezi-Moghadam**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS

Dana Foundation

UCR Biomed Sci grants

Title: CCL21 is neither sufficient nor necessary for lymphocytic influx into the CNS, but does direct T cell localization during early Toxoplasma gondii infection of the CNS

Authors: S. NOOR, D. D. LO, E. H. WILSON, *M. J. CARSON;
Div. of Biomed. Sci., Univ. of California Riverside, RIVERSIDE, CA

Abstract: The CNS is immune privileged but not immune isolated. For example, ~30% of the world's (and ~20% of the US) population have lifelong CNS infections of the intracellular protozoan parasite, Toxoplasma gondii that do not cause overt CNS dysfunction. Chronic influx of IFNg producing lymphocytes into the CNS is absolutely essential to prevent lethal Toxoplasmosis-inflicted brain damage such as that observed in individuals with severe T cell deficiency. Recently, lymphocytes expressing CCR7 (the receptor for CCL21) have been

demonstrated to play essential roles in controlling T. gondii infection (Noor et al. 2010). Here we examine how expression of CCL21 within the murine CNS regulates lymphocytic influx, migration and activation. Therefore, we generated transgenic mice in which the astrocytic promoter GFAP drives constitutive CCL21 expression that was ~2-fold higher than that the levels induced in the CNS of wild-type mice with chronic T. gondii infections. Atlhough CCL21 expression was bioavailable and able to support homeostatic T cell proliferation in cervical lymph nodes draining the CNS in GFAP-CCL21 mice, CCL21 expression by itself was insufficient to cause T cell accumulation in the CNS. By contrast, transgenic expression of CCL21 within the pancreas was sufficient to trigger and organize T cells into neo-lymphoid structures. CCL21 does appear to play a CNS-specific immunomodulatory role because ~2-fold higher numbers of CD4+ T cells were found in the CNS of GFAP-CCL21 mice than in the CNS of wild-type mice following T. gondii infection. By using CCL21 KO mice, we also found that lymphocyte influx into the CNS following T. gondii infection did not require CCL21. However, in the absence of CCL21, lymphocytes were found to accumulate preferentially in perivascular instead of parenchymal spaces following infection. Taken together these data suggest that CCL21 may play a CNS-specific role in directing essential lymphocytc responses able to control chronic CNS infections without disrupting CNS function.

Disclosures: S. Noor, None; **D.D. Lo**, None; **E.H. Wilson**, None; **M.J. Carson**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant NS061973

Title: The role of regulatory T lymphocytes in neuronal survival after CNS injury

Authors: *J. KIPNIS[1], J. T. WALSH[2];
[1]Neurosci., Univ. Virginia, CHARLOTTESVLE, VA; [2]Univ. of Virginia, Charlottesville, VA

Abstract: Though an active immune response and inflammation have been traditionally viewed as being a maladaptive response to CNS injury, there is mounting evidence that a well-controlled adaptive immune response is necessary for achieving optimal neuroprotection. Self-reactive T lymphocytes, specifically, are able to augment neuroprotection after CNS injury, however this autoimmune response, without a proper regulation, might turn into destructive and, thus,

outweigh the benefit. Naturally occurring regulatory (CD4+CD25+Foxp3+) T cells (Treg) have been shown to restrict autoimmune responses and, thus, to limit neuroprotection. The precise mechanism, however, underlying the role of Treg cells in neuroprotective immune response after CNS injury in still not well understood. We show here that CNS injury leads to activation of effector T cells in the CNS-draining deep cervical lymph nodes and to downregulation of the suppressive function of Treg cells. Moreover, we show that TLR signaling in involved in CNS-mediated alleviation of Treg suppressive function. Depletion of Treg in a short term allows a more efficient neuroprotective response, however, a prolonged depletion of Treg results in an increased neuroinflammation and enhanced neuronal degeneration. Most interestingly, our results indicate that the Treg role in CNS injury differs among different mouse strains and, thus, while depletion of Treg in one mouse strain is neuroprotective, it could be neurodestructive in a different strain. Our results propose that neuroprotective therapies based on modulation of Treg numbers and function need to be taken with an extreme caution since both prolonged depletion of Treg as well as their boost could result in either neurodestruction and exacerbation of neuronal damage, depending on the genetic background of the individual.

Disclosures: J. Kipnis, None; **J.T. Walsh**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.11

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant R01 NS037570

Title: Oligodendrocyte-sequestered antigens lead to T cell recruitment to the CNS

Authors: *M. G. HARRIS, B. CLARKSON, C. LING, J. KARMAN, M. SANDOR, Z. FABRY;
Univ. Wisconsin, MADISON, WI

Abstract: Multiple sclerosis (MS) is the most common demyelinating disease and is thought to be mediated by immune effector cells specific to myelin antigens. Oligodendrocytes are the only myelinating cells in the CNS and, therefore, play a crucial role in neuronal survival and function. Contrary to the traditional understanding that the primary destruction of myelin is by macrophages, evidence shows that oligodendrocyte death precedes macrophage infiltration and phagocytosis in newly forming MS lesions (Barnett and Prineas, 2004). Further, oligodendrocyte

death is critical to the onset of experimental autoimmune encephalomyelitis (EAE), the animal model of MS (Hisahara et al., 2000; Hovelmeyer et al., 2005). This is likely important in disease pathogenesis, as dying oligodendrocytes are a rich source of antigens that become liberated following neural insult, becoming targets for further immune cell attack. Our laboratory has designed a novel transgenic mouse model to test the hypothesis that multiple sequestered oligodendrocyte antigens are sampled by immune cells during EAE, leading to the priming and recruitment of antigen-specific T cells and the amplification of the disease. In these mice (referred to as CNPCre-OVA-PCC mice), inducible Cre recombinase function allows for triple antigenic epitope expression in oligodendrocytes.
Following induction of triple non-myelin antigen expression, EAE was induced with MOG_{35-55} peptide in the CNPCre-OVA-PCC mice. To test the priming and homing of antigen-specific T cells into the CNS, 9 days following EAE induction CFSE-labeled antigen-specific CD4+ and CD8+ T cells were adoptively transferred into the mice. We show here that when neuroinflammation is established in EAE, antigen-specific CD8+ T cells are primed and home to the CNS of the transgenic animals more than in the non-transgenic littermates. These data suggest that initial neuroinflammation may be causing oligodendrocyte death leading to the subsequent release of cytoplasmic antigens and the recruitment of effector T cells.

Disclosures: M.G. Harris, None; **B. Clarkson**, None; **C. Ling**, None; **J. Karman**, None; **M. Sandor**, None; **Z. Fabry**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Muscular Dystrophy Association

NIH Grant (1 R03 NS070050-01)

Texas Methodist Foundation

Title: The effects of $CD4^+CD25^+$ regulatory T cells on microglia in a mouse model of inherited ALS

Authors: W. ZHAO, D. BEERS, J. HENKEL, B. LIAO, *S. H. APPEL;
Methodist Neurol Inst., HOUSTON, TX

Abstract:
Activated microglia and infiltrating immune cells are neuropathological hallmarks in amyotrophic lateral sclerosis (ALS), a rapidly progressing motoneuron disease leading to death. The absence of $CD4^+$ T cells accelerates disease progression and shortens survival in transgenic mice overexpressing the G93A mutation of Cu^{2+}/Zn^{2+} superoxide dismutase (mSOD1), a model of inherited ALS. Cytotoxic markers of microglial activation (NOX2 and TNF-α) were upregulated in spinal cords of mSOD1/$CD4^{-/-}$ mice compared with their mSOD1/$CD4^{+/-}$ littermates. These data suggest that $CD4^+$ T cells can provide neuroprotection by suppressing cytotoxic activation of microglia; motoneuron injury is non-cell-autonomous in ALS and partially depends upon a well orchestrated dialogue between T cells and microglia. Previous studies have not defined the specific subpopulation of $CD4^+$ T cells and the mechanisms for this suppression. Therefore, the purpose of this study was to define the interactions between T cells and microglial activation in ALS. Primary adult microglia were prepared from spinal cords of mSOD1 or wild-type mice. $CD4^+CD25^+$ (Tregs) and $CD4^+CD25^-$ (Teffs) were isolated from spleen and lymph nodes of mSOD1 mice, and then co-cultured with microglia. Protein and mRNA were analyzed by ELISA and quantitative RT-PCR.
Our results showed that NOX2 mRNA expression, a cytotoxic index of microglial activation, was elevated in adult mSOD1 microglia compared with wild-type microglia. When co-cultured with mSOD1 Tregs, microglial NOX2 mRNA was decreased. In contrast, mSOD1 Teffs had minimal effect on microglial NOX2 mRNA levels; T cells expressed minimal NOX2 mRNA. mSOD1 Tregs produced more IL-4 protein, an anti-inflammatory cytokine, than mSOD1 Teffs. IL-4 inhibitory antibody blocked the suppression of microglial activation induced by mSOD1 Tregs. Although CTLA-4 is involved in cell-contact pathways of Tregs, inhibitory antibodies to CTLA-4 did not block the suppression of microglial activation by mSOD1 Tregs. IL-10 and TGF-β blocking antibodies were also introduced into microglia and T cells cocultures. However, these two antibodies did not reverse the suppressive effect of mSOD1 Tregs on microglial activation.
In conclusion, mSOD1 Tregs inhibit microglial activation by secreting IL-4, but independent of CTLA-4, IL-10 and TGF-β engagement. These results suggest that mSOD1 Tregs suppress microglial toxicity, thus providing motoneuron protection in ALS. Ability of utilizing the $CD4^+CD25^+$ Tregs to decrease cytotoxicity of microglial activation may offer a novel therapeutic option for ALS.

Disclosures: W. Zhao, None; **D. Beers**, None; **J. Henkel**, None; **B. Liao**, None; **S.H. Appel**, None.

Nanosymposium

427. Neuroinflammation and CNS Injury

Location: Room 31C

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 427.13

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH EY 05690

Alseres Pharmaceuticals, Inc.

Miriam and Sheldon Adelsion Medical Research Foundation

Patterson Family Trust

Title: Oncomodulin links intraocular inflammation to optic nerve regeneration

Authors: *Y. YIN, H.-Y. GILBERT, T. KURIMOTO, L. I. BENOWITZ;
F.M. Kirby Neurobio. Center, Children's Hospital, Harvard Med. Sch., BOSTON, MA

Abstract: The immune response that accompanies central nervous system (CNS) injury can affect neurological outcome in both positive and negative ways. In the optic nerve, a CNS pathway that normally fails to regenerate when damaged, intraocular inflammation dramatically enhances the survival of injured retinal ganglion cells (RGCs) and enables these cells to regenerate axons well beyond the site of optic nerve damage. Within a day of either injuring the lens or injecting zymosan into the eye, inflammatory cells enter the vitreous and express high levels of oncomodulin (Ocm) mRNA and protein. Ocm binds to RGCs with high affinity and specificity in a cAMP-dependent manner. In the presence of mannose, an abundant constituent of the vitreous, and agents that elevate intracellular cAMP, Ocm induces extensive outgrowth from RGCs in culture. The binding site of Ocm to its receptor lies in its N-terminus, although its axon-promoting activity requires both N- and C-terminal regions. The latter region includes two active Ca2+-binding sites, but Ca2+-binding per se is not required for the axon-promoting effects of Ocm. The downstream signaling activated by Ocm involves Ca2+-calmodulin kinase II, CREB phosphorylation, and gene transcription. In vivo, intravitreal injection of microspheres that release Ocm and a cAMP analog leads to dramatic axon regeneration into the distal optic nerve in mature rats. Conversely, inflammation-induced regeneration is suppressed by either an Ocm-derived peptide that prevents Ocm from binding to its receptor or a neutralizing antibody. These results show that Ocm plays a central role in mediating the effects of intraocular inflammation on optic nerve regeneration.

Disclosures: Y. Yin, None; **H. Gilbert**, None; **T. Kurimoto**, None; **L.I. Benowitz**, None.

Nanosymposium

428. Neurotoxicity and Neurodegeneration IV

Location: Room 23A

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 428.1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 NS052677

Title: Metal chelators coupled with nanoparticles as potential therapeutic agents for Alzheimer's disease

Authors: M. A. SMITH[1], G. LIU[2], P. MEN[2], X. ZHU[1], R. J. CASTELLANI[3], *G. PERRY[4]; [1]Dept. of Pathology, Case Western Reserve Univ., Cleveland, OH; [2]Dept. of Radiology, Univ. of Utah, Salt Lake City, UT; [3]Dept. of Pathology, Univ. of Maryland, Baltimore, MD; [4]Col. of Sci., The Univ. of Texas At San Antonio, SAN ANTONIO, TX

Abstract: Alzheimer's disease (AD) is a devastating neuro-degenerative disorder characterized by the progressive and irreversible loss of memory followed by complete dementia. Despite the disease's high prevalence and great economic and social burden, an explicative etiology or viable cure is not available. Great effort has been made to better understand the disease's pathogenesis, and to develop more effective therapeutic agents. However, success is greatly hampered by the presence of the blood-brain barrier that limits a large number of potential therapeutics from entering the brain. Nanoparticle-mediated drug delivery is one of the few valuable tools for overcoming this impediment and its application as a potential AD treatment shows promise. Our groups are developing novel nanoparticle delivery mechanisms for chelation agents as possible therapeutics for AD because several metals are found excessive in the AD brain and may play a role in the disease development. Specifically, we are focused on a novel approach involving transport of iron chelation agents into and out of the brain by nanoparticles. This approach may provide a safer and more effective means of simultaneously reducing several toxic metals in the AD brain. It may also provide insights into the mechanisms of AD pathophysiology, and prove useful in treating other iron-associated neurodegenerative diseases such as Friedreich's ataxia, Parkinson's disease, Huntington's disease and Hallervorden-Spatz Syndrome. It is important to note that the use of nanoparticle-mediated transport to facilitate toxicant excretion from diseased sites in the body may advance nanoparticle technology, which is currently focused on targeted drug delivery for disease prevention and treatment.

Disclosures: **M.A. Smith:** Speakers Bureau/Honoraria; Medivation, Pfizer. Ownership Interest; Neuropharm, Neurotez, Pancea, Voyager. Consultant/Advisory Board; Anavex, Medivation, Neurotez, Aria Neurosciences. **G. Liu:** None. **P. Men:** None. **X. Zhu:** Medivation. **R.J. Castellani:** None. **G. Perry:** Ownership Interest; Neurotez. Consultant/Advisory Board; Takeda.

Nanosymposium

428. Neurotoxicity and Neurodegeneration IV

Location: Room 23A

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 428.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Chinese Science & technology Ministry

Peace Hospital

Uppsala University

Swedish medical research Council

Title: Engineered nanoparticles from metals induce upregulation of nitric oxide and exacerbate pathophysiology of spinal cord injury in the rat

Authors: *L. FENG[1], A. SHARMA[2], H. S. SHARMA[2];
[1]Neurol., Bethune Intl. Peace Hosp., Hebi Province, China; [2]Surgical Sciences, Anesthesiol. & Intensive Care medicine, Univ. Hopital, Uppsala Univ., Uppsala, Sweden

Abstract: Previous reports from our laboratory suggest that nanoparticles exposure exacerbates pathophysiology of hyperthermia and spinal cord injury (SCI). The possible mechanisms of nanoparticles induced exacerbation of neuropathological changes are still not well understood. Since SCI upregulates a potent free radical gas, nitric oxide production, it appears that nanoparticles that are known to induce oxidative stress may aggravate spinal cord pathology probably mediated through mechanism involving nitric oxide. This hypothesis was examined in this investigation in a rat model of SCI.
Rats were treated with either Cu or Ag nanoparticles (50-60 nm, 50 mg/kg, i.p.) daily for 7 days. On the 8th day a focal SCI was made in their right dorsal horn of the T10-11 segment using a unilateral incision (3 mm deep and 5 mm long) under Equithesin anesthesia. Five h after injury, the animals were perfused with 4 % neutral paraformaldehyde through cardiac puncture preceded with a brief saline rinse. The spinal cord was dissected out and the C4, T5, T9, T10-11 and T12 segments were dissected out. About 30 µm thick Vibratome sections were cut from each spinal cord segment and examined for all isoforms of the nitric oxide synthase (NOS) responsible for nitric oxide production, i.e., neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS) or endothelial nitric oxide synthase (eNOS) using commercial antibodies and standard protocol. Saline treated spinal cord injured animals were used as controls. In addition, neuronal and myelin damage of the spinal cord was also examined using Nissl and luxol fast blue (LFB) staining respectively.

Rats treated with saline showed a mild to moderate upregulation of nNOS in the injured spinal cord as well as around the lesion site, e.g., T9 and the T12 segments. On the other hand, eNOS expression was most pronounced in the injured and the T12 segments. The iNOS expression was most marked in the T9 segment. The remote segments such as C5 and T4 did not exhibit any isofroms of NOS expression. Interestingly, when SCI was performed on Cu or Ag nanoparticles treated rats, massive expression of nNOS and eNOS were seen in all the spinal cord segments (from C5 to T12). In these rats iNOS expression was also seen in all the segments except C5. The intensity of NOS immunostaining was most marked in the Cu treated injured rats. Damage to motoneruons and loss of LFB staining was most common in the areas showing NOS expression in all SCI groups. This indicates that nanoparticles exposure could exacerbate NOS upregulation that could be instrumental in exacerbation of spinal cord pathology.

Disclosures: L. Feng, None; **A. Sharma**, None; **H.S. Sharma**, None.

Nanosymposium

428. Neurotoxicity and Neurodegeneration IV

Location: Room 23A

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 428.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: SSNN, Romania

Cluj-Napoca University

Uppsala University

EOARD, London, UK

Medical Res Council 2710, Sweden

Title: Cerebrolysin treatment reduces oxidative stress and pathophysiology of brain injury caused by engineered nanoparticles following heat stress in the rat

Authors: *D. F. MURESANU[1], R. PATNAIK[2], A. SHARMA[3], H. SHARMA[3];
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Abstract: Previous reports from our laboratory show that chronic exposure of nanoparticles e.g., Ag, Cu or Al (50 mg/kg, i.p. once daily for 7 days) leads to breakdown of the blood-brain barrier (BBB) and brain pathology by inducing oxidative stress and increased nitric oxide production in the rat. These rats when subjected to additional heat stress, their brain pathology and oxidative stress parameters were exacerbated. This indicates that military personnel exposed to desert environmental heat could be more prone to stress induced brain dysfunction. Thus, exploration of new therapeutic measures is needed to induce neuroprotection in nanoparticles induced brain damage in heat stress situations. Keeping these views in mind we explored the role of a potent neuroprotective agent Cerebrolysin (a select combination of several neurotrophic factors) in reducing nanoparticles induced brain pathology.
Cerebrolysin was administered in Male Sprague Dawley rats (body weight 200-250 g, age 18 to 22 weeks old) 5 ml/kg (i.v.) once daily for 14 days. On day 7th of the cerebrolysin treatment, these animals start receiving nanoparticles (Ag, Al or Cu) in separate groups for another 7 days. The control group received saline instead of Cerebrolysin under identical conditions. Cerebrolysin treated rats when tested on the 14th day did not show any sensory and cognitive dysfunction on Rota rod performance, grid walking, inclined plane angle and stride length tests using standard procedures unlike the saline treated animals. Cerebrolysin treated rats also did not show any significant increase in Myeloperoxidase (MP) and Malondialdehyde (MD) levels in nanoparticles treated rats as compared to saline treated animals. Furthermore, no decline in glutathione (GH) level was seen in cerebrolysin treated animals after nanoparticles exposure in contrast to saline treated rats. No increase in the number of nNOS positive neurons or appearance of dark or distorted nerve cells were seen in the cortex, hippocampus, cerebellum, thalamus and hypothalamus in Cerebrolysin treated rats after nanoparticles exposure. When these nanoparticles treated animals were subjected to 4 h heat stress at 38° C, the brain pathology, oxidative stress or nNOS expression were near normal levels noted in the cerebrolysin treated group. Whereas, saline treated animals showed massive neuronal damage, increased oxidative stress production and nNOS expression. Taken together these observations are the first to demonstrate that chronic Cerebrolysin treatment is neuroprotective following nanoparticles exposure and is also able to thwart further exacerbation following hyperthermia induced heat stress.

Disclosures: D.F. Muresanu: None. **R. Patnaik:** None. **A. Sharma:** None. **H. Sharma:** None.

Nanosymposium

428. Neurotoxicity and Neurodegeneration IV

Location: Room 23A

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 428.4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Peptide-engineered polylactide-co-glycolide (PLGA) nanoparticles for brain delivery of drugs: In vivo experiments and proof of concept

Authors: *G. TOSI[1], R. A. FANO[2], L. BADIALI[3], L. BONDIOLI[1], B. RUOZI[1], A. VERGONI[3], F. RIVASI[2], R. BENASSI[4], M. VANDELLI[1], F. FORNI[1];
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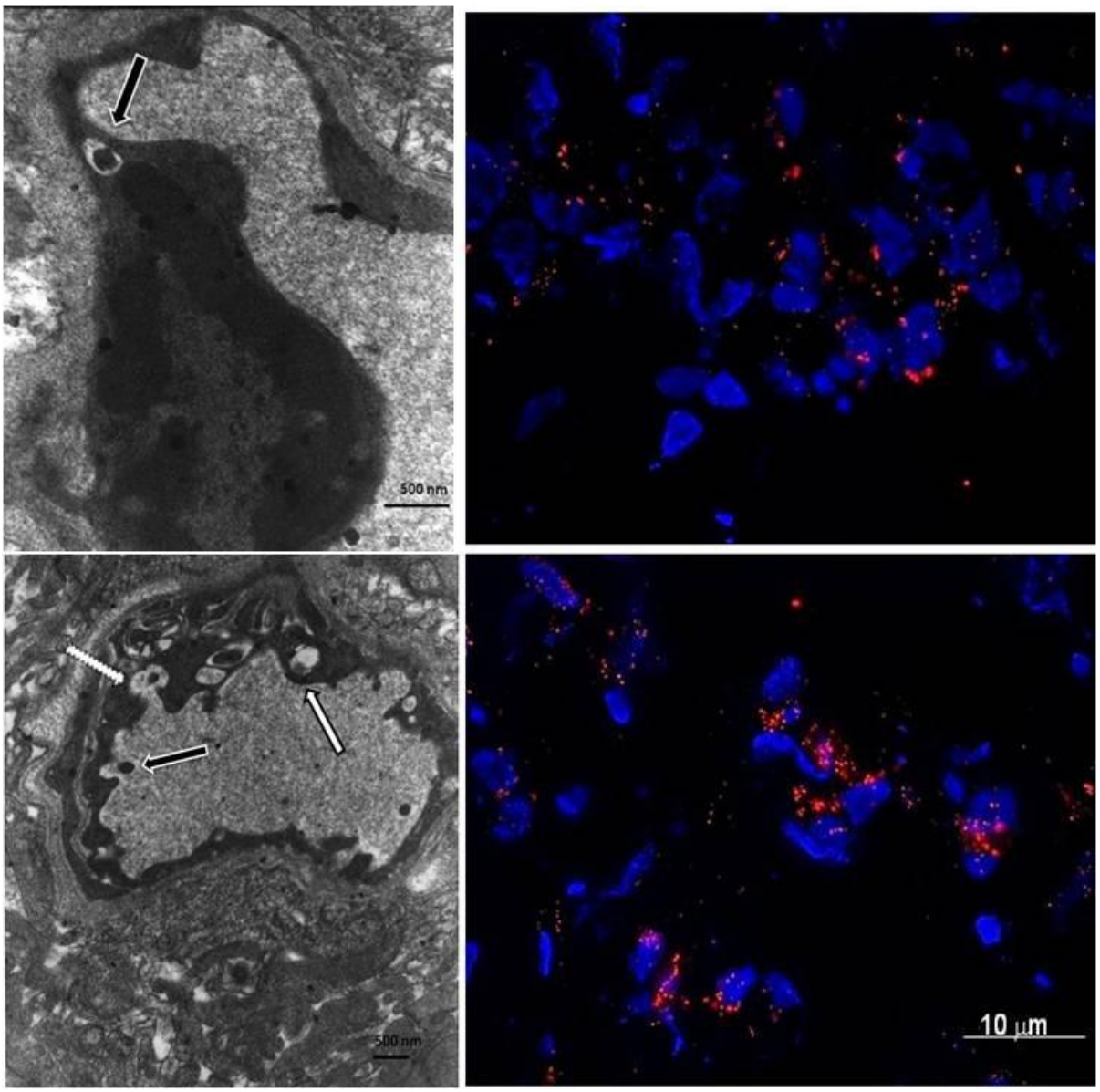
Abstract: Drug delivery to the Central Nervous System (CNS) represents a huge challenge for all neuroscientists owing to the presence of the Blood-Brain Barrier (BBB) hampering the influx to the brain of most of the drugs, enzymes, gene materials. Nanotechnology, based on polymeric nanoparticles (Np) and liposomes, could be an useful tool for the delivery of the drugs in the brain if they are planned for crossing the BBB. This goal can be achieved specifically engineering the Np surface in order to take advantage of the BBB crossing pathways, such as endocytosis or transcytosis. We applied this approach modifying polylactide-co-glycolide (PLGA) Np with two different peptides to produce highly selective nanosystems able to enter the brain after i.v. administration in the rats [Costantino L. et al. (2005). J Control Rel 108, 84-96; Tosi G. et al. (2007) J. Control Rel 122, 1-9]. The administration of decorated Np with a simil-opioid peptide (planned and synthesized in our laboratories) allows a variety of P-glycoprotein substrate to cross the BBB at a rate of 15-20% of the injected dose, as microscopy technique (confocal, fluorescent), biodistribution and pharmacological studies proved [Vergoni A.V. et al. (2009) Nanomedicine (NBM) 5, 369-377] These systems cross the BBB via an endocytic mechanism pointed out by an electron microscopy procedure (fig. 1). On the contrary, the Np decorated with a Leptin fragment should be able to take advantage of specific BBB-leptin receptors (Ob-R). In vivo experiments pointed out the efficacy of these leptin modified Np in the brain delivery and the trancytosis mechanism of the BBB crossing (fig. 2). Any anoretic effect of the Leptin-fragment covering the Np was exclude by food-intake experiments.
Figure 1. Left: Electron microscopy image of multiple mechanisms of simil-opioid-Np interaction with BBB endothelial cells; Right: Fig. 2. Brain images after iv administration of Leptin-derived peptide- Np. Red spots are due to Np labeled with TRICT and blue-spots are brain nuclei with DAPI.



Disclosures: G. Tosi: None. **R.A. Fano:** None. **L. Badiali:** None. **L. Bondioli:** None. **B. Ruozi:** None. **A. Vergoni:** None. **F. Rivasi:** None. **R. Benassi:** None. **M. Vandelli:** None. **F. Forni:** None.

Nanosymposium

428. Neurotoxicity and Neurodegeneration IV

Location: Room 23A

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 428.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: GIC(IT491-10) and SAIOTEK(S-PE08UN52) Basque Government

Title: Age-dependent upregulation of neuronal nitric oxide synthase, heat shock protein 72 kD responses and neurotoxicity following acute methamphetamine administration

Authors: *J. V. LAFUENTE[1], R. PATNAIK[2,3], A. SHARMA[2], H. S. SHARMA[2];
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Abstract: The possibility that methamphetamine induced neurotoxicity depends on upregulation of nitric oxide synthase and stress protein responses that could be age related was examined in young and adult rats. Sprague Dawley male rats aged 7-10 weeks (150-200 g) and 25-32 weeks (300-400 g) were treated with methamphetamine (METH 40 mg/kg, i.p.) separately and allowed to survive 4 h. The control animals from both the groups were received 0.9 % saline in identical manner. After 4 h, control and experimental rats were anesthetized with Equithesin (3 ml/kg, i.p.) and the brains were perfused in situ with 4 % neutral paraformaldehyde through cardiac puncture preceded with 0.9% saline. After perfusion, the brains and spinal cord were removed and processed for neuronal nitric oxide synthase (nNOS), heat shock protein 72 kD (HSP 72 kD) immunoreactivity, histopathological stains, e.g., Nissl staining for neuronal damage and Luxol fast blue (LFB) for myelin damage according to standard protocol. During 4 h meth administration, the animals body temperature, behavioral functions, heart tare, blood pressure and respiration rate were observed. Immediately before termination of experiment, the blood-brain barrier permeability was measured using Evans blue and [131] Iodine extravasations in the brain as well as using albumin immunoreactivity.
METH treatment resulted in a significantly greater overexpression of nNOS and HSP in several brain areas such as cerebral cortex, hippocampus, thalamus, hypothalamus, cerebellum and brain stem in young rats as compared to adult animals. The most marked overexpression of nNOS and HSP were seen in the hippocampus followed by thalamus, cortex, cerebellum, hypothalamus and brain stem. These brain areas were also showed pronounced increase in Evans blue and radioiodine extravasation as well as leakage of endogenous albumin as seen using immunohistochemistry. Several neurons were found dark and distorted in these areas and loss of myelin as seen using LFB is frequent. These cellular and axonal disturbances correlated well with the upregulation of nOS and HSP expression. Taking together, it appears that METH could induce sever stress response in the brain as seen by upregulation of HSP activity and is crucial to induce oxidative stress leading to increased nNOS expression. These two events could lead to breakdown of the BBB as seen by leakage of albumin in the neruopil. Obviously, all these events will lead to neurotoxicity. Our results further show that these effects of METH are age-related.

Disclosures: H.S. Sharma, None; **R. Patnaik**, None; **A. Sharma**, None; **J.V. Lafuente**, None.

Nanosymposium

428. Neurotoxicity and Neurodegeneration IV

Location: Room 23A

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 428.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Air Force Material Command, USAF, under grant number FA8655-05-1-3065

EOARD, London Office, UK

Swedish Medical Research Council 2710

Uppsala University

Swedish Ministry of Education & Research

Astra-Zeneca, Mölndal, Sweden

Göran Gustafsson Foundation, Sweden

Title: Diabetes aggravates brain pathology caused by SiO2 nanoparticles exposure

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Abstract: Silica dust can easily enter human body system in persons exposed to short term or long-term desert environment. However, it is not well know whether inhalation of silicon dioxide (SiO2), the main component of silica dust could induce neurotoxicity. Previous reports from our laboratory show that chronic exposure of SiO2 exacerbates pathophysiology of hyperthermic brain injuries in rats. However, this is still uncertain whether Human populations suffering from various cardiovascular or endocrine disorders could be more sensitive to SiO2 exposure. Since our military personnel are often exposed to SiO2 in desert environment it would be interesting to find out whether diabetes could exaggerate the symptoms of SiO2 exposure in terms of brain dysfunction. Thus, in present investigation, the possibility that diabetes aggravates nanoparticles induced blood-brain barrier breakdown, edema formation and brain pathology was examined in a rat model. Engineered nanoparticles from metals Ag and Cu (50-60 mn) were administered (50

mg/kg, i.p.) once daily for 7 days in normal and streptozotocine induced diabetic rats. On the 8th day, blood-brain barrier (BBB) permeability to Evans blue and radioactive iodine ([131]-I-sodium) was examined in 14 brain regions. In these brain regions alterations in regional CBF was also evaluated using radiolabelled ([125]-I) carbonized microspheres (o.d. 15±6 μm). Regional brain edema and ion analysis were done in 8 selected brain regions. Normal histopathology was used to detect neuronal damage using Nissl staining. Nanoparticles treatment in diabetic rats showed much more profound disruption of the BBB to Evans blue albumin and radioiodine in almost all the 14 regions examined as compared to the normal animals. In these diabetic animals reduction in regional cerebral blood flow was more pronounced than normal rats. Edema development as seen using water content and increase in Na+ and a decrease in K+ ion were most marked in diabetic rats as compared to normal animals after nanoparticles treatment. Cell changes in the regions of BBB disruptions were also more pronounced in diabetic rats compared to normal animals after nanoparticles treatment. Taken together these observations are the first to show that diabetic rats are more susceptible to nanoparticles induced cerebrovascular reactions in the brain and neuronal damage. The possible mechanisms and significance of the present findings will be discussed.

Disclosures: H.S. Sharma, None; **R. Patnaik**, None; **A. Sharma**, None.

Nanosymposium

428. Neurotoxicity and Neurodegeneration IV

Location: Room 23A

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 428.7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Iron overload and melanin systems in the retina - a new mechanism for retinal degeneration?

Authors: *A. E. MILWARD[1], S. HOLLINS[1], R. GRAHAM[2], M. VAN BALEN[1], D. TRINDER[2], M. CAIRNS[1], D. JOHNSTONE[1];
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Abstract: Iron is essential for vision but excess iron may contribute to retinal diseases such as age-related macular degeneration (AMD), which causes vision impairment or blindness in ~20-30 million people worldwide. High body iron of dietary or genetic etiology is relatively common in Western countries, affecting ~10% of people. Additionally, ~0.5% of people of Anglo-Celtic descent carry dual mutations in the *HFE* gene, predisposing to the iron overload disorder hemochromatosis. An *Hfe* knockout mouse model of hemochromatosis shows retinal

degeneration but the mechanisms are unknown. We aimed to identify mechanisms which might underlie degeneration by investigating changes in retinal gene expression in the *Hfe* knockout mouse model of hemochromatosis compared to wildtype mice, using genome-wide microarray and real-time reverse transcription-PCR. To optimize reproducibility we used four different normalization and analysis approaches and focused on the group of genes (n=171) that showed significant expression changes (p <0.05) in the *Hfe* knockout retina by all four approaches. There were various changes for genes involved in vision and diseases such as retinitis pigmentosa and AMD (e.g. complement component 2). Strikingly, within the group of 171 genes, there was up-regulation of at least 12 genes encoding proteins important in melanin synthesis and melanosomes, specialized melanin-containing organelles present in retinal pigment epithelial (RPE) cells. These included genes involved in all stages of melanosome biogenesis (e.g. melanoma marker Silver/Pmel17 and melan-A, both increased over 20-fold, p<0.016) and in melanin synthesis (e.g. dopachrome tautomerase and R-Ras, increased up to 7.5-fold, p<0.025). We therefore next investigated the direct effects of iron on melanin synthesis using a human RPE cell line (ARPE-19). Ferric ammonium citrate at 1 mM - a pharmacological but sublethal dose used in similar studies in the literature - increased melanin content of cells 2-fold within 6 days of treatment (p<0.01). Melanin binds iron and melanosomes may initially serve a protective function by sequestering excess iron but the eye is not equipped for bulk iron storage and tissue damage may occur if the storage capacity is exceeded. Increased melanin and melanosome abnormalities have been observed in several eye diseases including AMD. We hypothesize that i. iron can contribute to this and that these changes may provide an early warning marker of potentially harmful iron exposure and ii. monitoring retinal melanin accumulation and retinal iron chelation therapy will assist in early detection and prevention of retinal degeneration in people with iron overload.

Disclosures: S. Hollins, None; **R. Graham**, None; **M. Van Balen**, None; **D. Trinder**, None; **M. Cairns**, None; **D. Johnstone**, None; **A.E. Milward**, None.

Nanosymposium

428. Neurotoxicity and Neurodegeneration IV

Location: Room 23A

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 428.8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: RIVM - Institutional Support

Western University of Health Sciences - Institutional Support

Title: Inhalation of diesel engine exhaust and neuroinflammation

Authors: *A. G. CAMPBELL[1], F. CASSEE[2], M. GERLOFS-NIJLAND[2];
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Abstract: Gene-environment interactions are proposed to play a role in initiation and progression of neurodegenerative diseases. One of the factors associated with neurodegeneration is an increase in inflammatory events. Our preliminary studies show that exposure to particulate matter (PM), present in ambient air pollution, increases markers of inflammation in rodent brains. Using rats as a model, we show that there is regional variation in response after exposure to diesel engine exhaust (DEE). Levels of the pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin-1alpha (IL-1α) were greatest only in the striatum of rats exposed to DEE compared to control animals exposed to filtered air. Our results indicate that exposure to diesel engine exhaust induces inflammatory responses in the brain in a region-specific manner. To what extent the observed changes may impact the normal function and cellular integrity of unique brain regions is the subject of our future studies.

Disclosures: A.G. Campbell, None; **F. Cassee**, None; **M. Gerlofs-Nijland**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.1

Topic: D.04. Vision

Support: NIH 9 RO1 EY019179-29

Stanford University Dean's Postdoctoral Fellowship

Title: Top-down modulation of bottom-up stimulus competition in the owl optic tectum

Authors: *S. P. MYSORE, E. I. KNUDSEN;
Neurobio., Stanford Univ., STANFORD, CA

Abstract: The selection of the next target for gaze or spatial attention is influenced both by the internal goals of the animal (top-down influences) and the physical properties of the stimuli in the world (bottom-up influences). The neural mechanisms underlying the interaction of top-down

and bottom-up influences for stimulus selection remain poorly understood. Here, we investigate the effect of top-down signals on bottom-up stimulus competition in the owl optic tectum, a structure critically involved in competitive selection for gaze and attention.
We evoke top-down signals by electrically microstimulating the forebrain gaze control center (arcopallial gaze fields, equivalent of the mammalian frontal eye fields), a technique previously shown to mimic the effects of top-down attention. We achieve bottom-up stimulus competition by simultaneously presenting two stimuli (both visual, or one visual and one auditory) whose relative strengths are systematically varied. We show that activation of the forebrain circuitry improves the discriminability of the strength of the stimulus at the activated location relative to the strength of a competing stimulus, specifically when the two stimuli are nearly equal in strength. For this condition, the variability of responses to the receptive field stimulus is also consistently reduced. Opposite effects are observed for responses to the competing stimulus (at the non-activated location). These effects are independent of the sensory modality of the competing stimulus. Together, the results show that top-down input improves the signaling of the strongest stimulus in a complex multisensory environment, especially when such discrimination is the hardest.

Disclosures: S.P. Mysore: None. **E.I. Knudsen:** None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.2

Topic: D.04. Vision

Support: NIH Grant EY018216

Title: Attention-gated spatial coding in the human pulvinar is tied to functional connectivity in visual cortex

Authors: *J. FISCHER, D. WHITNEY;
UC Berkeley, Berkeley, CA

Abstract: The pulvinar nucleus of the thalamus is widely believed to play an important role in visual attention, but its specific function remains elusive. Theories variously posit that the pulvinar is involved in binding object representations across visual areas, selecting relevant visual information from surrounding clutter, and others, but there is little experimental evidence for any particular theory. Thus, at present, even the basic role of the pulvinar in visual processing

is unclear. Here, in an fMRI experiment, we examined spatial coding in the human pulvinar while several visual stimuli were presented simultaneously. Subjects attended to relevant cues within some of the stimuli while ignoring distracting cues within the others. We found an absolute gating of spatial information in the pulvinar by attention: both hemispheres selectively encoded the locations of the attended stimuli, while containing no measurable spatial information about the ignored stimuli. Further, the strength of this attentional gating in the pulvinar was tightly correlated with the strength of the signal coherence between the attended locations in V1 and V2. Our results provide a link between spatial coding in the pulvinar and cortico-cortical signal coupling, lending support to a role of the pulvinar in binding information across visual areas.

Disclosures: J. Fischer, None; **D. Whitney**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.3

Topic: D.04. Vision

Support: NWO Vici

Title: Automatic spread of attentional response modulation according to Gestalt criteria in primary visual cortex

Authors: *P. R. ROELFSEMA[1,2], L. STANISOR[1], A. WANNIG[1];
[1]Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [2]Dept. of Integrative Neurophysiol., Ctr. for Neurogenomics and Cognitive Research, VU Univ. Amsterdam, Amsterdam, Netherlands

Abstract: Visual attention can select spatial locations, features and objects. Theories of object-based attention suggest that attention enhances the representation of all parts of an object, even parts that are not task-relevant. However, the automaticity of the spread of attention to parts of an object that are not task-relevant has been disputed because previous studies did not always rule out the possibility of strategic attention shifts.
Here we investigated if attention spreads automatically to task-irrelevant features in three macaque monkeys by monitoring neuronal activity in area V1 with chronically implanted electrode arrays. We trained the monkeys to make eye movements to one of two (relevant) image elements and also presented two task-irrelevant image elements that could be grouped with the

relevant elements or not. One of these irrelevant image elements was placed in the receptive field of the V1 neurons to investigate if attentional response modulation spreads from the relevant to the irrelevant elements according to a number of Gestalt-grouping cues. Our first experiment tested the spread of attention from relevant to irrelevant contours that were either collinear or orthogonal (good continuation). The second experiment tested if attention spreads from relevant to irrelevant elements with the same or a different colour (similarity). Our third experiment tested the combined influence of colour-similarity and collinearity and the fourth experiment tested the effect of element motion in the same or in a different direction (common fate).
When the task-irrelevant image elements in the receptive field were grouped with one of the relevant contour elements, then the selection of this element for an eye movement response influenced V1 activity. Activity was stronger if eye movement target was grouped with the element in the receptive field. In contrast, the effects of eye movement selection were comparatively weak if the relevant and irrelevant image elements were not related by grouping cues. In addition we found that the effects of grouping cues were additive: the strength of the attentional spread in case of grouping by collinearity and colour similarity was the sum of the spread caused by either grouping cue alone.
We conclude that enhanced neuronal activity spreads automatically from attended image elements to elements that are not yet attended but are related to them by Gestalt grouping cues. Our results support the hypothesis that enhanced neuronal activity can highlight all the image elements that belong to a single perceptual object, and that it can thereby act to bind them together in perception.

Disclosures: P.R. Roelfsema, None; **L. Stanisor**, None; **A. Wannig**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.4

Topic: D.04. Vision

Title: Unmasking the contribution of low-level features to the guidance of attention

Authors: *J. P. OSSANDON[1], S. ONAT[1], D. CAZZOLI[2], T. NYFFELER[2], R. MÜRI[2], P. KÖNIG[1];
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Abstract: To study the contribution of low-level visual features to the guidance of overt attention, we explored their influence on free-viewing behavior when cortical control of attention is impaired in different ways.
In a first study, we recorded eye movements of 15 patients with subacute left-sided visual neglect and 8 control subjects while they explored 32 natural scenes. In a second study, the posterior parietal cortex (PPC) of ten healthy subjects was selectively inhibited by repetitive transcranial magnetic stimulation (rTMS). Subjects' eye movements were recorded while they explored 96 natural scenes in 4 rTMS conditions: no-rTMS, right PPC-rTMS, left PPC-rTMS, and bilateral PPC-rTMS. We found systematic differences between conditions in both exploratory patterns and in the correlation between fixated locations and a compound low-level feature comprising luminance contrast, color contrast and edge content:
(1)Neglect patients, as expected, exhibit a severe spatial bias with a 67% of the fixations located on the right hemifield compared to only 49% for control subjects ($P = 0.001$). Surprisingly however, the locations selected on the left side of the image were characterized by significantly higher feature values than the regions selected in the right hemifield or the location selected by control subjects ($F = 5.2$, $P = 0.04$).
(2)rTMS over the right PPC also induces a significant right-bias on exploration (56% of the exploration on the right vs 52% in the no-rTMS condition, $P = 0.003$).To disentangle between the effect of spatial bias and the influence of low level features we investigated bilateral PPC-rTMS. It does not produce an asymmetry of exploration. Nevertheless, when compared to the no-rTMS condition, it results in the selection of locations with higher feature values in both hemifields ($F = 6.73$, $P = 0.01$).
In summary, correlations of low-level visual features with selected fixation points are increased upon inhibiting/lesion of parietal cortex. This suggests that inhibiting PPC function unmasks the contribution of low-level image features. Consequently, we conjecture that the affected regions of PPC mediate not bottom-up mechanisms but high level saliency and other presumably subcortical structures mediate the influence of low-level features. Finally, our results highlight potential contributions of low-level features to modify the behavioral deficit in hemineglect.

Disclosures: J.P. Ossandon, None; **S. Onat**, None; **P. König**, None; **R. Müri**, None; **T. Nyffeler**, None; **D. Cazzoli**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.5

Topic: D.04. Vision

Support: NIH, NEI, NIBIB Grant R01EB000843

Title: Cortical attentional field maps for individual visual areas from V1 to V7

Authors: *A. M. PUCKETT[1], E. A. DEYOE[2], J. R. MATHIS[2];
[1]Med. Col. Wisconsin, MILWAUKEE, WI; [2]Radiology, Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Visual attention is known to modulate a variety of cortical visual areas in humans but details of these patterns for individual visual areas other than V1 are scant. We used fMRI to compare the spatial topography of both attention-driven and target-driven activation within the retinotopic maps of cortical visual areas V1,V2,V4v and V7. Subjects (4) attended to one of 12 continuously present, disc targets arranged in a circle at one of 4 eccentricities (2^o,4^o,8^o,16^o) about a central marker that was fixated at all times. Each disc varied randomly between 2 just-discriminable luminances. Subjects pressed buttons to indicate the luminance of the attended target while undergoing conventional BOLD fMRI at 3T, 2.5 mm cubic voxels. To assess target driven sensory effects, subjects performed the luminance discrimination at the fixation point while individual target discs were flashed in the periphery. Conventional retinotopic mapping was also obtained and used in conjunction with the attention- and target-driven data to back-project the cortical patterns of activation onto a diagram of the subject's visual field (Functional Field Map). This allows visualization of the target representations and the "attentional window" as they would appear within the subject's field of view. Attention directed to a cued target activated most occipital visual areas with the weakest effects in V1/V2 compared to target-driven activation. In higher visual areas, attention effects became more robust with the highest amplitude effects found in V4v and V7 where attention effects dominated target-driven activity. Functional field maps for both target- and attention-driven tasks were most spatially focused in V1/V2. The attentional effects were not as focused in V4v but were still strongly localized to the attended quadrant. In V7, spatial specificity was poor but attentional effects were dominant and strongly lateralized to the attended target hemifield but less specifically to the quadrant. In sum, the topography of attention and target driven activation varies in spatial specificity and magnitude across individual visual areas suggesting that there is not one "attentional window" but many, each associated with a different visual area.

Disclosures: A.M. Puckett, None; **E.A. DeYoe**, None; **J.R. Mathis**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.6

Topic: D.04. Vision

Support: Postdoctoral fellowship from the Danish Medical Research Councils (DB)

Title: Eye-position dependent functional connection from somatosensory to visual cortex involved in visuospatial attention

Authors: *D. BALSLEV[1], F. Å. NIELSEN[2], T. KASSUBA[1];
[1]MR-department, Copenhagen Univ. Hosp., Hvidovre, Denmark; [2]IMM, Tech. Univ. of Denmark, Lyngby, Denmark

Abstract: Whereas the link between the oculomotor command and attention is established, less is known about how the recently discovered proprioceptive eye-position signal in the somatosensory cortex (S1) (Wang et al., Nat Neurosci, 2007; Balslev et al., Hum Brain Mapp, in press) impacts on the allocation of attention in space. 1Hz rTMS over S1 alters the proprioceptive signal inducing illusory perceptions of gaze direction (Balslev and Miall, J. Neurosci, 2008). This is accompanied by a pseudoneglect further from the perceived direction of gaze for targets presented at equal retinal eccentricity (Balslev et al., J Cogn Neurosci, in press). The neural substrate for this effect is unknown. Here we used fMRI to identify brain areas where visually-evoked activity changes after S1-rTMS.
Healthy subjects (n=19) were scanned after 30 min of 1Hz rTMS of either left S1 or a control area in the left (L) motor cortex (M1). They fixated on a cross in monocular vision with the right (R) eye, while maintaining an eye rotation by 5 degrees to L or R of the sagittal plane. Targets flashed at 5 degrees to either L or R from fixation.
When the eye was rotated to L, S1-rTMS shifted perceived eye position to R and increased visual detection for R vs. L targets. In this condition S1-rTMS also increased the BOLD response for R vs. L targets bilaterally in BA 17 and 18. This increase was significantly larger for S1 vs. M1-rTMS, ruling out an unspecific effect of rTMS. Furthermore, psychophysiological interaction analysis showed that the superior temporal/inferior parietal areas correlated their activity with the visual cortex. Thus, for any increase in visual activity during R target presentation, these areas increased their activity more after S1-rTMS than M1-rTMS. The left posterior parietal cortex is part of a network that orients attention to the R body hemispace. We suggest therefore that top-down signals from the L temporo-parietal to the visual cortices may mediate the effect of eye proprioceptive signal on visual attention, increasing visual detection and BOLD response for the R targets, which in this condition, appeared in the R body hemispace.
When the eye was rotated to R, S1-rTMS had opposite results. It increased visual detection and bilateral visual cortex activity for targets presented now in the L vs. R hemifield. This effect reversal suggests that the connection between S1 and occipital cortex is modulated by gaze direction, further strengthening the association between eye position and visual cortex activity. These findings demonstrate a functional connection between the eye proprioceptive representation in S1 and the visual cortex serving the allocation of attention in space.

Disclosures: D. Balslev, None; **F.Å. Nielsen**, None; **T. Kassuba**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.7

Topic: D.04. Vision

Support: BBSRC

Title: Cholinergic influence on attentional modulation in extrastriate cortex of the rhesus monkey

Authors: *M. A. GIESELMANN, A. THIELE;
Inst. Neurosci., Newcastle Univ., Newcastle upon Tyne, United Kingdom

Abstract: Neurons of the visual cortex enhance their firing rates when subjects attend to the visual stimulus in their receptive field. We have recently shown that attention induced firing rate changes in primary visual cortex (V1) depend on cholinergic muscarinic, but not nicotinic receptors (Herrero et al., 2008). Given the differences in cholinergic receptor distribution between macaque striate and extrastriate areas (Disney et al., 2006), the known involvement of nicotinic receptors in a variety of cognitive functions (Sarter, 2005), the role of cholinergic mechanisms in gamma frequency oscillations (Rodriguez et al., 2004), and the recently established differences between V1 and extrastriate visual area V4 regarding attention induced LFP gamma frequency synchronization (Chalk et al., 2010), we decided to investigate the mechanisms by which acetylcholine promotes attentional modulation in macaque area V4. We recorded from 121 V4 units in one monkey. The monkey performed a task that required top-down spatial attention. A cue was used to direct attention to a specific location in the visual field, and the animal subsequently had to detect a small contrast change in a monochromatic drifting grating at the cued location, ignoring changes in a grating at an uncued location. One of the gratings was always centered at the receptive field of the neuron under study, the other in the opposite hemifield. We recorded neuronal activity under control conditions and when cholinergic agonists (acetylcholine, 36 units) or antagonists (scopolamine, 56 units; mecamylamine, 29 units) were applied. We used brief alternating blocks of drug ejection and retention. The effect of drug application on attentional modulation was quantified as the area under the receiver operating characteristics curve. We found that across the population of recorded units a general activation of cholinergic receptors by means of acetylcholine significantly ($p<0.05$) enhanced the attentional firing rate modulation. Blocking muscarinic cholinergic receptors significantly reduced attentional modulation. Surprisingly, but in line with our V1 study, we found no systematic effect of inactivating nicotinergic receptors. This suggests that attention induced

firing rate modulations in primate visual V1 and V4 depend on similar cholinergic mechanisms.
Disney, A.A. et al. J. Comp. Neurol. 499, 49-63, 2006
Sarter, M. et al. Nat. Rev. Neurosci. 6, 48-56, 2005
Rodriguez, R. et al. J. Neurosci. 24, 10369-10378, 2004
Chalk, M. et al. Neuron 66, 114-125, 2010
Herrero, J.L. et al. Nature 454, 1110, 2008

Disclosures: M.A. Gieselmann, None; **A. Thiele**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.8

Topic: D.04. Vision

Support: NIH Grant EY12925

Title: Spatial attention affects perceived stimulus position

Authors: *P. BINDA[1,2], C. MORRONE[3,4], S. O. MURRAY[5], G. M. BOYNTON[5];
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Abstract: Focusing attention in the periphery enhances visual processing at the attended location. An underlying mechanism may be an increased spatial resolution of visual maps (Fisher & Whitney, Curr Biol 2009), which could distort visual spatial representations. Here we report a shift in the perceived location of a stimulus when it is attended, but not when attention is continuously directed at fixation.
Two salient visual stimuli (black 3 x 0.5 deg vertical bars, 100% contrast) were flashed (50 ms) in the right peripheral visual field, one above and one below the horizontal meridian, at variable Stimulus Onset Asynchrony (SOA). Subjects judged the horizontal location of the second stimulus relative to the first. When the stimuli were simultaneously presented, localization was accurate as both stimuli appeared to be located equal distances from fixation. When the stimuli were asynchronous, the second stimulus was perceived as more peripheral than the first one. The mislocalization was strong: up to 20% of the stimulus eccentricity (between 5 and 20 deg). The size of perceived displacement increased with SOA up to about 250 ms and remained constant for all the longer SOA tested (up to 3 s).

Crucially, the mislocalization effect vanishes if spatial attention is allocated at fixation. Subjects were required to perform simultaneously two tasks: a demanding primary task at fixation (reporting the sum of two digits flashed simultaneously with the peripheral stimuli) and the relative localization of the peripheral stimuli. The localization of the peripheral stimuli was uninfluenced by their order of appearance and the two stimuli appeared to be equal distances from fixation. However, the localization bias was observed again when the primary task was moved to the location of the stimuli, i.e. when the peripheral stimuli were replaced by the digits, and subjects had to sum them and localize the second digit relative to the first one.
Our results indicate that the location of spatial attention plays a critical role in determining the perceived position of stimuli flashed in the visual periphery. In some visual areas, receptive fields change position and size when spatial attention is directed away from fixation (Womelsdorf et al., Nat Neurosci 2006). Also, receptive field shifts have been reported when an eye movement is intended (Duhamel et al., Science 1992). Our findings are consistent with the hypothesis that a covert shift of spatial attention causes distortions of visual space, possibly resulting from shifting receptive fields of neurons that represent the attended location.

Disclosures: P. Binda, None; **C. Morrone**, None; **S.O. Murray**, None; **G.M. Boynton**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.9

Topic: D.04. Vision

Support: GOA 2005/18

IUAP P6/29

GSKE

Title: Anticipatory climbing activity present in the local field potential but not in spike rates in macaque area LIP

Authors: *P. JANSSEN, W. VANDUFFEL, E. PREMEREUR;
Lab. voor Neuro Psychofysiol, KU Leuven, Leuven, Belgium

Abstract: Neurons in the Lateral Intraparietal area (LIP) are modulated by elapsed time during saccade tasks. We recorded LIP activity in two rhesus monkeys during different tasks. In the

delayed visually-guided saccade (VGS) task, four possible grey go-cues and one green saccade target were presented. One of the go-cues appeared in the LIP receptive field (RF), and the target appeared either in the RF or in the opposite hemifield. After a variable delay, one of the go-cues dimmed, instructing the monkeys to make a saccade to the target. The go-time was a random draw from a Weibull function defined between 500 and 2000 ms (unimodal hazard function). In the memory-guided saccade task (MEM), a single saccade target appeared briefly either in- or outside the RF of the neuron, and the monkeys had to saccade to the remembered target position after the fixation point dimmed. In the passive fixation task (FIX), either a colored grating or no visual stimulus was presented in the RF. LIP activity was recorded from 133 LIP sites showing spatially-selective saccadic activity during the VGS-task. Both monkeys were anticipating the timing of the go-cue: reaction time decreased monotonically for longer trial durations. However, the average single unit (SUA) (N=81) and multi unit activity (MUA) (N=52 sites) did not show climbing activity: after the initial visual transient evoked by target onset, the spike rate remained constant throughout the trial (200-1000 ms). Anticipatory climbing activity was present in LFP power, but only in lower frequencies: low-gamma (25-50Hz), beta (12-25Hz) and alpha (8-12Hz). Higher frequency bands followed the SUA pattern. Furthermore we observed significant (permutation test, $p < 0.001$) stimulus-locked increases in all frequency bands of the LFP in the MEM (N= 88 sites) and FIX (N= 95 sites) tasks, even when no visual stimulus was present in the RF. Remarkably, the increase in low-gamma power was virtually identical when a grating appeared in the RF compared to no stimulus (0-700 msec, $p > 0.05$), but was stronger when the saccade target appeared in the RF (VGS and MEM: $p < 0.001$). Higher frequency bands did show significant activity increases ($p < 0.001$) when a grating appeared in the RF. Thus, anticipatory climbing activity can be observed in the LFP power at frequencies below 50Hz without clear modulations in the SUA or MUA activity. Furthermore our observations suggest a functional distinction between the different frequency bands in the LFP: contrary to higher gamma bands, increases in low-gamma power do not reflect visual stimulation. Finally, our data show that LIP contains populations of neurons without climbing activity, suggesting that LIP is a heterogeneous area.

Disclosures: P. Janssen, None; **W. Vanduffel**, None; **E. Premereur**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.10

Topic: D.04. Vision

Support: GSKE

IUAP P6/29

GOA 2005/18

Title: Frontal eye field microstimulation modulates local field potential power in macaque area LIP

Authors: *E. PREMEREUR[1], W. VANDUFFEL[1,2,3], P. R. ROELFSEMA[4], P. JANSSEN[1]; [1]Lab. voor Neuro- & Psychofysiologie, K.U.Leuven, Leuven, Belgium; [2]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA; [3]Dept. of Radiology, Harvard Med. Sch., Massachusetts, MA; [4]Dept. of Vision and Cognition, Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: Electrical microstimulation of macaque Frontal Eye Fields (FEF) mimics the effects of spatial attention on both behavioral performance and V4 single-cell activity. FEF microstimulation also induces fMRI activations in many visual areas including the Lateral IntraParietal Area (LIP) (Ekstrom et al., Science, 2008). We investigated the effect of FEF microstimulation on LIP single unit, multi unit and local field potential (LFP) activity in two rhesus monkeys during three different tasks. In the delayed visually-guided saccade (VGS) task with multiple go-cues, four possible grey go-cues and one green saccade target appeared after a brief period of fixation. One of the go-cues was always in the LIP receptive field (RF), and the target was presented either inside the RF or in the opposite hemifield. After a variable delay, one of the go-cues dimmed, instructing the monkeys to make a saccade to the target. In the memory-guided saccade task (MEM), a single saccade target appeared briefly either in- or outside the RF of the neuron, and the monkeys had to saccade to the remembered target position after the fixation point had dimmed. In the passive fixation task (FIX), either a colored grating or no visual stimulus was presented in the RF of the neuron. LIP activity was recorded only from sites showing spatially-selective saccadic activity during the VGS-task. FEF was stimulated for 500 msec after target or distractor onset, at 1/3 of the saccade threshold. The FEF saccade vector was directed towards the LIP RF. FEF microstimulation did not cause significant changes in spiking activity in LIP in any of the tasks (n = 58 SUA sites, 39 MUA sites), but did cause significant increases in low-gamma power (25-50Hz) (n = 97 sites, 0-500 msec, permutation test, $p < 0.01$) when the saccade was directed towards the LIP RF, and significant increases in alpha power (8-12 Hz) ($p < 0.01$) when the saccade was directed away from the RF (VGS task). Alpha increases were also present in the MEM task, and again only when the monkey planned the saccade away from the RF ($p < 0.01$). These LFP effects were absent when the FEF movement vector and the LIP RF were not aligned (n = 36 sites, $p > 0.4$). Modulations in low gamma power have been associated with selective attention, and increases in alpha power have been associated with disengagement of visuospatial attention. Thus subthreshold FEF-microstimulation causes spatially-selective modulations in the LFP power spectrum in LIP. These modulations are consistent with the allocation of spatial attention.

Disclosures: E. Premereur, None; **W. Vanduffel**, None; **P.R. Roelfsema**, None; **P. Janssen**, None.

Nanosymposium

429. Visual Cognition: Attentional Modulation of Neuronal and Network Activity

Location: Room 5B

Time: Monday, November 15, 2010, 1:00 pm - 3:45 pm

Program Number: 429.11

Topic: D.04. Vision

Support: NIH 11R01EY016281-02

Title: A network model of multiplicative attention modulation

Authors: *S. MIHALAS[1], E. NIEBUR[2];
[1]Mind Brain Inst., Johns Hopkins Univ., BALTIMORE, MD; [2]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Gain modulation of neuronal firing rate has been shown to be important for a large number of computations in the brain including attentional scaling. While shunting inhibition does not lead to a change in gain, many theoretical studies have found several alternative ways to perform such a change. At a network level, gain modulation can be obtained via lateral connections. However previous network models typically produce gain modulation in a narrow range of inputs, and do not distinguish between excitatory and inhibitory neurons. A gain modulation can be obtained by two nonlinearities: a log at the level of the input synapses and an exponentiation at the level of the spike generating mechanism. However such a model will multiply all the inputs. A third method uses the noise level of balanced excitatory and inhibitory neurons to change the gain, with a higher noise producing lower gain. However electrophysiological measurements show the attentional mechanisms almost universally increasing both the average firing rate and the gains.
We implemented a model which contains both excitatory and inhibitory neurons and obtains the gain modulation via lateral connections. We studied the effect of different patterns of lateral connections: Using a biologically plausible pattern of connections, in which excitatory connections are specific and the inhibitory connections are nonspecific, we obtain reliable multiplicative effects of the firing rates of the excitatory neurons if the spread of attentional input is much larger than that of the feedforward inputs. If the spread of attentional input is similar in scale to the feedforward input, the gain modulation is unreliable. Thus we observe a tradeoff between the minimal size of the focus of attention and the reliability of its gain modulation. This pattern of connections also leads to positive attentional modulations for the inhibitory neurons and both positive and negative attentional modulation for the excitatory neurons, results which are in concordance to those observed neurophysiologically.

Disclosures: S. Mihalas, None; **E. Niebur**, None.

Nanosymposium

430. TRP Channels and Pain Transduction

Location: Room 33C

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 430.1

Topic: D.08. Pain

Support: NSF Predoctoral Fellowship

Title: TRPA1 is required for Chloroquine-evoked itch

Authors: *S. WILSON[1], D. M. BAUTISTA[2], A. BIFOLCK-FISHER[3], X. DONG[4], Q. LIU[4]; [1]Dept. of Mol. & Cell Biol., [2]Mol. and Cell Biol., [3]Univ. of California, Berkeley, Berkeley, CA; [4]Johns Hopkins, Baltimore, MD

Abstract: A major side effect of the anti-malarial drug, chloroquine, is intolerable itch. MrgprA3 acts as a chloroquine receptor and is required for chloroquine-evoked itch. However, the signaling pathways and transduction channels that are activated downstream of MrgprA3 are unknown. Here we demonstrate that the ion channel TRPA1 is required for chloroquine-evoked itch. Activation of MrgprA3 leads to the opening of TRPA1 channels, both in sensory neurons and NG108 cells expressing these proteins. Pharmacological or siRNA-mediated knockdown of TRPA1 significantly attenuates chloroquine-evoked responses in sensory neurons. Likewise, chloroquine-evoked responses are greatly diminished in sensory neurons isolated from TRPA1-/- animals. Finally, TRPA1-/- mice display little to no scratching in response to chloroquine. These data show that TRPA1 is the main transduction channel for chloroquine-evoked itch. Antihistamine-insensitive chronic itch is a major detriment of chloroquine-based malaria therapies. Thus, TRPA1 may represent a new target for the treatment of chronic itch.

Disclosures: S. Wilson, None; **D.M. Bautista**, None; **A. Bifolck-Fisher**, None; **X. Dong**, None; **Q. Liu**, None.

Nanosymposium

430. TRP Channels and Pain Transduction

Location: Room 33C

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 430.2

Topic: D.08. Pain

Support: DAAD postdoctoral fellowship for Schmidt M, D/07/41089

NIH grant DEO16927

Title: TRPA1-associated proteins and their role in nociception

Authors: *M. SCHMIDT[1], E. C. PETERS[2], A. E. DUBIN[1], T. J. EARLEY[1], M. J. PETRUS[2], A. PATAPOUTIAN[1];
[1]The Scripps Res. Inst., LA JOLLA, CA; [2]Genomics Inst. of the Novartis Res. Fndn., San Diego, CA

Abstract: Transient receptor potential A1 (TRPA1) ion channels are expressed in nociceptive neurons of dorsal root ganglia (DRG) and trigeminal ganglia (TG) where they serve as noxious stimulus detectors and are critically involved in acute and inflammatory pain. We have recently shown that TRPA1-mediated nocifensive behavior can be sensitized in vivo by activating TRPA1 with its ligand mustard oil (MO). Interestingly, this stimulus also increased TRPA1 membrane levels and function in vitro. These data suggest that TRPA1 translocation to the membrane might represent one of the mechanisms controlling TRPA1-mediated nociceptive signaling upon acute activation or inflammatory signals. However, little is known about proteins that interact with TRPA1 and presumably modulate its trafficking and function.
We established a mass spectrometry-based proteomics approach to identify and study proteins associated with endogenous TRPA1 channels in sensory neurons. We report on the identification of proteins that interact with TRPA1 and control its membrane trafficking and function. We anticipate that this study will contribute to our molecular understanding of the processes which give rise to pain conditions.

Disclosures: M. Schmidt: None. **E.C. Peters:** Employment; Genomics Institute of the Novartis Research Foundation. **A.E. Dubin:** None. **T.J. Earley:** None. **M.J. Petrus:** None. **A. Patapoutian:** None.

Nanosymposium

430. TRP Channels and Pain Transduction

Location: Room 33C

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 430.3

Topic: D.08. Pain

Title: TRPA1 is a key contributor to cold hypersensitivity

Authors: *M. M. MORAN[1], S. MURPHY[1], M. HEIRY[1], L. BARRETT[2], T. J. EARLEY[3], C. A. COOK[1], M. J. PETRUS[4], M. ZHAO[1], M. D'AMOURS[1], N. DEERING[1], G. J. BRENNER[5], M. COSTIGAN[2], J. A. CHONG[1], N. J. HAYWARD[1], C. M. FANGER[1], A. ARDEM PATAPOUTIAN[3,4], C. J. WOOLF[2], D. DEL CAMINO[1];
[1]Hydra Biosci., CAMBRIDGE, MA; [2]F.M. Kirby Neurobio. Ctr., Children's Hosp. Boston, Boston, MA; [3]Dept. of Cell Biol., The Scripps Res. Inst., La Jolla, CA; [4]Genomics Inst. of the Novartis Res. Fndn., San Diego, CA; [5]Neural Plasticity Res. Group, Dept. of Anesthesia and Critical Care,, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: TRPA1 is a calcium permeable, non-selective cation channel expressed by nociceptors. It serves as a broad irritancy receptor for a variety of reactive chemicals, both endogenous and exogenous. Whether TRPA1 plays an additional role in cold sensation remains controversial. Here, we demonstrate that even mild cooling markedly increases TRPA1 currents in a recombinant system, but only following agonist activation. In the absence of an agonist, noxious cold has a clear but very small effect on current amplitude. Extending this finding in acutely isolated neurons from the rat dorsal root ganglia, cooling significantly potentiates agonist-evoked TRPA1 currents. Cooling alone, however, is not sufficient to produce measurable TRPA1 currents. We conclude that TRPA1 is likely a key mediator of cold hypersensitivity in pathological conditions where pro-inflammatory activators of the channel are present, but plays only a minor role in acute cold sensation. Supporting this, cold hypersensitivity can be induced in wild-type but not Trpa1-/- mice by subcutaneous administration of a non-noxious concentration of the TRPA1 agonist 4- HNE. Furthermore, the selective TRPA1 antagonist HC-030031 reduces cold hypersensitivity in rodent models of inflammatory and neuropathic pain without altering normal cold sensation in naïve animals.

Disclosures: M.M. Moran: Employment; Hydra Biosciences Full-time. **S. Murphy:** Hydra Biosciences Full-time. **M. Heiry:** None. **L. Barrett:** None. **T.J. Earley:** None. **C.A. Cook:** Hydra Biosciences Full-time. **M.J. Petrus:** None. **M. Zhao:** None. **M. D'Amours:** Hydra Biosciences Full-time. **N. Deering:** Hydra Biosciences Full-time. **G.J. Brenner:** None. **M. Costigan:** None. **J.A. Chong:** Hydra Biosciences Full-time. **N.J. Hayward:** Hydra Biosciences Full-time. **C.M. Fanger:** Hydra Biosciences Full-time. **A. Ardem Patapoutian:** None. **C.J. Woolf:** Consultant/Advisory Board; Hydra Biosciences. **D. del Camino:** Employment; Hydra Biosciences Full-time.

Nanosymposium

430. TRP Channels and Pain Transduction

Location: Room 33C

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 430.4

Topic: D.08. Pain

Support: NIH Grant NS054069

NIH Training Grant GM067587-06

Wellcome Fund Career Award in Biomedical Sciences

McKnight Endowment Fund Scholar Award

Title: TRPM8, but not TRPA1, is required for neural and behavioral responses to acute noxious cold temperatures and cold-mimetics in vivo

Authors: *W. M. KNOWLTON[1], A. FISHER[2], D. M. BAUTISTA[2], D. D. MCKEMY[1]; [1]USC, Los Angeles, CA; [2]Univ. of California, Berkeley, Berkeley, CA

Abstract: Somatosensory neurons in the skin detect environmental stimuli, converting external cues such as temperature, pain, and mechanosensory inputs into neural activity that is relayed to second-order neurons in the spinal cord. The detection of cold is proposed to be mediated by the ion channels TRPM8 and TRPA1, yet there is significant debate regarding the role of each channel in cold-evoked pain. To address this issue, we generated mice lacking functional copies of both channels and examined *in vitro* neuronal cold-evoked calcium responses and *in vivo* behaviors and neural activity in response to painful cold and noxious cooling compounds. In calcium imaging experiments on cultured trigeminal neurons from TRPM8/TRPA1 double knockout mice (DKO), we find no additional deficits in calcium responses to cold beyond those seen in neurons from TRPM8-null ($TRPM8^{-/-}$) mice. Identical to $TRPM8^{-/-}$ mice, DKO animals display deficits in preference behaviors for warmer temperatures over colder ones until cold reaches the extreme noxious range. In contrast to wildtype mice that avoid touching cold surfaces, both $TRPM8^{-/-}$ and DKO mice display no such avoidance and freely explore noxious cold surfaces, even at 5°C. Nocifensive behaviors to intraplantar injection of the cold mimetic icilin are absent in $TRPM8^{-/-}$ and DKO mice, but are retained in TRPA1-nulls ($TRPA1^{-/-}$). Lastly, stimulus-evoked neural activity in the spinal cord, measured by expression of the immediate early gene c-fos induced by hindpaw stimulation with noxious cold, menthol, or icilin, is reduced to nearly background levels in $TRPM8^{-/-}$ and DKO mice, but remains at wildtype levels in $TRPA1^{-/-}$ animals. Our results show that noxious cold signaling is impaired in animals lacking TRPM8, and that the additional loss of TRPA1 has no effect on residual cold responses. This suggests that acute cold sensing is exclusive to TRPM8 and that TRPA1 is not required for this sensation in mammals.

Disclosures: W.M. Knowlton, None; **A. Fisher**, None; **D.M. Bautista**, None; **D.D. McKemy**, None.

Nanosymposium

430. TRP Channels and Pain Transduction

Location: Room 33C

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 430.5

Topic: D.08. Pain

Title: Pore turret is part of the temperature-sensing structure of thermoTRP channels

Authors: F. YANG[1], Y. CUI[1,2], X. CAO[1,2], K. WANG[2], *J. ZHENG[1];
[1]Physiol& Membrane Biol, Univ. of California at Davis, DAVIS, CA; [2]Dept Neurobio., Peking Univ. Hlth. Sci. Ctr., Beijing, China

Abstract: Temperature-sensitive thermoTRP channels constitute the major cellular sensors for hot and cold stimuli in both innocuous and noxious ranges. These cation-permeable channels respond to temperature changes with exquisite sensitivity and speed, while structurally similar cation channels exhibit benign temperature sensitivity. Our recent work focuses on understanding structural element(s) that bestow high temperature sensitivity on thermoTRPs. We have identified the pore turret as part of the temperature-sensing structure. Heat causes conformational changes in the turret, which can be monitored directly with site-directed fluorophores and FRET or indirectly with current. Structural perturbations to the turret by mutation and chemical modification lead to substantial changes in the channel's temperature response, while replacing the turret sequence eliminates temperature-induced activation. This heat activation pathway appears to also convey noxious stimulus by acid, while chemical activation of TRPV1 by capsaicin is transduced by a separate pathway.

Disclosures: F. Yang, None; **J. Zheng**, None; **Y. Cui**, None; **X. Cao**, None; **K. Wang**, None.

Nanosymposium

430. TRP Channels and Pain Transduction

Location: Room 33C

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 430.6

Topic: D.08. Pain

Support: This work was supported by a CIHR grant (MOP-57832).

MCL was successively supported by a NSERC scholarship and a FRSQ MD-PhD training award.

Title: Ciliary neurotrophic factor regulates thermoreceptors of the transient receptor potential family and modulates thermoception and nociception

Authors: *M.-C. LETELLIER[1], J.-S. WALCZAK[1], S. CRABÉ[1], M. GINGRAS[2], A. J. TORMO[1], F. BERTHOD[2], G. ELSON[3], P. BEAULIEU[1], J.-F. GAUCHAT[1];
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Abstract: Cold-induced sweating syndrome (CISS) and Crisponi syndrome (CS) are two autosomal recessive diseases associated with abnormal thermoregulation and sensorimotor attempts. CISS patients start to suffer from hyperhidrosis in cold conditions and from anhidrosis in high environmental temperature at sexual maturity; some patients present a relative pain insensibility. CS manifests at birth with respiratory distress. During the first year of life, patients have contractures crisis with hyperthermia episodes, often leading to death. Surviving patients tend to develop a CISS. CISS and CS patients carry mutations in the genes encoding the subunits of the composite cytokine CLC/CLF (cardiotrophin-like cytokine/cytokine receptor-like factor), a ciliary neurotrophic factor (CNTF) receptor ligand. These observations suggest that CLC/CLF regulates both thermoregulation and nociception, two neurobiological processes involving thermoTRP, a subfamily of transient receptor potential (TRP) channels, known to sense temperatures. We therefore investigated, using behavioural tests, whether intraperitoneal injection of CNTF modulates thermoception, nociceptive sensibility to thermoTRP agonists and inflammation-induced hyperalgesia in mice. We used agonists of cold receptors TRPM8 (TRP subfamily M, member 8) and TRPA1 (TRP subfamily A, member 1) and of the heat receptor TRPV1 (TRP subfamily V, member 1). CNTF was used rather than CLC/CLF to avoid effects on immune cells, as CLC is known to activate B cells through an unidentified receptor. In physiological conditions, CNTF increases cold sensibility, causes TRPM8 agonist allodynia and TRPA1 agonist hyperalgesia, but have no effect on sensibility mediated by TRPV1. This modulation of cold sensibility is paralleled by increases in trigeminal ganglia of TRPA1 RNA and protein expression. When neurogenic inflammation is induced by carrageenan, CNTF prevents inflammatory heat and TRPV1 agonist hyperalgesia. These results suggest that an abnormal thermoTRP regulation contributes to the CISS and CS phenotypes and that CNTF receptor is a potential target in pain treatment.

Disclosures: M. Letellier, None; **J. Walczak**, None; **S. Crabé**, None; **M. Gingras**, None; **A.J.**

Tormo, None; **F. Berthod**, None; **G. Elson**, None; **P. Beaulieu**, None; **J. Gauchat**, None.

Nanosymposium

430. TRP Channels and Pain Transduction

Location: Room 33C

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 430.7

Topic: D.08. Pain

Support: NIH grant 5R01NS055159

Title: The role of phosphoinositides in the desensitization of TRPM8 currents

Authors: *T. ROHACS, Y. YUDIN, E. ZAKHARIAN, V. LUKACS;
Pharmacol. & Physiol., UMDNJ, New Jersey Med. Sch., NEWARK, NJ

Abstract: The Transient Receptor Potential Melastatin 8 (TRPM8) ion channel is a major sensor of environmental cold temperatures. TRPM8 requires the presence of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) for activity. To conclusively demonstrate that PIP_2 acts on the channel directly, we purified the TRPM8 protein and reconstituted it into planar lipid bilayers. We show that the reconstituted channel is activated by cold, menthol and icilin, and its activity requires the presence of PIP_2. The specificity profile of the reconstituted channel for phosphoinositides was very similar to that observed in cellular systems.
In the presence of extracellular Ca^{2+} TRPM8 currents diminish in response to repeated applications of menthol and cold. This phenomenon is called desensitization or adaptation. We and others have shown that Ca^{2+} influx through TRPM8 activates a Ca^{2+} sensitive phospholipase C (PLC) isoform, leading to depletion of PIP_2. Here we show that co-expression of any of the three PLC delta isoforms but not PLC beta or gamma accelerated Ca^{2+}-induced inhibition of TRPM8. Both depletion of the lipid and activation of protein kinase C (PKC) downstream of PLC activation have been proposed to underlie Ca^{2+} dependent desensitization of TRPM8. Here we show that desensitization is accelerated by omission of MgATP from the patch pipette in whole-cell patch clamp experiments. Recovery from desensitization was slowed down by inhibition of phosphatidyinositol 4-kinases by high concentrations of wortmannin. Furthermore, inclusion of PIP_2, but not its precursor PIP into the whole-cell patch pipette inhibited menthol-induced desensitization. To demonstrate that depletion of PIP_2, without activation of PLC, is sufficient to inhibit TRPM8, we show that TRPM8 activity is inhibited by dephosphorylation of PIP_2 both by a chemically inducible 5-phosphatase and a voltage sensitive 5-phosphatase. To further address the role of PKC, we tested the effects of two PKC activators on menthol-induced

TRPM8 currents. In our hands neither the cell permeable diacylglycerol analogue 1-oleoyl-2-acetyl-sn-glycerol OAG (100 μM), nor the phorbol ester PMA (100 μM) inhibited menthol-induced currents. Our data support the role of PIP_2 depletion, but not that of PKC in TRPM8 desensitization.

Disclosures: T. Rohacs, None; **Y. Yudin**, None; **E. Zakharian**, None; **V. Lukacs**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.1

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS047715

NIH Grant NS065718

Title: Using in vivo structure-function analysis to reveal how force activates the MEC-4 channel responsible for touch sensation in C. elegans

Authors: A. L. EASTWOOD, *M. B. GOODMAN;
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Abstract: The C. elegans MEC-4 channel, a member of the DEG/ENaC superfamily of channels, is the first force-gated ion channel identified that can be activated by gentle touch and studied in vivo in its native environment (O'Hagan et al., Nat Neurosci, 2005, 8, 43-50). To gain insight into the way in which this channel translates mechanical energy into ion flux, we incorporated mutant MEC-4 channels into worms lacking the wild-type MEC-4 protein and its co-subunit MEC-10 using the Mos1-mediated single copy insertion (MosSCI) strategy (Frøkjaer-Jensen et al., Nat Genet, 2008, 40, 1375-83). We focused on two highly conserved sequences in the channel: (1) the GxxxG motif in the pore-lining helix, and (2) the LxxxφG sequence in the extracellular domain that is physically adjacent to the top of the transmembrane domain in the homologous ASIC1a crystal structure (Gonzales et al., Nature, 2009, 460, 599-604). The second conserved motif may potentially couple the extracellular domain to the transmembrane domain and act as a gating hinge. To study the role of the GxxxG motif, each glycine was mutated to the slightly larger alanine, both individually and in adjacent pairs. Behavioral assays characterizing the responsiveness of the mutant worms to gentle touch show that these subtle mutations were enough to disrupt the touch sensitivity of the worm. Thus, the pore-lining GxxxG motif is critical

for channel function. To study the role of the potential gating hinge, the aromatic tyrosine in the LxxxφG sequence, the asparagine at the top of the first transmembrane helix, and the glutamate at the top of the second transmembrane helix were mutated to the much smaller alanine individually, in pairs, and all together. In several ASIC family members, the aromatic tryptophan and the residue at the top of the first transmembrane helix interact in a manner critical for gating (Li et al., J Biol Chem, 2009, 284, 4689-94). Behavioral assays on the gating hinge mutants suggest that these residues are less critical for MEC-4 channel function since all of these mutants were touch sensitive. These results indicate that mechanical stimuli open the MEC-4 channel in a manner that differs from the way in which protons are believed to open ASICs.

Disclosures: A.L. Eastwood, None; **M.B. Goodman**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.2

Topic: D.09. Tactile/Somatosensory

Support: NIH T32 GM08600

Title: Ion channel contributions to auditory mechanosensation in Drosophila melanogaster

Authors: *J. C. CALDWELL[1], J. JACOBS[2], E. SIVAN-LOUKIANOVA[2], D. F. EBERL[2], W. D. TRACEY, Jr.[1,3,4];
[1]Anesthesiol., Duke Univ. Med. Ctr., Durham, NC; [2]Biol. Sci., Univ. of Iowa, Iowa City, IA; [3]Cell Biol., [4]Neurobio., Duke Univ., Durham, NC

Abstract: Sensation of environmental mechanical forces unites the senses of touch, hearing, balance and pain. Although the spectrum of stimuli detected by these different sub-modalities is broad, at the center of each specialized process is a molecular mechanism to transduce the mechanical stimuli and convert them to electrochemical signals. Evidence suggests that force sensing ion channels are present in each of these systems and these channels play a central role in force transduction. However, the molecular identity of the vertebrate and, indeed, invertebrate, auditory mechanotransducer remains elusive. The vertebrate auditory hair cell mechanotransduction channel has a very large singe-channel conductance, an inwardly rectifying current-voltage relationship and a high selectivity for calcium. While the biophysical properties of the hair cell mechanotransducer are well understood, relatively little else is known about ion channel contributions to mechanosensation. We therefore turned to the genetically tractable

model organism Drosophila melanogaster with the goal of identifying ion channels involved in hearing. To achieve this, we utilized RNAi against ion channel subunits and performed tissue specific knock-down in the auditory organ. We then directly tested the effect of knock-down on audition using an established electrophysiological paradigm.
The Drosophila auditory system is housed in the second segment on each of the two bilateral antennae that are situated between the compound eyes. Present in each segment is a large array of ~175 ciliated chordotonal neurons that are activated by deflection of a receiver known as the arista. When a fly's antenna vibrates in response to a biologically relevant courtship song, the chordotonal neurons stretch and relax and action potentials travel along the antennal nerve and are interpreted in the brain as sound. In our experiments, we recorded the sound evoked potentials of the antennal nerve in the RNAi knockdown animals.
We performed recordings from RNAi lines targeting 201 of the 203 predicted Drosophila ion channels. The effect of knock-down in the adult progeny was probed by electrophysiology for each of these subunits and we have found 10 ion channel genes which are required for auditory mechanosensation. Future studies will look at whether mechanical forces directly gate these channels or if the candidates are simply required downstream in the mechanosensory pathway.

Disclosures: J.C. Caldwell, None; **J. Jacobs**, None; **E. Sivan-Loukianova**, None; **D.F. Eberl**, None; **W.D. Tracey**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.3

Topic: D.09. Tactile/Somatosensory

Support: NS40538

NS070711

Title: TRPA1 mediates mechanical currents in the plasma membrane of mouse sensory neurons

Authors: *D. VILCEANU, C. L. STUCKY;
Cell Biology, Neurobio. and Anat., Med. Col. of Wisconsin, MILWAUKEE, WI

Abstract: Mechanosensitive channels serve as essential sensors for cells to interact with their environment. The identity of mechanosensitive channels that underlie somatosensory touch transduction in vertebrates is still a mystery. One promising mechanotransduction candidate is

the Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel. To determine the role of TRPA1 in generation of mechanically-sensitive currents in the sensory neuron plasma membrane, we used dorsal root ganglion (DRG) neuron cultures from adult mice and applied rapid focal mechanical stimulation (indentation) to the soma membrane. Small neurons (diameter <27 um) were studied because TRPA1 is functionally present in these neurons which largely give rise to C fiber nociceptive afferents in vivo. Small neurons were classified by isolectin B4 binding.
Mechanically-activated inward currents were classified into two subtypes: Slowly Adapting and Transient inward current. First, significantly more IB4 negative neurons (84%) responded to mechanical stimulation than IB4 positive neurons (51%). Second, among current profiles, 89% of Slowly Adapting currents were present in IB4 negative neurons whereas only 11% were found in IB4 positive neurons. Third, importantly, in IB4 negative neurons from TRPA1-/- mice Slowly Adapting currents were completely absent. Consistent with this, Slowly Adapting currents were abolished in wild type IB4 negative neurons stimulated with mechanical force in the presence of a TRPA1 antagonist, HC-030031. In addition, the amplitude of the Transient mechanically-activated currents in IB4 positive neurons from TRPA1-/- mice was reduced by over 60% compared to TRPA1+/+ controls; however, a similar reduction did not occur in wild type neurons treated with the TRPA1 antagonist, HC-030031.
These parallel genetic and pharmacological data demonstrate that TRPA1 mediates the Slowly Adapting mechanically-gated currents in IB4 negative C fiber type sensory neurons from adult mice. The TRPA1 protein may also contribute to a complex that mediates Transient mechanically-gated currents in IB4 positive C fiber type neurons.

Disclosures: D. Vilceanu, None; **C.L. Stucky**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.4

Topic: D.09. Tactile/Somatosensory

Support: NSF Grant T32 GM007048-35

Title: The star-nose mole as a model system for studying molecular mechanisms of mechanotransduction

Authors: *K. GERHOLD[1], T. MORITA[2], D. B. LEITCH[3], K. CATANIA[3], D. M. BAUTISTA[2];

[2]Mol. and Cell Biol., [1]Univ. of California, Berkeley, Berkeley, CA; [3]Vanderbilt Univ., Nashville, TN

Abstract: The star-nosed mole, *Condylura cristata*, has an extremely sensitive touch organ around its nose called the star. The star is specialized for probing the soil to locate small prey items with speeds of up to 15 touches/second (Catania and Remple, 2005). It consists of 22 fleshy appendages covered with thousands of touch papillae called Eimer's organs. Each Eimer's organ is densely innervated with trigeminal nerves, one merkel-cell neurite complex and one lamellated corpuscle. We investigated the anatomical, functional and molecular mechanisms underlying tactile sensitivity of the star. Using immunohistochemistry we demonstrate that the star is innervated primarily by NF200 positive fibers (>80%) and displays little substance P reactivity, consistent with a significant enrichment of light touch receptors over nociceptors. This enrichment may result from extensive branching of a small number of innervating neurons, like in the mouse cornea, or it could be due to an enrichment of light touch cells at the level of the ganglia. To examine this, we compared trigeminal ganglion (TG) that innervate the star to dorsal root ganglion (DRG) that innervate the body. We demonstrate that the TG is significantly enriched for NF200 positive neurons compared to the DRG (TG:57.2±9.6% vs DRG:36.1±6.2%; $p<.01$) and contains a lower percentage of peripherin positive nociceptive neurons compared to DRG (TG:13.4±7.4% vs DRG:35.0±6.5%; $p<.01$). We next compared the functional properties of neurons cultured from TG and DRG. We show that the TG contain a higher percentage of neurons responsive to mechanical stimuli (osmotic stretch: 54.9% TG vs 33.9% DRG; radial stretch: 79.1% TG vs 28.7%DRG. Likewise, TG neurons are less sensitive noxious stimuli (capsaicin: 14.7% TG vs 36.1% DRG), suggesting a functional enrichment of light touch receptors. The diffuse localization of touch receptors throughout the skin of mammals has hampered the discovery of molecules underlying somatosensation. Thus, the high concentration of touch receptors in the star provides a unique opportunity for identifying mechanotransduction molecules. Towards this end, we used transcriptome analysis, to identify transcripts enriched in the trigeminal ganglion of the star-nosed. Parallel sequencing was performed on star-nosed mole cDNA libraries generated from mole TG and brain mRNA. A reference transcriptome was generated by sequencing from RNA isolated from a wide array of neuronal and non-neuronal tissues (26,742 transcript models, mean = 328.75+-284.13 bp, max=4449 bp). We then aligned ~10 million reads per library to the reference transcriptome to identify genes enriched in the TG.

Disclosures: K. Gerhold, None; **D.M. Bautista**, None; **T. Morita**, None; **K. Catania**, None; **D.B. Leitch**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.5

Topic: D.09. Tactile/Somatosensory

Support: Burroughs Wellcome Career award in Biosciences

Sloan Research Fellowship

Title: Tingling alkylamides target distinct subsets of mechanosensitive somatosensory neurons

Authors: *M. TSUNOZAKI[1], R. C. LENNERTZ[2], C. L. STUCKY[2], D. M. BAUTISTA[1]; [1]Dept. of Mol. and Cell Biol., UC Berkeley, Berkeley, CA; [2]Dept. of Cell Biology, Neurobiology, and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Somatosensory mechanotransduction allows us to feel and distinguish between a soft feather and a pinprick against our skin; however, among sensory modalities, mechanotransduction is the least understood. While recent studies have advanced our understanding of the cellular and molecular mechanisms of thermosensation, mechanotransduction in mammals remains enigmatic. Thus, a key goal of somatosensory research is to identify components of the mechanotransduction machinery. Here, we describe a multi-faceted approach to identify candidate mechanotransducers and to probe the *in vivo* roles of such candidates in somatosensation. First, we are studying the mechanisms of action of natural plant products that specifically target distinct subsets of myelinated mechanosensitive fibers. We find that tingling alkylamides from *Zanthoxylum* plants activate light touch receptors and silence responses of mechano-nociceptors. In contrast, thermal sensitivity is unaffected by alkylamides. Consistent with its *in vitro* effects, alkylamides raise von Frey thresholds when applied to the hind paw of mice. Thus, *Zanthoxylum* alkylamides are novel tools to identify molecular components of this signaling pathway. Second, we are developing new behavioral assays to measure responses to light touch. Classical tests for mechanosensation, including the von Frey assay, probe responses to harsh mechanical stimuli; however, equivalent assays for light touch are lacking. We have recently adapted the startle reflex assay to quantitate responses to innocuous mechanical force. In the tactile startle response, a puff of air applied to the mouse induces a robust startle reflex, including eye blinking and whole-body muscle contraction, which can be measured quantitatively. The use of this assay to test a variety of transgenic mice lacking channels or fiber subsets will be discussed.

Disclosures: M. Tsunozaki, None; **R.C. Lennertz**, None; **C.L. Stucky**, None; **D.M. Bautista**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.6

Topic: D.09. Tactile/Somatosensory

Support: Deutsche Forschungsgemeinschaft

Deutsche Akademische Austausch Dienst (DAAD)

Title: Generation of Cav3.2 reporter mice to characterize ultrasensitive skin mechanoreceptors

Authors: Y. ANDREA-BERNAL-SIERRA, A. KOZLENKOV, *G. R. LEWIN;
Dept of Neurosci., Max-delbruck-Center For Mol. Med., Berlin, Germany

Abstract: Low-voltage-activated Ca2+ currents or so-called T-type currents can be formed by one of three subunits designated $Ca_v3.1$, $Ca_v3.2$ or $Ca_v3.3$. Gene deletion studies in mice have demonstrated important roles for T-type channels in a wide variety of physiological functions, in the cardiovascular and nervous systems. However, given the high degree of sequence similarity and lack of pharmacolgical agents that discriminate between different T-type channels it has been problematic to reliably identify the cell types that express each subunit. Here we generated two knock-in mouse strains in which the endogenous $Ca_v3.2$ promotor was used, either to drive expression of EGFP, or the Cre recombinase. For the generation of $Ca_v3.2^{EGFP}$ and $Ca_v3.2^{cre}$ mice, EGFP-lox-Neo/Kan-lox or EGFP-lox-Neo/Kan-lox targeting cassettes were integrated into the start codon of the first exon of the mouse *$Ca_v3.2$* gene using homologous recombination in 129SV/J ES cells. We have previously shown that expression of $Ca_v3.2$ in the dorsal root ganglia is exclusive to a specific ultra-sensitive mechanoreceptor type called D-hair receptors. Initial results with the $Ca_v3.2^{EGFP}$ mice indicated that large sensory neurons can be visualized with antibodies directed against EGFP. We used the $Ca_v3.2^{cre}$ in combination with a Tau^{mGFP} reporter mouse in which expression of a membrane targeted GFP is driven by the Tau promoter (Hippenmeyer, et al. 2005 PLoS Biol. 2005 May;3(5):e159). Here, we obtained strong fluorescence signals in a sub-population of DRG neurons as well as subsets of neurons in the forebrain. The mice may potentially be used to define the precise anatomical nature of the peripheral endings of D-hair receptors. We also cultured DRG neurons from the latter mice and found fluorescently labeled cells suitable for electrophysiological examination. The two new mouse strains generated here will enable us to characterize in detail a single and homogenous sub-population of mechanoreceptors. In principle we may be able to examine the central and peripheral projection of these neurons under circumstances where $Ca_V3.2$ channel function is ablated or not. These mice will be very useful to examine the factors that control the development of a specific mechanoreceptor type in vivo.

Disclosures: Y. Andrea-Bernal-Sierra, None; **A. Kozlenkov**, None; **G.R. Lewin**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.7

Topic: D.09. Tactile/Somatosensory

Support: NSERC

CIHR

FRSQ

Title: Touché: A recessive zebrafish mutation which abolishes generator potentials in cutaneous mechanoreceptors

Authors: S. E. LOW, J. RYAN, M. LACHANCE, *L. SAINT-AMANT;
Pathologie et biologie cellulaire, Univ. De Montréal, Montreal, QC, Canada

Abstract: The process by which light-touch in vertebrates is transformed into an electrical response in cutaneous mechanosensitive neurons is a largely unresolved question. To address this question we undertook a forward genetic screen in zebrafish (*Danio rerio*) to identify mutants exhibiting abnormal touch-evoked behaviors, despite the presence of sensory neurons and peripheral neurites. One family, subsequently named *touché*, was found to harbor a recessive mutation which produced offspring that were unresponsive to light-touch, but responded to a variety of other sensory stimuli. The optogenetic activation of motor behaviors by *touché* mutant sensory neurons expressing ChannelRhodopsin-2 suggested that the synaptic output of sensory neurons was intact, consistent with a defect in sensory neuron activation.
To explore sensory neuron activation we developed an *in vivo* preparation permitting the precise placement of a combined electrical and tactile stimulating probe upon eGFP positive peripheral neurites. In wild type larva electrical and tactile stimulation of peripheral neurites produced action potentials detectable within the cell body. In a subset of these sensory neurons an underlying generator potential could be observed in response to subthreshold tactile stimuli. A closer examination revealed that the amplitude of the generator potential was proportional to the stimulus amplitude. When assayed *touché* mutant sensory neurons also responded to electrical stimulation of peripheral neurites similar to wild type larva, however tactile stimulation of these neurites failed to uncover a subset of sensory neurons possessing generator potentials. Collectively these findings suggest that *touché* is required for generator potentials, and that generator potentials underlie responsiveness to light-touch in zebrafish.

Disclosures: S.E. Low, None; **J. Ryan**, None; **L. Saint-Amant**, None; **M. Lachance**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.8

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant DC02281

HHMI

Title: Structural determinants of tip-link-cadherin function in hearing and deafness

Authors: M. SOTOMAYOR[1], W. WEIHOFEN[3], R. GAUDET[3], *D. P. COREY[2];
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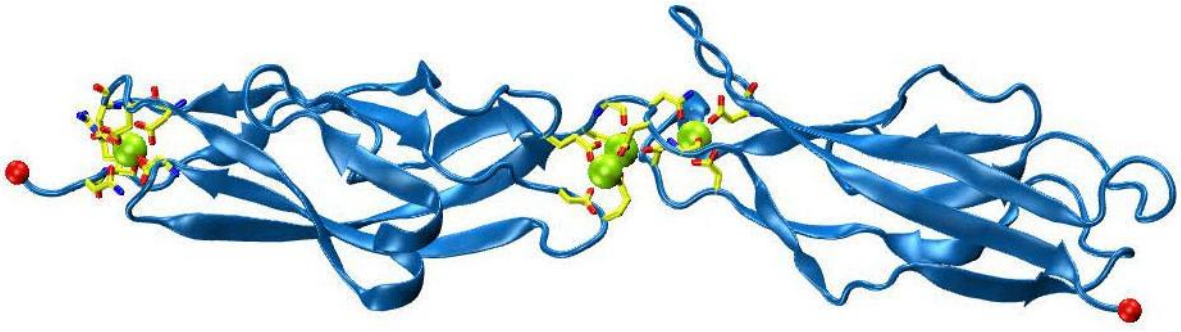
Abstract: The hair-cell tip link, a fine filament directly conveying force to mechanosensitive transduction channels, is likely composed of two proteins, protocadherin-15 and cadherin-23, whose mutation causes deafness. However, their molecular structure, elasticity, and deafness-related structural defects are unknown. We present crystal structures of extracellular tip-link cadherin repeats involved in hereditary deafness and tip link formation. In addition, we show that the deafness mutation D101G, in the linker region between the repeats EC1 and EC2 of cadherin-23, causes a slight bend between repeats and decreases Ca^{2+} affinity. Molecular dynamics simulations suggest that tip-link cadherin repeats are stiff and that either removing Ca^{2+} or mutating Ca^{2+}-binding residues reduces rigidity and unfolding strength. The structures define an uncharacterized cadherin family and, with simulations, suggest mechanisms underlying inherited deafness and how cadherin-23 may bind with itself and with protocadherin-15 to form the tip link.



Disclosures: M. Sotomayor, None; **W. Weihofen**, None; **R. Gaudet**, None; **D.P. Corey**, None.

Nanosymposium

431. Receptors: Cellular and Molecular Mechanisms of Transduction

Location: Room 1B

Time: Monday, November 15, 2010, 1:00 pm - 3:15 pm

Program Number: 431.9

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS047715

NIH Grant EB006745

Helen Hay Whitney Fellowship

NSF Graduate Research Fellowship

Swiss National Science Foundation

Stanford Medical School Dean's Fellowship

Title: DEG/ENaC and TRP channels have distinct roles in C. elegans mechanonociception

Authors: *S. L. GEFFENEY[1], J. G. CUEVA[1], D. A. GLAUSER[1], J. C. DOLL[2], T. H.-C. LEE[1], M. MONTOYA[1], S. KARANIA[1], A. NAIM[1], A. GARAKANI[3], B. PRUITT[2], M. B. GOODMAN[1];
[1]Molec, Cell Physiol, Stanford Univ. Sch. Med., STANFORD, CA; [2]Mechanical Engin., Stanford Univ., Stanford, CA; [3]Reify Corp., Cambridge, MA

Abstract: The majority of nociceptors are polymodal, excited by multiple stimuli including noxious force. Because nociceptors are distributed diffusely in the skin and culturing them separates the cell body from its sensory terminal, little is known about sensory receptor potentials or the underlying sensory receptor currents generated by these neurons *in situ*. This fact complicates efforts to identify the channels responsible for events at the sensory terminals of nociceptors. Our goal is to identify the sensory transduction channels that allow nociceptors to respond to mechanical stimuli using ASH, a ciliated, polymodal nociceptor-like neuron in *Caenorhabditis elegans*, as a model system. In ASH, we are able to record mechanoreceptor currents (MRCs) in a preparation that leaves the sensory terminal intact. This neuron is an

appropriate model of nociceptor function: ASH activity and animal withdrawal are induced by multiple noxious stimuli including mechanical, osmotic and chemical stimuli. As in mammalian nociceptors, ASH expresses members of two ion channel gene families (DEG/ENaC and TRP channels) that have been identified as candidate mechanosensory transduction channels.
MRCs in ASH are carried by DEG/ENaC channels and not by TRP channels. We determined the identity of the ion channels that carry MRCs using genetic dissection and by defining the properties of the channels. ASH expresses two members of the DEG/ENaC gene family: *unc*-8 and *deg-1*. MRCs are decreased by approximately ten-fold in *deg-1;unc-8* double knock-out mutants and inhibited by the DEG/ENaC channel-blocking drug amiloride. Additionally, MRCs reverse polarity near the equilibrium potential for Na^+ ions and decreased in Na^+-free saline. Thus, MRCs are carried by amiloride-sensitive, Na^+ channels, likely formed by the products of the *deg-1* and *unc-8* genes.
TRP channels play a distinct role in the cellular response to mechanical stimulation of ASH. Unlike DEG/ENaC channels, they are not required for the *in vivo* generation of MRCs since such currents are unaffected in *osm-9* and *ocr-2* single mutants as well as in *osm-9ocr-2* double mutants. Instead, the TRP channels are required for a large depolarization-activated current. This current is blocked by Gd^{3+} and eliminated by null mutations in *osm-9*, *ocr-2* or in both genes. Thus, TRP channels are dispensable for force-activated currents in ASH, but required for a Gd^{3+}-sensitive, voltage-activated current. The model emerging from these data is that DEG/ENaC proteins form neurosensory mechanotransduction channels in ASH and that TRP proteins are required for voltage-activated currents that amplify electrical responses to mechanical stimulation.

Disclosures: S.L. Geffeney: None. **J.G. Cueva:** None. **D.A. Glauser:** None. **J.C. Doll:** None. **T.H. Lee:** None. **M. Montoya:** None. **S. Karania:** None. **A. Naim:** None. **A. Garakani:** Ownership Interest; Reify Corporation. **B. Pruitt:** None. **M.B. Goodman:** None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.1

Topic: E.08. Biological Rhythms and Sleep

Title: Neural pathways are responsible for relaying the direct effects of light to the sleep and alertness system

Authors: J. HUBBARD[1], E. RUPPERT[1], J. TSAI[2], J. HANNIBAL[3], G. HAGIWARA[2], D. COLAS[2], *B. F. O'HARA[4], H. C. HELLER[2], P. FRANKEN[5], P. BOURGIN[1,2];

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Abstract: Light influences sleep and vigilance either indirectly, through a well-defined circadian pathway that involves the suprachiasmatic nucleus (SCN), or directly through a series of non-visual, non-circadian effects. These non-circadian effects of light will determine the timing and quality of sleep and alertness through an interaction with the circadian and homeostatic drives. This mediation is facilitated by melanopsin (Opn4), a retinal photopigment which is crucial for conveying non-visual light information to brain target areas that remain to be identified. We recently demonstrated that induction of c-Fos immunoreactivity by light (a marker of neuronal activity) in the SCN was reduced in mice which lacked melanopsin (Opn4-/-), suggesting a possible participation of the SCN as a relay for the direct effects of light.
To test this hypothesis, we extensively analysed sleep EEG, in melanopsin deficient mice (Opn4-/-) under 3 SCN conditions: intact, sham and "arrhythmic" (verified with actimetry) lesioned animals. Sleep recordings were performed under the following light-dark regimens: standard LD12h:12h, single 1h L- or D-pulses during the 12h D- or 12h L-period, respectively, and a 24 hour period under short LD1h:1h cycles. After completion of the protocol, the tracer cholera toxin B was injected into the eyes of the mice. A functional anatomical study of the SCN and retinohypothalamic tract was then performed (triple immunohistochemistry (IHC) staining of the main SCN neurotransmitters, AVP and VIP, and cholera toxin.)
Preliminary analysis shows that mice with lesioned SCNs have disrupted sleep was consistent with the removal of the circadian drive. Results also indicate that after removal of the SCN, the sleep-promoting effect of light and alerting effect of darkness, as evidenced under the 1h:1h LD ultradian cycle, are partly abolished in both genotypes in a way similar to the disturbances observed with intact Opn4-/- mice. The anatomical analysis shows a complete lesion of the SCN that has spared the surrounding brain structures. Staining of the retinohypothalamic fibers to the VLPO and other areas were conserved in lesioned animals, with a comparable distribution to those observed in sham and intact animals.
Comprehensive analysis is currently underway to confirm these initial findings which suggest that the SCN is one of several possible relays mediating the direct effects of light on sleep and alertness and plays a role beyond its function as circadian master clock.

Disclosures: J. Hubbard, None; **B.F. O'Hara**, None; **E. Ruppert**, None; **P. Bourgin**, None; **J. Tsai**, None; **G. Hagiwara**, None; **D. Colas**, None; **J. Hannibal**, None; **H.C. Heller**, None; **P. Franken**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.2

Topic: E.08. Biological Rhythms and Sleep

Title: Role of melanopsin in sleep regulation: The direct effects of light interact with the circadian and homeostatic drive

Authors: P. BOURGIN[1,2], J. TSAI[2], E. RUPPERT[1], J. HUBBARD[1], J. HANNIBAL[3], G. HAGIWARA[2], D. COLAS[2], *N. F. RUBY[2], H. C. HELLER[2], P. FRANKEN[4];
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Abstract: Light can influence sleep and alertness either indirectly through a well-characterized circadian pathway or directly through poorly understood mechanisms. Melanopsin (Opn4) is a retinal photopigment crucial for conveying non-visual light information to the brain. Our goal is to determine the mechanisms by which the direct effects of light affect sleep and alertness.
In various light-dark regimens (including 12h:12h light-dark (LD), single 1h L- or D-pulses during the 12h D- or 12h L-period, respectively; and one day under short LD1h:1h cycles), we analyzed sleep-wake time and the EEG in melanopsin-deficient (Opn4-/-) mice (n=10). We also performed a 6 hr sleep deprivation starting at light onset.
In contrast to wild type, single light pulse failed to induce sleep in Opn4-/- mice at this time of the day and the D-pulse-induced increase in EEG theta and gamma activity (EEG correlates of alertness and cognition) was delayed. Analysis of the LD1h:1h cycle revealed that only in Opn4-/- mice the light and dark effects greatly depended on circadian time. In addition to these acute light effects, Opn4-/- mice slept 1h less during the 12h L-phase. Despite this reduction in sleep time, EEG delta activity, a marker of sleep need, was decreased in Opn4-/- mice for most of the (subjective) D-period and the level of delta power reached after 6h sleep deprivation was significantly lower in Opn4-/- mice. This indicates that lack of melanopsin alters sleep homeostasis and experiments are under way to further determine the mechanisms by which melanopsin can affect the homeostatic control of sleep.
The findings that melanopsin-mediated direct effects of light, the circadian drive and sleep homeostasis interact together to determine the timing and quality of sleep and waking calls for a re-evaluation of the role of light on human behavior and performance.

Disclosures: P. Bourgin: None. **J. Tsai:** None. **E. Ruppert:** None. **J. Hubbard:** None. **J. Hannibal:** None. **G. Hagiwara:** None. **D. Colas:** None. **N.F. Ruby:** None. **H.C. Heller:** None. **P. Franken:** None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.3

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant HL-086662

Title: Slow wave sleep is preferentially regulated during sleep fragmentation in mice

Authors: *R. VIJAY, N. KAUSHAL, D. GOZAL;
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Abstract: Sleep fragmentation (SF) is an important component of many sleep disorders, including obstructive sleep apnea. However, sleep homeostatic responses during acute and prolonged SF have not been explored. C57BL/6 mice (n=6) were chronically implanted with telemetric transponders for sleep recordings. Following surgical recovery, 24 hr baseline telemetric sleep recordings were conducted, after which mice were subjected daily to 12h SF during the light period (7:00 am - 7:00 pm) using a custom-designed and validated apparatus. During day 1, animals showed increased Wake, decreased SWS and REM for the first 4h, after which Wake and SWS gradually returned to baseline. REM sleep however remained lower throughout the SF procedure and showed a rebound during the dark period (when SF was ceased). Delta power during SWS was markedly higher and SWS latency lower when compared to BL. On day 8 of daily SF, animals exhibited no differences in Wake, SWS or REM sleep, even though the total number of arousal and waking events during the SF period remained identical to day 1 SF. Furthermore, EEG delta power was markedly higher throughout the SF-day 8 procedure, and SWS latency was lower when compared to both day 1 SF and BL. These results support the hypothesis that SF elicit discrepant SWS and REM homeostatic responses.

Disclosures: R. Vijay, None; **N. Kaushal**, None; **D. Gozal**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.4

Topic: E.08. Biological Rhythms and Sleep

Support: NIH grant HL-071097

Title: Protein levels of the $GABA_A$ receptor β1 subunit and GABA precursor, GAD, are increased in the perifornical region of the posterior hypothalamus following sleep deprivation

Authors: D. V. VOLGIN[1], G. M. STETTNER[1], *R. J. ROSS[2,3], L. KUBIN[1];
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Abstract: Neuronal activity in the posterior hypothalamus (PH) promotes wakefulness (von Economo, 1930), whereas exogenous activation of $GABA_A$ receptors ($GABA_AR$) in this region facilitates sleep (Sallanon et al., 1989). These data prompted us to hypothesize that regulation of $GABA_AR$ expression in the PH may contribute to the homeostatic regulation of sleep. We previously determined that mRNA levels of some $GABA_AR$ subunits in the PH, including the β1 subunit, are increased following sleep deprivation (SD) independently of some of them also varying with circadian time. We now measured β1 subunit of $GABA_AR$ and GAD protein levels in the PH at different circadian times and following SD. Adult, male, Sprague-Dawley rats were anesthetized and rapidly decapitated at: 9 am, 3-4 pm, or at 3-4 pm following 6 h of gentle SD (n=9-10/group). Tissue micropunches (700 μm) were extracted from the perifornical (PF) and dorsomedial (DM) PH regions from 500 μm-thick slices. For each sample, proteins were separated by SDS-PAGE and subjected to Western blots. Membranes were double-stained for the β1 subunit and β-actin and then re-stained for GAD. Distinct bands were quantified densitometrically (ImageJ) and the amounts of β1 subunit and GAD65/67 were expressed relative to the density of the band for β-actin in each sample. In the PF region, protein levels of both the β1 subunit and GAD65/67 were higher at 9 am than at 3-4 pm (1.28±0.06 (SE) vs. 1.08±0.04, p=0.01; and 1.22±0.06 vs. 0.78±0.08, p=0.0004, respectively), and were also higher in the samples from SD rats than in the samples collected at 3-4 pm from freely sleeping rats (1.43±0.12 vs. 1.08±0.04, p=0.01; and 1.34±0.18 vs. 0.78±0.08, p=0.007, respectively). In contrast, in the DM region, only β1 subunit protein levels (but not GAD65/67) were higher at 9 am than at 3-4 pm and these were not altered by SD (0.99±0.14 vs. 0.53±0.03 at 3-4 pm without SD and 0.48±0.03 with SD; $p<0.01$ for both vs. 9 am). The absolute levels of β-actin measured in arbitrary units did not differ significantly among the study groups. These data suggest that GABAergic inhibition increases in a regionally-selective manner in the PH in relation to the magnitude of sleep drive. The increased expression of $GABA_A$ receptors and GAD in the PF region may contribute to sleep rebound following SD.

Disclosures: D.V. Volgin, None; **R.J. Ross**, None; **G.M. Stettner**, None; **L. Kubin**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.5

Topic: E.08. Biological Rhythms and Sleep

Support: CNPQ

Title: Increased pain perception and attenuated opioid anti-nociception is associated with a reduced tyrosine hydroxylase staining in periaqueductal grey matter in paradoxical sleep deprived rats

Authors: *G. O. SKINNER, F. DAMASCENO, O. M. M. S. ALMEIDA;
DFP, Univ. Do Estado Do Rio De Janeiro - UERJ/Centro Biomédico, Rio De Janeiro, Brazil

Abstract: Several studies have demonstrated that paradoxical sleep deprivation (PSD) leads to changes in pain sensitivity, but the mechanisms that govern these changes are poorly understood. GABAergic, serotonergic and dopaminergic neurons in the periaqueductal grey matter (PAG) are involved in pain modulation and opioid induced anti-nociception. In this study, we evaluated the effects of PSD on thermal pain sensitivity, morphine-induced anti-nociception and dopaminergic functionality in the PAG by assessing tyrosine hydroxylase (TH) immunoreactivity. Rats deprived of the paradoxical phase of sleep for 96 h and controls received either vehicle (saline) or morphine (2.5, 5 or 10 mg/kg, i.p.) and were tested with a hot plate at 46°C one hour late. TH immunoreactivity was evaluated in the ventrolateral PAG. The paw withdrawal latency response to the hot plate was significantly lower in PSD than in controls rats and it was modified by morphine only at the highest dose (10 mg/kg). Analgesic effects were observed in control groups for all doses. The number of the cellular bodies that were immunopositive marked for TH in vlPAG was reduced in animals undergoing PSD, as compared to controls. These data suggest that the PSD may increase sensitivity to harmful stimuli and change the nociceptive response induced by morphine. This alteration is apparent from the reduction in TH levels in the PAG.

Disclosures: G.O. Skinner, None; **F. Damasceno**, None; **O.M.M.S. Almeida**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.6

Topic: E.08. Biological Rhythms and Sleep

Support: CNPq Grant 140864

Title: Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) activity in dorsolateral periaqueductal gray matter of hyperalgesic paradoxical sleep deprived rats

Authors: *F. DAMASCENO, G. D. SKINNER, P. C. ARAÚJO, O. M. M. S. ALMEIDA; UERJ - IBRAG/Centro Biomédico, Rio de Janeiro, Brazil

Abstract: Paradoxical sleep deprivation (PSD) has been found to promote hyperalgesia in animals and human beings. PSD and sciatic nerve injury in rats seem to share the same spinal pain mechanisms, such as reversal of mechanical hypersensitivity by nitric oxide synthase (NOS) inhibition. Given that both nitric oxide (NO) and the dorsolateral periaqueductal gray matter (dlPAG) area of the brainstem are thought to be involved in hyperalgesia, we evaluated the activity of nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d), which served as an indicator of NOS activity on the dlPAG of paradoxical sleep-deprived rats. We also measured the pain-related behavior response of the rats after mechanical and chemical noxious stimuli. Data revealed that PSD led to increased pain-related behavior (+27%, $p \leq 0.05$) in phase I of a chemical test (formalin) and a lower paw withdrawal threshold (-47%, $p \leq 0.05$) in a mechanical test (von Frey filaments), confirming its hyperalgesic effect. In addition, the number of NADPH-d positive cells in dlPAG was higher after PSD than that in control animals (+59%, $p \leq 0.05$). Taken together, our data suggest that the hyperalgesia observed in PSD rats after mechanical and chemical noxious stimuli might be associated with increased NOS activity in dlPAG, presumably influencing the descending antinociceptive pathway.

Disclosures: F. Damasceno, None; **G.D. Skinner**, None; **P.C. Araújo**, None; **O.M.M.S. Almeida**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.7

Topic: E.08. Biological Rhythms and Sleep

Support: Merck & Co., Inc.

Defense Advanced Research Projects Agency (DARPA) and the Army Research Office (ARO), award number DAAD19-02-1-0038

Title: Identification and validation of NTSR1 as a candidate gene for affect and sleep

Authors: *K. MRAZEK[1], M. HOTZ VITATERNA[1], C. OLKER[1], J. MILLSTEIN[2], A. L. GOTTER[3], C. J. WINROW[3], J. J. RENGER[3], F. W. TUREK[1];
[1]Ctr. for Sleep and Circadian Biol., Northwestern Univ., EVANSTON, IL; [2]Statistical Genet., Sage Bionetworks, Seattle, WA; [3]Neurosci. Dept., Merck Res. Labs., West Point, PA

Abstract: Our large-scale sleep-wake phenotype and genotype analysis of 269 adult male mice from a [C57BL/6J X (BALB/cByJ X C57BL/6J F1)] N2 segregating cross lead to the identification of 52 unique quantitative trait loci (QTL). Included in this data set was a QTL for wake, rapid eye movement (REM) sleep amount, and REM bout length that mapped to chromosome 17. Furthermore, we analyzed over 40,000 expressed transcripts in three brain regions (frontal cortex, hypothalamus, and thalamus), which lead to the identification of a candidate gene, Neurotensin Receptor 1 (NTSR1). NTSR1 has an expression QTL (eQTL) logarithm of the odds (LOD) score of 3.385 in the hypothalamus, and expression levels of NTSR1 significantly correlated with wake and REM amounts mapped to the chromosome 17 QTL.
Neurotensin is a neuropeptide that interacts with the dopaminergic system, and has been previously shown to have affects on anxiety and despair behaviors. We have measured these behaviors in the NTSR1 knockout mouse, and studies are currently underway to examine sleep. NTSR1 knockouts have demonstrated an increase in anxious behavior over wild type controls as measured by the open field activity test, with significant differences in distance traveled ($p<0.01$), percent of time spent in the center ($p<0.05$), and percent of time spent in the corners ($p<0.01$). NTSR1 knockouts also show an increase in despair behavior compared to wild type controls as measured by the tail suspension test, with significant differences in bouts of immobility ($p<0.01$) and a trend for differences in seconds of immobility ($p=0.058$).
Anxiety and depression have long been associated with alterations in sleep. Despite a wealth of evidence for a genetic component for depression and anxiety, the specific genes and gene networks associated with the affective disorders remain largely unknown. We have previously demonstrated associations between anxiety and despair behavior and sleep architecture and amount, and here we are presenting that a knockout of a candidate gene in the region of a QTL for sleep characteristics known to have comorbidity in humans with affective disorders shows the same behavioral characteristics. Taken together, these findings indicate that a combined phenotype QTL and gene expression level eQTL approach is a powerful method for identifying genes involved in the regulation of complex behaviors, including sleep and related affective disorders.

Disclosures: K. Mrazek, None; **M. Hotz Vitaterna**, None; **C. Olker**, None; **J. Millstein**, None; **A.L. Gotter**, None; **C.J. Winrow**, None; **J.J. Renger**, None; **F.W. Turek**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.8

Topic: E.08. Biological Rhythms and Sleep

Support: 5F32GM086207-02

Title: Identification of genes regulating the Sleep-Feeding conflict in Drosophila

Authors: *A. C. KEENE[1], E. R. DUBOUE[2], D. M. MCDONALD[2], J. BLAU[3];
[1]Biol. dept, New York Univ., New York, NY; [2]Biol., [3]New York Univ., New York City, NY

Abstract: Sleep is affected by a number of environmental factors including light, heat, stress, social interaction and nutrition. Recent studies using *Drosophila* have identified genes and neurons regulating sleep, but the involvement of environmental factors remains relatively unexplored. In mammals there are strong interactions between the neural systems controlling sleep and feeding: sleep deprivation increases appetite and weight gain, while starvation suppresses sleep.
Our findings demonstrate that *Drosophila* potently suppress sleep during starvation and this occurs independently of light cues. It was previously shown that the mushroom bodies are required for sleep, but we find that they are dispensable for this sleep-feeding interaction. We find that disrupting function of the circadian genes *Clock* (*Clk*) and *cycle* (*cyc*) increases sleep-suppression during starvation. Silencing *Clk/cyc* expressing neurons phenocopies the hyper-responsiveness of *Clk* and *cyc* mutants. While disrupting *Clk* function in all circadian neurons hypersensitizes sleep to starvation, there is no effect of this manipulation in pacemaker neurons, suggesting that a subpopulation of non-PDF expressing *Clk/cyc* cells promote sleep during starvation. Thus we have uncovered an additional role for these clock genes in modulating an interaction between two homeostatically regulated behaviors.
We have also initiated a large-scale RNAi-based screen by pan-neuronally targeting neurally expressed genes. To date we have screened over 1,400 for sleep during sated and starved states. We are currently characterizing lines that result in flies that are hypersensitive to food deprivation or flies that fail to suppress sleep in response to food deprivation. We hope that these experiments will provide a novel approach investigating interactions between innate behaviors and further our understanding of the neural mechanisms underlying hierarchical control of homeostatically regulated behaviors.

Disclosures: A.C. Keene, None; **E.R. Duboue**, None; **D.M. McDonald**, None; **J. Blau**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.9

Topic: E.08. Biological Rhythms and Sleep

Title: The role of casein kinase 1 epsilon (CK1ε) in mouse sleep

Authors: *L. ZHOU[1], M. VITATERNA[1], A. LOUDON[2], F. TUREK[1];
[1]Ctr. for Sleep and Circadian Biol., Northwestern Univ., EVANSTON, IL; [2]Univ. of Manchester, Manchester, United Kingdom

Abstract: Sleep regulation has been considered as a two-process model which consists of a homeostatic process and a circadian timing process. Casein kinase 1 epsilon (CK1 ε) has been well known for its key role in circadian regulation. The CK1 ε tau mutation induces pronounced changes in circadian organization in rodents; however the CK1 ε knock-out mice show very subtle changes. Given the assumption that the homeostatic process and the circadian timing process are interacting with each other, we hypothesized that the CK1 ε mutants bearing circadian dysfunction would also exhibit sleep phenotypes. In the present study, we used both CK1 ε tau mutation and CK1 ε knock-out mice to directly determine the role of CK1 ε in sleep architecture in male mice. The mouse baseline sleep and recovery sleep after 6 hours of sleep deprivation (using EMG and EEG) were recorded and hand-scored. The tau mutation affected a number of sleep parameters, primarily related to rapid eye movement (REM) sleep. In LD 12:12, heterozygous and homozygous tau mutants had similar amount of baseline non- rapid eye movement (NREM) sleep as wild-type mice, but had 30 and 60%, respectively, increases in base line REM sleep than wild-type mice. Interestingly, the increase of REM sleep in tau mutants was primarily concentarted in dark phase. The sleep phenotype in CK1 ε knock-out mice was very subtle; however after sleep deprivation the sleep recovery was less in CK1 ε knock-out mice than wild-type mice. These preliminary results indicate that genetic alterations of the circadian system have widespread influence on many aspects of sleep and wake architectures, supporting the hypothesis that the homeostatic process and a circadian timing process are interacting with each other.

Disclosures: L. Zhou, None; **M. Vitaterna**, None; **A. Loudon**, None; **F. Turek**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.10

Topic: E.08. Biological Rhythms and Sleep

Support: Fidelity

Fondation Lejeune

Title: App imbalance causes sleep disturbances in a genetic model of Down syndrome

Authors: *D. COLAS[1], B. CHULUUN[1], G. C. HAGIWARA[1], C. GARNER[2], C. HELLER[1]; [1]Biol., [2]Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: Down syndrome (DS) results from the triplication of part of the chromosome 21 and is associated with learning disabilities. Sleep disturbances are also seen in DS and mice models of this condition are characterized by abnormal sleep architecture and EEG quality (Colas et al., 2003, 2004, 2008). The sleep-wake cycle can be described by several parameters (circadian, architecture, spectral quality) which are under strong genetic control. We thus questioned the possible involvement of different part of the triplicated genomic area in the observed phenotypes and our prior studies indicated that App gene was a likely candidate. Sleep-wake and EEG spectral quality were studied in mice models of DS: Ts65Dn bear a triplication of part of the mouse chromosome 16 (synthenic to the human chromosome 21) including App, Ts65/App++- bear the same triplication but for App which is present in normal dosage. These TS mice are compared to their normal diploid littermates (2N). Recordings were obtained in 3 months old animal, in baseline conditions. Ts65Dn are characterized by delayed sleep onset, decreased sleep amounts during the dark period, increased REM-sleep amounts during the light period, the presence of abnormally high theta activity in NREM and REM sleep resulting in fragmented sleep. Normalization of App in Ts65/App++- rescues most of the abnormal features seen in Ts65Dn. Thus, App plays a prominent role in sleep and EEG abnormalities in DS mice models as early as 3 months of age. Impaired sleep quality by increased theta activity in Ts65Dn could account, in part, for the long term memory deficits seen in this model.

Disclosures: D. colas, None; **B. Chuluun**, None; **G.C. Hagiwara**, None; **C. Garner**, None; **C. Heller**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.11

Topic: E.08. Biological Rhythms and Sleep

Title: Spontaneous brain rhythms predict sleep stability in the face of noise

Authors: *T. DANG-VU[1], S. MCKINNEY[1], O. BUXTON[2], J. SOLET[3], J. ELLENBOGEN[1]; [1]Neurology, Sleep Med., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [2]Med., Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA; [3]Psychology, Cambridge Hlth. Alliance, Harvard Med. Sch., Cambridge, MA

Abstract: *Introduction*
Illuminating the conditions under which sounds can disturb sleep - and who is most vulnerable to them - is critical to protecting healthful sleep. This implies the identification of potential predictors for individual sensitivity to noise during sleep.
Given that the neural processing of sound during sleep is thought to be modulated by spindles, we thus hypothesized that a sleeping brain's rate of spindle production signals its unique sensitivity to sound.
Methods
Twelve healthy human volunteers (mean age 26.3 ±7.5[SD]) were studied in the laboratory for three consecutive nights, during which sleep was monitored with a full polysomnographic battery.
The first night was a quiet night, during which no sound was presented. The second and third nights were noisy nights: fourteen common sounds, each 10 seconds in duration, were presented to the sleeping participant. Sounds were initiated at 40 dB and presented every minute in 5 dB increments until an arousal was observed on the electroencephalogram (EEG). Sound intensities at which an arousal occurred were averaged across sound types during stage 2 sleep in order to obtain a single (mean) arousal threshold per individual.
Sleep spindles were detected during stage 2 sleep of the quiet night using an automatic algorithm applied to central EEG channels (C3, C4). The density of spindles was computed as the number of spindles per minute.
Results
We found that spindle densities on the quiet night were positively correlated with arousal thresholds during the two noisy nights ($r = 0.77$; $p = 0.003$). Using Cox regression, we also found that those with higher spindle rates on a quiet night had greater sleep stability during noisy nights: hazard ratios of 0.39 from C3 ($p = 0.001$) and 0.51 from C4 ($p= 0.002$).
Conclusion
These results demonstrate that it is possible to predict an individual's ability to maintain sleep in the face of external sound. Those with more abundant spindles are more resistant to sounds during sleep. Therefore an assessment of the sleeping brain's spontaneous activity, as reflected by spindle density, can serve as a biological marker for predicting individual resistance to acoustic stimulation during sleep. This finding might aide physicians in the care of patients by

anticipating who is vulnerable to sounds and implementing strategies to mitigate them. This finding might also serve as a launching point for experiments looking to enhance the biological properties of the brain - with drug or device - that render it more resilient to noise-induced disruption.

Disclosures: T. Dang-Vu, None; **S. McKinney**, None; **O. Buxton**, None; **J. Solet**, None; **J. Ellenbogen**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.12

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NR011230

Title: Reduced cortical thickness with depressive symptoms in patients with obstructive sleep apnea

Authors: *P. M. MACEY[1], A. S. MOYADI[2], R. KUMAR[2], M. A. WOO[3], R. M. HARPER[2]; [1]UCLA Sch. of Nursing, Los Angeles, CA; [2]Neurobio., [3]UCLA Sch. of Nursing, UCLA, Los Angeles, CA

Abstract: Obstructive sleep apnea (OSA) patients show high levels of mood disruption, with approximately half of patients experiencing depressive symptoms. Multiple sites of neural injury appear in OSA; these sites include regions associated with mood regulation, such as the hippocampus and anterior cingulate cortex (ACC). We hypothesized that the extent of depressive symptoms would correlate with the magnitude of neural changes in cortical sites implicated in depression, and specifically, that cortical thickness of the ACC would decrease with increasing depressive symptoms.
We collected two high-resolution T1-weighted anatomical brain scans from 47 recently-diagnosed, untreated, largely moderate-to-severe OSA patients (apnea/hyponea index mean ± stdev: 32.9 ± 21.1 events/hour; age 46.7 ± 9.1 years; female:male 12:35) using a 3.0 Tesla magnetic resonance imaging scanner. Subjects also completed the Beck Depression Inventory-II (BDI) questionnaire, which provides a measure of severity of depressive symptoms. We used SPM5 and custom software to average T1-weighted scans, calculate total intracranial volume, based on gray matter, white matter and cerebrospinal fluid segmentations of the T1-weighted images. We calculated and analyzed cortical thickness using FreeSurfer software, and performed

surface-based analysis using a general linear model to assess the relationship between cortical thickness and BDI scores (threshold: t-statistic > 2), with total intracranial volume and sex included as covariates.
Cortical thickness decreased with higher BDI scores in multiple cortical regions, including the ACC. The left side showed a greater reduction, consistent with earlier measures of tissue alterations in OSA. Other affected areas included the left posterior cingulate (isthmus region), scattered areas in the superior and inferior parietal cortices, and the superior frontal cortex. Depressive symptoms are associated with decreased cortical thickness in the ACC, as well as other cortical regions in OSA subjects. These findings suggest that the elevated depressive symptoms in OSA are not simply a consequence of poor sleep; rather, a biologic basis appears to exist for at least some of the mood disturbances common in this population. The question remains as to whether the brain changes precede the onset of depressive symptoms, or whether the depression leads to neural changes. Similarly, the question of whether depression follows from OSA or develops concurrently with the respiratory disease is also unresolved.

Disclosures: P.M. Macey, None; **A.S. Moyadi**, None; **R. Kumar**, None; **M.A. Woo**, None; **R.M. Harper**, None.

Nanosymposium

432. Mechanisms and Physiological Factors that Regulate Sleep

Location: Room 6A

Time: Monday, November 15, 2010, 1:00 pm - 4:15 pm

Program Number: 432.13

Topic: E.08. Biological Rhythms and Sleep

Support: NSF Graduate Research Fellowship

Mary Elisabeth Rennie Epilepsy and Epilepsy-related Research Grant

Title: Two-dimensional mapping of EEG states for higher resolution sleep scoring

Authors: *B. LOPOUR[1], S. TASOGLU[2], H. E. KIRSCH[3], J. W. SLEIGH[4], A. J. SZERI[2]; [1]Neurobio., Univ. of California - Los Angeles, Los Angeles, CA; [2]Mechanical Engin., Univ. of California - Berkeley, Berkeley, CA; [3]Neurol., Univ. of California - San Francisco, San Francisco, CA; [4]Anesthetics, Waikato Clin. Sch., Hamilton, New Zealand

Abstract: The current standard for scoring of electroencephalogram (EEG) data allows sleep to be categorized into five discrete stages. This information is extremely valuable, but it is often too limited. For example, in studying the complex relationship between seizures and sleep, we might

ask: Was the subject descending to deeper stages of sleep when the seizure occurred, or arising from them? How quickly was the subject moving through each stage? Was a transition between stages imminent at the time of the seizure? We have found that a mathematical model of the human sleep cycle can be used to obtain a detailed, two-dimensional mapping of EEG sleep stages and may provide insight into such intriguing questions.
We have associated human EEG data to a mathematical cortical model via locally linear embedding (LLE), a method of dimensionality reduction. Our analysis, based on LLE, can distinguish between traditional sleep stages when applied to human data; it reliably separates REM and non-REM sleep and maps the EEG to a low-dimensional output space where the sleep state changes smoothly over time. In addition, the concept of strongly connected components enables us to incorporate automatic outlier rejection for the EEG data. Then, by using LLE on a hybrid data set containing both sleep EEG and signals generated from the mesoscale cortical model, we are able to quantify the relationship between the data and the mathematical model. This enables us to take any sample of sleep EEG data and associate it with a position among the continuous range of sleep states provided by the model; we can thus infer a trajectory of states as the subject sleeps. This method gives consistent results for various subjects over a full night of sleep and can be done in real time. If this analysis is applied to subjects with epilepsy, it may be possible to use the trajectory of sleep states to predict (and perhaps prevent) seizures.

Disclosures: B. Lopour, None; **S. Tasoglu**, None; **H.E. Kirsch**, None; **J.W. Sleigh**, None; **A.J. Szeri**, None.

Nanosymposium

433. Reward and Ultrasonic Vocalization

Location: Room 2

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 433.1

Topic: F.03. Motivation and Emotion

Support: NSERC Grant 155055

CIHR Grant MOP-10516

Title: A subtype analysis of amphetamine-induced 50-kHz ultrasonic vocalizations in adult rats: Effects of dopaminergic and noradrenergic drugs

Authors: *J. WRIGHT, M. R. S. DOBOSIEWICZ, P. B. S. CLARKE;
Pharmacol. and Therapeut., McGill Univ., Montreal, QC, Canada

Abstract: *Rationale*: Recently, we have shown that the 50-kHz ultrasonic vocalizations (USVs) emitted by adult rats can be classified into at least 14 distinct subtypes (Wright et al., 2010, Psychopharmacology). Furthermore, both social context and systemic amphetamine (AMPH) administration differentially altered the rats' call profile (i.e. the proportional contribution of each subtype to the total number of calls). These findings add to evidence that call subtypes may differ in their behavioural significance and neurochemical basis. *Objective*: The main objective of this study was to investigate the potential contribution of dopamine (DA) and norepinephrine (NE) transmission to the production of the 14 subtypes of 50-kHz USVs that we previously defined. *Methods*: Male adult Long-Evans rats were pre-treated with the D2 antagonists haloperidol (0.1 and 0.2 mg/kg, IP) or sulpiride (20 and 40 mg/kg, SC), or the D1 antagonist SCH 23390 (5, 10 and 20 mg/kg, SC), or vehicle. Rats were then treated with AMPH (1 mg/kg, IP) or saline and recorded for 20 min. In a separate experiment, USVs were recorded from rats tested under various doses of the NE reuptake inhibitor nisoxetine (4, 8 and 16 mg/kg, IP). *Results*: No antagonist given alone significantly altered call number or call profile; however, the baseline rate of calling (i.e. in the drug-free condition) was very low. All doses of nisoxetine failed to produce any change in USVs, altering neither the total number of calls nor the call profile. Similarly, the atypical D2 antagonist, sulpiride, did not affect AMPH-induced call number, or have any effect on the call profile. However, both SCH 23390 and haloperidol markedly and dose-dependently decreased USVs emitted under AMPH. Haloperidol altered the call profile such that the proportion of AMPH-induced trills was dose-dependently reduced, while step-ups were dose-dependently increased. In contrast, flat (constant-frequency) calls emitted under AMPH were proportionally unchanged, regardless of antagonist pre-treatment. *Conclusions*: The failure of the NET blocker nisoxetine to elicit USVs suggests that AMPH-induced calls are likely not mediated through changes in noradrenergic transmission. While most amphetamine-induced 50-kHz USVs appear to depend on dopaminergic transmission, flat calls do not. Whether sulpiride's lack of effect is reflective of antipsychotic atypicality requires further study.

Disclosures: J. Wright: None. **M.R.S. Dobosiewicz:** None. **P.B.S. Clarke:** None.

Nanosymposium

433. Reward and Ultrasonic Vocalization

Location: Room 2

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 433.2

Topic: F.03. Motivation and Emotion

Support: DFG grant Schw 10-1

Title: Rat 50-kHz calls in anticipation of food reward

Authors: *R. K. SCHWARTING, J. C. BRENES;
Philipps-University of Marburg, Marburg, Germany

Abstract: Rats can emit 50 kHz ultrasonic vocalizations, which are thought to represent their subjective emotional or motivational state. These calls have been observed in response to positive affective stimuli such as sex, food, drug of abuse, electrical brain stimulation, play, and tickling. Although 50 kHz calls could gauge an appetitive motivational state, the role of these calls in incentive motivation tasks has not been systematically investigated. This study, therefore, sought to determine whether anticipation of food reward could elicit 50 kHz calls. For this aim, male Wistar rats were tested in a home cage where they learned to associate the 1.5-h daily feeding session with a preceding tone-cue. Food-paired rats rapidly showed conditioned anticipatory activity, specially digging, and decreased latencies to eat, but not a substantial increase in 50 kHz calling throughout the days of testing.
The prolonged engagement in stereotyped behavior could presumably account for the lower call rate observed in these animals.
Conversely, a remarkable reduction in anticipatory activity and food intake, and a huge increase in 50-kHz calling during all test phases were detected as soon as the food-paired rats were sated.
In a second experiment, rats were trained to run through a runway maze to access their daily food. This food was delivered in the same single home cage used as in the previous experiment, which in case of this modified test was attached to the end of the runway goal arm. In this task, the rats were free to move between both compartments. Here, food-paired rats showed progressively more 50-kHz calls in both test compartments.
Interestingly, the increase in call rate was mainly observed between eating bouts, i.e. when animals revisited the maze. A high and consistent individual variability was observed along days and tests in both groups. Our data somewhat support the assumption that 50-kHz calls can index an appetitive motivational state in the rat; however this effect became noticeable just when certain homeostatic and testing conditions were given.

Disclosures: R.K. Schwarting, None; **J.C. Brenes**, None.

Nanosymposium

433. Reward and Ultrasonic Vocalization

Location: Room 2

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 433.3

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA014640

NIH Grant DA014640-05S1

Bruce-Jones Graduate Fellowship

The University of Texas Waggoner Center for Alcohol and Addiction Research

Title: Drug-free weekends enhance cocaine and cue-associated ultrasonic vocalizations

Authors: *C. L. DUVAUCHELLE[1], M. I. ABDALLA[2], N. H. THAKORE[3], T. SCHALLERT[4], E. Y. MAIER[3];
[1]Pharmacol. & Toxicology, [2]Biol., [3]Col. of Pharm., [4]Psychology, Univ. Texas at Austin, AUSTIN, TX

Abstract: Cues and environments extensively paired with cocaine intake are known to enhance cocaine craving in humans and can trigger relapse. However, since drug craving is an intense emotional response, it is challenging to model in animals. Ultrasonic vocalizations (USVs) are postulated to reflect the emotional states of animals. In rats, short 50-kHz USVs are evoked by anticipation of positive events, such as mating and food delivery and low-frequency 22-kHz USVs are associated with cues predicting negative events, such as foot-shock cue and predator odor. In drug dependence studies, rats are often tested daily with short breaks (such as weekends) spent untested in their home cages. The present study explored whether the salience of cocaine-access cues and cocaine reinforcement is increased after skipping weekend cocaine and cue exposures. The present study found that over the course of several weeks of cocaine self- or yoked-administration pre-drug cues signaling forthcoming access or delivery of cocaine elicited marked amounts of anticipatory 50-kHz USVs, and that weekend deprivation from cues and cocaine further exaggerated the level of calling (more calls on Mondays compared to Fridays). In addition, 50-kHz USVs evoked by cocaine administration were significantly greater on Mondays compared to Fridays. During the extinction phase, 50-kHz USVs during the pre-drug intervals were also significantly increased after 2 days without exposure to the cocaine environment. Locomotor activity and lever response rates did not reflect corresponding differences after weekend deprivation. No 22-kHz USVs were detected during any phase of the experiment. These results suggest a particular sensitivity of USVs to reflect deprivation-induced cocaine craving after only a short period of drug abstinence. These findings may have clinical implications, in that intermittently avoiding cocaine, cues or context may enhance drug cue salience and resistance to extinction.

Disclosures: C.L. Duvauchelle, None; **E.Y. Maier**, None; **M.I. Abdalla**, None; **N.H. Thakore**, None; **T. Schallert**, None.

Nanosymposium

433. Reward and Ultrasonic Vocalization

Location: Room 2

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 433.4

Topic: F.03. Motivation and Emotion

Support: DFG Schw 559/10-1

Title: Ultrasonic communication in rats: Effects of nucleus accumbens ibotenic acid lesion on the production of ultrasonic vocalizations and social approach behavior?

Authors: M. C. CARVALHO[1,2], M. L. BRANDÃO[2], R. K. W. SCHWARTING[1], *M. WÖHR[1];
[1]Philipps-University of Marburg, Marburg, Germany; [2]Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: Rats emit ultrasonic vocalizations (USVs). In the adult rat, two distinct USV types are known, which serve as situation-dependent affective signals. 22-kHz USVs are emitted in aversive situations such as fear-conditioning or social defeat, whereas 50-kHz USVs are emitted in appetitive situations such as rough-and-tumble play or mating. Recently, it was shown that 22-kHz USVs and 50-kHz USVs induce call-specific behavioral responses in the recipient. While 22-kHz USVs induce freezing behavior, indicating an alarming function, 50-kHz USVs induce social approach behavior, supporting the notion that they serve as social contact calls. The opposite behavioral responses are paralleled by distinct patterns of brain activation: 22-kHz USVs induce activation in amygdala and central gray; 50-kHz USVs are followed by activation in the nucleus accumbens (NAcc). NAcc is well known for its critical role in motivated behavior, where it is thought to serve as a key structure for transforming motivation into action - important for psychomotor activation and approach behavior, both critically modulated by its dopaminergic input. NAcc is also efficient in eliciting 50-kHz USVs, but not 22-kHz USVs, e.g. by local administration of the catecholaminergic agonist amphetamine. Therefore, one can assume that dopaminergic activation in the NAcc is necessary for both 50-kHz calling and approach towards 50-kHz USVs. Here, we tested this hypothesis by comparing 50-kHz calling and social approach behavior elicited by 50-kHz USV in rats with bilateral neurotoxic lesions of the NAcc (n=14; 0.4µg/0.4µL ibotenic acid) with sham lesioned controls (n=14). After verifying the accuracy of injection sites by magnet resonance tomography, rats were tested for novelty-induced psychomotor activation and 50-kHz calling in a cage test. Then, social approach behavior elicited by playback of 50-kHz USVs was measured. Results showed a reduction in psychomotor activation in lesioned rats exposed to novel environments. Differences herein vanished after repeated testing. During the cage test, lesioned and control rats emitted 50-kHz USVs. Detailed spectrographic analyses revealed no group differences. In response to playback of 50-kHz USVs, high levels of social approach behavior were detected irrespective of treatment. Lesioned rats spent about 18±5 s/min in front of the ultrasonic speaker. A slightly, but non-significant higher value was obtained for control rats: 24±5 s/min. Both groups spent less than 5 s/min away from the speaker. These findings contrast therefore with the concept that the NAcc serves to close the

functional link between mechanisms of detection and production of 50-kHz USVs.

Disclosures: M.C. Carvalho, None; **M.L. Brandão**, None; **R.K.W. Schwarting**, None; **M. Wöhr**, None.

Nanosymposium

433. Reward and Ultrasonic Vocalization

Location: Room 2

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 433.5

Topic: F.03. Motivation and Emotion

Title: 50-kHz ultrasonic vocalization in response to first-time amphetamine exposure predicts the development of pre-drug anticipatory vocalization

Authors: *A. AHRENS[1], C. NOBILE[1], C. L. DUVAUCHELLE[2], T. SCHALLERT[3]; [1]Dept. of Psychology, [2]Col. of Pharm., [3]Dept. of Psychology and Inst. for Neurosci., Univ. of Texas, Austin, TX

Abstract: RATIONALE: Recent evidence suggests that rat 50-kHz ultrasonic vocalizations (USVs) are linked to appetitive motivation and drug reinforcement. We have previously found that acute exposure to psychostimulants increases 50-kHz USVs, and this response is sensitized by repeated exposure. Increased USVs have also been observed during cued anticipation of cocaine self-administration, and during the expression of conditioned place preference for amphetamine. OBJECTIVE: The goal of this study was to extend our previous findings by determining whether multiple non-contingent exposures to amphetamine would increase the 50-kHz USVs elicited by the drug-paired context alone. METHODS: Rats received 15 intermittent i.v. infusions of saline or 1 mg/kg amphetamine (spaced every 2-3 days). In each of the 15 sessions, conditioned USVs elicited by the drug context were recorded for 10 minutes prior to infusions, and post-drug USVs and locomotor activity were recorded for 20 minutes after infusions. RESULTS: The conditioned 50-kHz USVs elicited by the amphetamine-paired context progressively increased across the 15 sessions, but only in rats that showed an initial increase in positive 50-kHz calls after their first exposure to amphetamine. A subset of rats did not show a positive initial reaction to amphetamine, and also did not develop conditioned USVs with repeated treatment. Post-drug locomotor activity did not differ between these high- and low-responder groups. CONCLUSIONS: These results suggest that a rat's immediate USV response to a drug can predict the reinforcing value that drug cues will later develop. This could have implications for understanding how individual differences and drug characteristics contribute to the development of drug addiction.

Disclosures: A. Ahrens, None; **C. Nobile**, None; **C.L. Duvauchelle**, None; **T. Schallert**, None.

Nanosymposium

433. Reward and Ultrasonic Vocalization

Location: Room 2

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 433.6

Topic: F.03. Motivation and Emotion

Support: NIH DA 023202

Fellowship from the Seattle Chapter of the ARCS Foundation

Title: Ultrasonic vocalizations as a measure of affective state during cocaine and sucrose self-administration in rats

Authors: J. R. BROWNING[1], D. A. BROWNING[1], A. MAXWELL[1], Y. DONG[1], H. T. JANSEN[1], J. PANKSEPP[1], *B. A. SORG[2];
[1]VCAPP, [2]Washington State Univ., PULLMAN, WA

Abstract: Ultrasonic vocalizations in the 50 kHz range (50 kHz USVs) are emitted by rodents during positive affective states and may be a relatively direct measure of drug-induced emotional and motivational drives to seek and approach rewarding stimuli such as drugs of abuse. The focus of the present study was to measure 50 kHz USVs in rats during different phases of cocaine or sucrose self-administration, including acquisition, extinction, cue- and cocaine- or sucrose-induced reinstatement to gain insight into the changes in affective state across the different stages of self-administration. For cocaine self-administration, the number of 50 kHz USVs increased from the beginning to the end of acquisition, and decreased during extinction. Both cue-and cocaine-induced reinstatement increased 50 kHz USVs above extinction levels. Interestingly, a significant correlation existed between the number of 50 kHz USVs and the number of active nosepokes during cue-induced reinstatement, indicating that USVs became conditioned to the cocaine-predictive cue. Rats trained for sucrose self-administration also elevated USVs during acquisition, but unlike in the cocaine experiment, extinction did not lead to a reduction in USVs. This suggests that during extinction of cocaine, but not sucrose self-administration, the decrease in USVs may represent a dysphoric state in which rats show decreased motivation to seek/approach the cocaine. Also unlike for cocaine, for sucrose self-administration, no correlation was found between USVs and active nosepokes during cue-induced reinstatement, suggesting that USVs do not become as easily conditioned to the sucrose-

predictive cue as they do to the cocaine-predictive cue. In support of this, most animals in the sucrose experiment did not show cue-induced reinstatement. For both cocaine and sucrose, rats with a higher number of 50 kHz USVs in response to the first session of self-administration acquired self-administration more rapidly and attained a higher level of maintenance responding. These findings suggest that the initial USV response to acute exposure may be a sensitive indicator of the reinforcing efficacy of rewarding stimuli and therefore a strong predictor of the vulnerability to acquire and maintain high levels of self-administration of these stimuli. In addition, USVs may provide a sensitive measure of the extent of dysphoria in the absence of a highly rewarding stimulus such as cocaine.

Disclosures: J.R. Browning, None; **B.A. Sorg**, None; **D.A. Browning**, None; **Y. Dong**, None; **H.T. Jansen**, None; **J. Panksepp**, None; **A. Maxwell**, None.

Nanosymposium

433. Reward and Ultrasonic Vocalization

Location: Room 2

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 433.7

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA014640

NIH Grant DA014640-05S1

Bruce-Jones Graduate Fellowship

The University of Texas Waggoner Center for Alcohol and Addiction Research

Title: Ultrasonic vocalizations reveal changes in emotional impact of cocaine and experience

Authors: *E. Y. MAIER[1], M. I. ABDALLA[2], T. J. SCHALLERT[3], C. L. DUVAUCHELLE[1]; [1]Col. of Pharm., [2]Biol., [3]Psychology, The Univ. of Texas, AUSTIN, TX

Abstract: Rat ultrasonic vocalizations (USVs) serve as social signals and are thought to reflect the expression of emotionality. For example, short high-frequency USVs in the 35 to 80-kHz range, termed “50-kHz USVs”, are emitted during social play, food presentation, mating and other appetitive stimuli. Thus, 50-kHz calls are thought to reflect a positive emotional state of the animal. Long low-frequency USVs in the 18 to 32-kHz range, termed “22-kHz USVs”, are elicited after aversive events such as the presentation of foot-shock cues, touch by an unfamiliar

human and during food cue extinction, to name a few. Therefore, the emission of 22-kHz USVs has been correlated with negative emotional responses. In addition, 50-kHz USVs are evoked by amphetamine and cocaine and 22-kHz calls are more easily induced during drug withdrawal (e.g., cocaine and opiates). In the present experiment, we report rat USV recordings during 60-min daily cocaine administration sessions (4 wks, 5 days/wk) followed by 4 weeks of daily extinction sessions. Animals received cocaine via self-administration or a "yoked" procedure during those sessions. Findings show cocaine rats elicited significantly more 50-kHz USVs than saline controls. The increase in cocaine-induced USVs was comparable between rats that self-administered cocaine and their yoked counterparts. The total number of cocaine-induced 50-kHz USVs peaked during the second week of sessions. However, 50-kHz USVs decreased gradually over the last two weeks, even though cocaine intake and locomotor activity increased over the same time period. In addition, 50-kHz USVs and locomotor activity during extinction rapidly dropped to control levels while lever responses in the cocaine self-administration group remained above control for the entire extinction phase. No 22-kHz USVs were detected at any stages of the experiment. The decrease in cocaine-induced 50-kHz USVs during enhanced cocaine intake suggests the development of tolerance to cocaine's positive effects with increased drug experience. In addition, the rapid drop in 50-kHz USVs during extinction indicates that USVs reflect a greater sensitivity to changes in reinforcement conditions than does non-reinforced lever responding. These data support the use of USVs as an appropriate and sensitive measure of emotional responses to drugs and conditioned environments.

Disclosures: E.Y. Maier, None; **M.I. Abdalla**, None; **T.J. Schallert**, None; **C.L. Duvauchelle**, None.

Nanosymposium

433. Reward and Ultrasonic Vocalization

Location: Room 2

Time: Monday, November 15, 2010, 1:00 pm - 3:00 pm

Program Number: 433.8

Topic: F.03. Motivation and Emotion

Support: NSF CAREER 0953106

Title: Drug evoked ultrasonic vocalizations are dependent on social context in the monogamous prairie vole

Authors: *S. T.-S. MA, S. L. HARKEY, A. BALEN, A. ZALZALA, B. ARAGONA;
Univ. of Michigan, Ann Arbor, MI

Abstract: Emerging evidence indicates that both social attachment and the intake of drugs of abuse are regulated by the mesolimbic dopamine (DA) pathway in the monogamous prairie vole, Microtus ochrogaster. This gives rise to an excellent animal model to study the interaction between social behavior and drug addiction. It has been demonstrated that ultrasonic vocalizations (USVs) can be used to approximate affective state in rodents and that this is also regulated by DA transmission. Currently, however, less is known about the vocalizations of prairie voles and if they are emitted in the ultrasonic range. Whether these vocalizations are influenced by the animal's social status or the exposure to drugs of abuse is also unknown. Therefore, our present aim is to characterize the USVs of prairie voles and attempt to establish this as a behavioral assay to investigate the effects of social experience and drugs of abuse in the prairie vole model. Our current data indicate that prairie voles vocalize in ultrasonic range (~35-kHz) and that USVs are similar between males and females. When isolated, both sexes vocalize less USVs compared to when present with cage-mate. Drugs of abuse (cocaine & amphetamine) caused elevation in USVs only when voles are in a social context (present with cage-mates). Locomotor activity wasn't significantly correlated to the amount of USVs produced. Together, these data provide an encouraging first step for the use of USVs to investigate interactions between social and drug reward in the prairie vole.

Disclosures: S.T. Ma, None; **S.L. Harkey**, None; **A. Balen**, None; **A. Zalzala**, None; **B. Aragona**, None.

Nanosymposium

434. Optical Approaches to Explore the Nervous System

Location: Room 7B

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 434.1

Topic: G.04. Physiological Methods

Support: California Institute for Regenerative Medicine, RN1-00577-1

Title: Light-activated neuronal silencing with a photolysable unnatural amino acid-incorporated inward rectifying potassium channel Kir2.1

Authors: *J.-Y. KANG, L. WANG;
CBPL-W, Salk Inst., La Jolla, CA

Abstract: The goal of this study is to develop a new method to control neuronal activity in an optical manner.
In the study of neuronal circuitry and functional characterization of brain subregions, enhancing

or
suppressing the activity of specific neuronal population is a powerful and straightforward method used in many
laboratories. With its high specificity and fast control over target neurons, the optical method is in the limelight
to regulate neuronal activity. There have been numerous approaches to devise active chromophore-containing
chemical compounds, and channel proteins that can modulate ion channel activity or neurotransmitter
distribution in response to light. Despite these efforts, highly effective methods to regulate neuronal activity at
a single-cell level are still unavailable. In eukaryotes, the inward rectifying potassium channel Kir2.1 regulates
neuronal excitability with its membrane hyperpolarizing ability. Studies have shown that Kir2.1 overexpression
can suppress the targeted neurons' excitability1,2. Here, I propose a UV/blue light-inducible version of Kir2.1 by genetically incorporating a photolysable channel blocker into Kir2.1. With the innovation of
an orthogonal tRNA/aminoacyl-tRNA synthetase (aaRS) pair, unnatural amino acids (UAAs) are genetically
encoded with high efficiency and fidelity. This technique is highly promising as over 60 different UAAs can be
incorporated to almost any residue in a protein of interest. Of a great interest, a single cell-level spatial
resolution can be acquired by incorporating two-photon photolysable UAA. This strategy can be easily applied
to other types of channel proteins to modulate their specific function, suggesting a new tool to study other
channel proteins or receptor proteins in the synapse. It will shed light on the research of synaptic mechanism
to understand neuronal connectivity and can eventually be applied to reveal the mechanisms of major
neuropsychiatric diseases.

Disclosures: J. Kang: None. **L. Wang:** None.

Nanosymposium

434. Optical Approaches to Explore the Nervous System

Location: Room 7B

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 434.2

Topic: G.04. Physiological Methods

Support: Max-Planck-Gesellschaft

Title: Novel tools to expand optogenetic avenues

Authors: *S. KLEINLOGEL[1], P. WOOD[1], R. DEMPSKI[2], U. TERPITZ[1], K. FELDBAUER[1], C. BAMANN[1], E. BAMBERG[1];
[1]Biophysical Chem., Max-Planck-Institute of Biophysics, Frankfurt, Germany; [2]Chem. and Biochem., Worcester Polytechnic Inst., Worcester, MA

Abstract: The "optogenetic" approach to control membrane excitability is now widely used. Although the toolbox is expanding continuously, several enticing possibilities still remain. Amongst them are microbial rhodopsin variants with 1) an intrinsic "bidirectional optical switch", to provide precise localized control of excitation and inhibition 2) expanded bandwidths of excitation and/or inhibition over the whole visible spectrum, 3) altered kinetics and 4) an altered ion permeability (e.g. for Ca^{2+}).
We present a novel genetic cassette, which allows stoichiometric and co-localized expression of two light-gated (or other) proteins in tandem. Our tandem operators combining channelrhodopsin-2 with either halorhodopsin or bacteriorhodopsin provide precise bi-directional local control of membrane excitability. The combination of channelrhodopsin-2 with channelrhodopsin-1 from *Volvox carterii* extended light sensitivity over the whole visible spectrum, which may prove particularly useful for gene-therapeutic restoration of vision. The combination of channelrhodopsin step-up mutants with bacteriorhodopsin allowed us to fine-tune their temporal responses further by speeding up their closing kinetics, which increases their scope of application. In a similar way, the combination of the archaeal rhodopsin pumps could further extend their working range with enhanced temporal and/or spectral characteristics.
We also present a novel microbial rhodopsin variant with increased Ca^{2+}-permeability. Despite being kinetically and spectrally identical to the wildtype, this variant exhibits an increased light-sensitivity and elicits potent depolarization in neurons.
In summary, we present sought-after tools for neuroscience which may prove particularly useful for single synapse experiments, precise bi-directional network control, vision restoration and investigations of Ca^{2+}-activated processes, to name only a few.

Disclosures: S. Kleinlogel, None; **P. Wood**, None; **U. Terpitz**, None; **K. Feldbauer**, None; **C. Bamann**, None; **E. Bamberg**, None; **R. Dempski**, None.

Nanosymposium

434. Optical Approaches to Explore the Nervous System

Location: Room 7B

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 434.3

Topic: G.04. Physiological Methods

Title: Shedding light on brain mitochondrial function in vivo under systemic or local oxygen deficiency

Authors: *A. MAYEVSKY[1,2,3], E. BARBIRO-MICHAELY[4,2], M. MANDELBAUM-LIVNAT[4,2], A. LIVNAT[4,2];
[1]Ramat-Gan, Israel; [2]The Leslie and Susan Gonda Multidisciplinary Brain research center, [3]The Mina & Everard Goodman Fac. of Life-Sciences, [4]The Mina & Everard Goodman faculty of life sciences, Bar-Ilan Univ., Ramat-Gan, Israel

Abstract: Normal mitochondrial function is a critical factor in maintaining cellular homeostasis in the brain as well as in various organs of the body. Due to the involvement of mitochondrial dysfunction in many pathophysiological conditions, real-time *in vivo* monitoring of the mitochondrial metabolic state is crucially important. This type of monitoring in animal models as well as in patients provides real-time data that can help interpret experimental results or optimize patient treatment. The monitoring of NADH redox state in the brain provides the most important information on the metabolic state of the mitochondria in terms of energy production and intracellular oxygen levels. This study presents the responses of the rat cerebral tissue metabolic and hemodynamic state, to systemic as well as focal ischemia. The models that were tested included systemic hemorrhage and middle cerebral artery occlusion (MCAO). Hemodynamic changes were evaluated by laser Doppler flowmetry and NADH by the fluorometric technique. Hemorrhage was induced in rats (n=9) by bleeding until mean arterial pressure (MAP) decreased to 40mmHg. Then after there was no further interference for 30 minutes after which the withdrawn blood was re-infused and monitoring proceeded for 2 hours. Focal cerebral ischemia (MCAO) was induced according to a modified standard protocol (n=9) with several modifications. The MCA was occluded for 10 minutes, following by 60 minutes of reperfusion. Cerebral blood flow (CBF) and NADH were recorded from the lateral site (core) and the medial site (penumbra) on the right hemisphere. In the hemorrhagic model, although the small intestine showed significant deteriorations both in tissue blood flow and mitochondrial function during the beginning of the hemorrhagic phase, the brain was protected and NADH remained stable. Whereas, following focal cerebral ischemia (MCAO) CBF levels decreased and NADH increased in both core and penumbra although the deterioration was larger in the core compared to the penumbra. In both models mitochondrial dysfunction was associated with decreased cerebral perfusion and full recovery was associated with full reperfusion.
In addition, the results from the hemorrhagic model points towards the advantage of monitoring NADH and tissue blood flow in less-vital organs, such as the small intestine, versus vital organs such as the brain, in order to detect systemic deterioration in an early phase before vital organs are affected.
In conclusion, these results demonstrate the potential of mitochondrial NADH monitoring for the evaluation of tissue viability under systemic as well as focal deterioration.

Disclosures: A. Mayevsky: None. **E. Barbiro-Michaely:** None. **M. Mandelbaum-Livnat:** None. **A. Livnat:** None.

Nanosymposium

434. Optical Approaches to Explore the Nervous System

Location: Room 7B

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 434.4

Topic: G.04. Physiological Methods

Support: ANR RIB FRETinvivo

FCT, Portugal

CNRS

UPMC

Title: Real-time in vivo monitoring of PKA activity in deep brain regions using fibered fluorescence microscopy with dual emission detection

Authors: M. BARBOSA-BRITO[1], L. CAVELLINI[1], J. ZHANG[2], D. PAUPARDIN-TRITSCH[1], *P. VINCENT[1];
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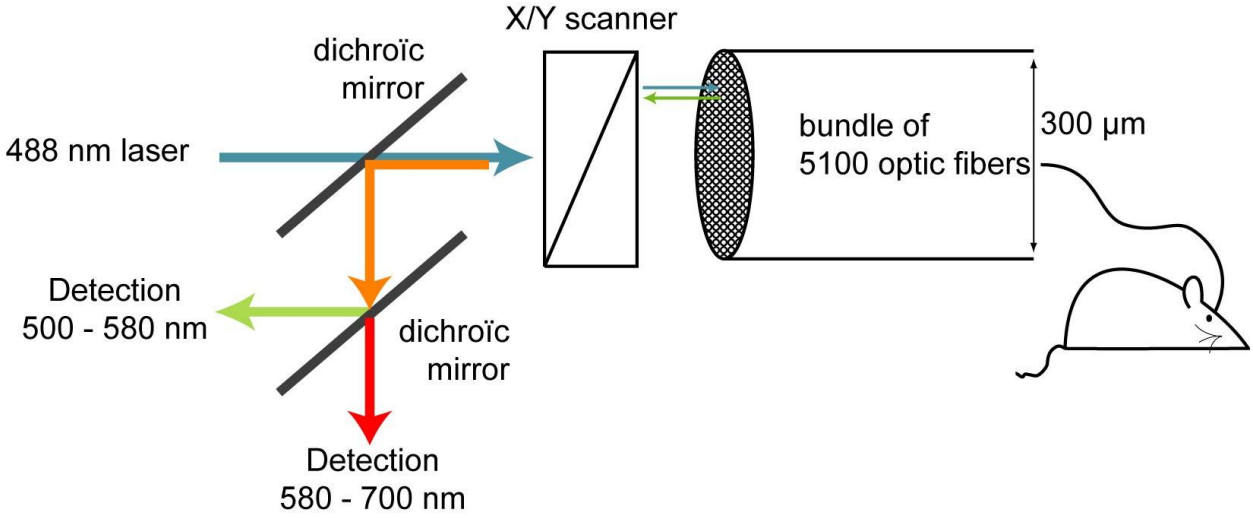
Abstract: Neurosciences use a large repertoire of imaging methodologies including optical methods that allow studies at the cellular level with high spatial and temporal resolution. In addition, biosensors have been developed to report in real-time an optical signal in response to specific cellular events. Those biosensors can be expressed using genetically encoded vectors increasing the specificity of the signal, giving the possibility to have a cellular report in vivo. Optical methods are mostly confined to superficial structures of the brain, due to the light scattering in the tissue. In 2006 we described a novel imaging approach to observe individual neurons in deep brain regions. This method uses a bundle of optical fibres (image guide) of only 300µm in diameter, which can be inserted into the brain tissue with negligible damage to the recorded structure. Indeed, using this approach, we reported calcium signals, for the first time recorded in deep brain (Vincent et al., EMBO reports, 2006).
In order to record signalling cascades with FRET biosensors, we use a novel instrument developed by Mauna Kea Technologies with simultaneous detection of two wavelengths,

allowing for ratiometric quantification.
We tested this instrument with rats injected with sindbis vectors expressing a FRET biosensor on striatal neurons.
Concerning FRET studies, FFM demands the use of a different FRET pair, which donor has to be excited with 488nm. Therefore we designed and validated a new FRET probe using the GFP/dTomato pair that reports PKA activation.
Combining this new cellular imaging technology with the genetically encoded FRET probes, we are now able to record, for the first time, specific cellular events occurring in deep brain and detected in real-time in vivo.



Disclosures: M. Barbosa-Brito, None; **L. Cavellini**, None; **J. Zhang**, None; **D. Paupardin-Tritsch**, None; **P. Vincent**, None.

Nanosymposium

434. Optical Approaches to Explore the Nervous System

Location: Room 7B

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 434.5

Topic: G.04. Physiological Methods

Title: Connecting brain and skin - Light tissue interaction in the near infrared spectrum studied with principal component analysis

Authors: *J. KAINERSTORFER[1], J. D. RILEY[2], F. AMYOT[3], L. NAJAFIZADEH[4], A. MEDVEDEV[5], E. WASSERMANN[3], J. GRAFMAN[3], A. H. GANDJBAKHCHE[2];

[2]NICHD, [3]NINDS, [1]NIH, BETHESDA, MD; [4]Henry M. Jackson Fndn., Rockville, MD; [5]Neurol., Georgetown Univ., Washington, DC

Abstract: Near Infrared Spectroscopy (NIRS) is an emerging imaging technology for measuring brain functions non-invasively on humans, by using near infrared light for illumination and measuring diffusely reflected light from the brain. Due to absorption and scattering changes, brain function can be imaged and mapped if multiple source detector pairs are used. The strength of this technique lays in the capability of assessing blood volume, oxygenation, and deoxygenation simultaneously, if a modified Beer-Lambert's Law is applied. Here we are proposing an alternative method, based on Principal Component Analysis (PCA), for extracting these values quantitatively, model independent and in real time. Using diffuse multi-spectral imaging on the skin, which is based on the same light-tissue interactions, we have already been showing that PCA can extract those values, with eigenvector 1 corresponding to blood volume and eigenvector 2 to blood oxygenation. We found that the calculated eigenvectors are the same for healthy volunteers, as well as Kaposi's sarcoma patients, with only 7degree angular shift. Given the stability in skin results, our data suggest that there is an underlying mechanism, which allows us to extract blood volume and oxygenation specific eigenvectors, which are wavelength dependent, but most importantly, tissue independent. In order to evaluate this hypothesis, we applied these eigenvectors to functional NIRS data from the brain. Brain data was acquired using a continuous wave instrument (CW5, TechEn, MA) with the imaging probes placed over the prefrontal cortex. The functional task used was a complexity task (Krueger, 2009) with twelve blocks of ten sentences each, each block lasting 30s, followed by 30s resting period. The subject was asked to indicate the level of complexity involved for the task displayed. We block averaged the data (30s) and performed baseline comparison (2s before block onset). For verification, image reconstruction for blood volume, oxygenation, and deoxygenation has been performed with HOMer (PMILab, MGH).
Reconstructed data suggests a local increase/decrease in blood oxygenation and blood volume in the anterior/posterior areas of the lateral prefrontal cortex, most likely due to language related activity. Eigenvector converted data shows a highly correlated similarity in spatial distribution, with eigenvector 1 and 2 corresponding to blood volume and oxygenation respectively. Our data therefore supports the hypothesis of using tissue independent eigenvectors for extracting blood volume and oxygenation. Future work will include a larger sample size, as well as diversification of the functional task used.

Disclosures: J. Kainerstorfer, None; **J.D. Riley**, None; **F. Amyot**, None; **L. Najafizadeh**, None; **A. Medvedev**, None; **E. Wassermann**, None; **J. Grafman**, None; **A.H. Gandjbakhche**, None.

Nanosymposium

434. Optical Approaches to Explore the Nervous System

Location: Room 7B

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 434.6

Topic: G.04. Physiological Methods

Title: Automated image capture and analysis of brdu labeled cells in rat brain sections

Authors: *L. S. BLEICHER, C. BARLOW, T. A. CARTER, A. R. DEARIE, K. EUM, B. T. FRANCHINI, E. K. HOFER, D. H. LEE, K. I. LORRAIN, J. C. PIRES, J. J. RODRIGUEZ, M. D. SAXE, B. S. STOVEKEN, P. C. WEDEL;
BrainCells Inc, SAN DIEGO, CA

Abstract: Recent discoveries linking neurogenesis to central nervous system disease have presented new opportunities for addressing the unmet medical needs of millions afflicted by brain-related illness. BrainCells Inc. is translating the science of neurogenesis into clinical practice with a technology platform built on this pioneering research. BrainCells is using the platform to build a pipeline of clinical stage programs.
BrainCells' proprietary neurogenic platform technology includes state-of-the-art human neural stem cell based and animal-based assays. These assays include measurements of the proliferation, migration, differentiation and survival of new neurons as well as traditional and leading-edge behavioral studies for CNS indications. Correlation between behavioral observations in disease models and direct histochemical analysis of neurogenesis is essential for progressing drug candidates into clinical studies.
One standard method for quantification of cellular proliferation involves the administration of the DNA labeling agent BrdU at the relevant time point, and determination of the number of labeled cells after sacrifice. Manual counting of sufficient BrdU labeled cells in rat brain tissue slices for robust statistical analysis of drug effects is highly time consuming, prone to inter-analyst variability and bias. To support our efforts on investigation of neurogenic agents we have developed an automated method for the key steps in this process.
Rat brain tissue slices appropriately fixed, stained and mounted on standard glass slides are imaged using an InCell 2000 automated microscopy system. The resulting images are analyzed using a Pipeline Pilot image and data analysis protocol suite which we have developed. Identification of the region of interest (Dentate Gyrus) is made based on staining patterns and shape analysis of objects labeled with NeuN. Within this region of interest cells containing BrdU are counted. Qualification of objects as cells or cell clusters is based on both intensity and size characteristics. Lastly the location of these newly generated cells is determined with respect to the Granular Cell Layer and the Molecular Layer.
Our method demonstrates several of the advantages of automated histological image analysis, resulting in faster, more reproducible and better annotated results. This presentation will focus on the methodology we have developed, comparison with manual counting results, its implementation and its advantages and pitfalls.

Disclosures: **L.S. Bleicher**, BrainCells Inc., Employment; **C. Barlow**, BrainCells Inc., Employment; **T.A. Carter**, BrainCells Inc., Employment; **A.R. Dearie**, BrainCells Inc., Employment; **K. Eum**, BrainCells Inc., Employment; **B.T. Franchini**, BrainCells Inc.,

Employment; **E.K. Hofer**, BrainCells Inc., Employment; **D.H. Lee**, BrainCells Inc., Employment; **K.I. Lorrain**, BrainCells Inc., Employment; **J.C. Pires**, BrainCells Inc., Employment; **J.J. Rodriguez**, BrainCells Inc., Employment; **M.D. Saxe**, BrainCells Inc., Employment; **B.S. Stoveken**, BrainCells Inc., Employment; **P.C. Wedel**, BrainCells Inc., Employment.

Nanosymposium

434. Optical Approaches to Explore the Nervous System

Location: Room 7B

Time: Monday, November 15, 2010, 1:00 pm - 2:45 pm

Program Number: 434.7

Topic: G.04. Physiological Methods

Support: UK Biological and Biotechnology Research Council (F021127)

UK Engineering Physical Sciences Research Council (F029241)

Title: Spike engineering with ChR2

Authors: *N. GROSSMAN[1], R. BERLINGUER PALMINI[2], K. NIKOLIC[2], M. GRUBB[3], J. BURRONE[3], P. DEGENAAR[2];
[1]London, United Kingdom; [2]Imperial Col., London, United Kingdom; [3]King's Col., London, United Kingdom

Abstract: Photostimulation technology that combines optics and genetics to interface with neural circuits is developing into one of the most important tools in neural stimulation. Using genetically encoded agents such as Channelrhodopsin-2 (ChR2), light sensitivity can be imparted onto otherwise ‘blind’ neuronal cells and an external light source can be used to remotely generate the action potentials. In contrary to conventional electrical stimulations, the ChR2 evoked currents are sensitive to the neural it drives. ChR2 behaves as a complex light-controlled, voltage-dependent current driver coupled to a dynamic-threshold voltage-oscillator (neuron). Despite the important of the opto-genetic technique, little is known today about the process in which it generates action potentials in neurons. Here, we use experimental and modeling techniques to explain the process and the underlying mechanisms that limit the light-to-spike process with ChR2. We show the interlinked relationship between ChR2 photocurrents and neural depolarization and the role of adaptation, saturation, negative feedback and shuttering speed in modeling the spiking response. In addition, we describe how smart engineering of the light pattern (intensity, pulse width) and the ChR2 properties (conductivity, stability and desensitization) can potentially help to maximize the reliability of this important technique.

Disclosures: N. Grossman, None; **K. Nikolic**, None; **R. Berlinguer Palmini**, None; **M. Grubb**, None; **J. Burrone**, None; **P. Degenaar**, None.

Nanosymposium

525. Development: Activity-Dependent Modulation of Connectivity II

Location: Room 25A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 525.1

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: F31NS066822

T32NS041231

R01NS047700

American Epilepsy Foundation Fellowship EF123098

Title: Neonatal exposure to phenobarbital delays maturation of synaptic transmission in striatum with behavioral consequences

Authors: *P. A. FORCELLI[1,2], M. J. JANSSEN[1], C. SWEENEY[1], S. VICINI[3,2], K. GALE[1,2]; [1]Dept of Pharmacol., Georgetown Univ., WASHINGTON, DC; [2]Interdisciplinary Program in Neurosci., Georgetown Univ., Washington, DC; [3]Dept of Physiol. and Biophysics, Georgetown Univ., WASHINGTON, DC

Abstract: At the end of the 2nd postnatal week in rats, marked and rapid changes occur in the innervation of striatum. However, maturation of GABA and glutamate transmission in the principal striatal output cells, medium spiny neurons (MSNs), during this period has not yet been described.
Here we examine development of synaptic responses in MSNs during the 2nd and 3rd postnatal week in corticostriatal slices from rat pups. We also determined if a single exposure to phenobarbital (PB) at postnatal day (P) 7 alters synaptic maturation from P10 to P18. IPSCs and mEPSCs were recorded from MSNs on P10, P14 and P18 using whole cell voltage clamp.
m and sIPSC frequency increased two-fold from P10 to P14 and by an additional 50% by P18. IPSC amplitude and decay constant decreased from P10 to P18. EPSC frequency also increased from P10 and P18, but the increase was restricted to the P14 to P18 period; amplitudes did not change during this period.

Following preexposure to PB (37.5 or 75mg/kg ip) at P7 (but not at P10), the increase in IPSC frequency from P10 to 18 was lost, while amplitude and decay were comparable to controls. The same treatment with PB disrupted the increase in EPSC frequency from P14 and P18, with EPSC amplitude unaffected.
To determine if the PB effect on maturation of MSN responses is related to the well-documented neurotoxicity of PB at P7, we pretreated pups with melatonin to protect against neuronal cell death induced by PB on P7, and examined IPSCs in MSNs on P14. In the presence of melatonin, PB treatment at P7 had no effect on IPSC frequency measured at P14.
In parallel studies, we examined effects of P7 exposure to PB (75 mg/kg) on behavior in postweanling rats (P21). We tested rats in a striatal-dependent reversal learning task and found PB exposed rats to be unimpaired on initial learning, but impaired during reversal learning when compared to vehicle exposed controls. In addition, although baseline locomotor activity did not differ between PB and vehicle exposed rats, PB exposed rats had a greater locomotor response to d-amphetamine than did controls.
Our data indicate that in MSNs from P10 to P18 in rat, both GABA-mediated inhibitory and glutamate-mediated excitatory synaptic transmission undergo marked maturational changes, with an earlier onset for the inhibitory synapses. This maturational pattern is susceptible to disruption by acute neurotoxic perturbations that occur earlier in the neonatal period, as evidenced by the effect of a single exposure to PB at P7. The fact that the same exposure leads to behavioral abnormalities 2 weeks later, raise the possibility that hindering normal progression of synaptic elaboration in striatum may lead to long-term functional deficits.

Disclosures: P.A. Forcelli: Research Grant; Epilepsy Foundation of America Fellowship EFA123098, F31NS066822. **M.J. Janssen:** None. **C. Sweeney:** None. **S. Vicini:** R01NS047700. **K. Gale:** T32NS041231.

Nanosymposium

525. Development: Activity-Dependent Modulation of Connectivity II

Location: Room 25A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 525.2

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: FNRS Grant 31003A_130625

Title: Developmental stage- and exposure time-dependent persistent effects of propofol anesthesia on neuronal circuitry development in the rat medial prefrontal cortex

Authors: *L. VUTSKITS[1], I. NIKONENKO[3], A. DAYER[2], A. BRINER[2];

[1]Univ. Hosp. of Geneva, 1211 Geneva, Switzerland; [2]Univ. Hosp. of Geneva, Geneva, Switzerland; [3]Univ. of Geneva Med. Sch., Geneva, Switzerland

Abstract: Background: Recent observations demonstrate that anesthetics can rapidly impair synaptogenesis during neuronal circuitry development. The questions, whether these effects are lasting and depend on the developmental stage at which these drugs were administered remain, however, to be explored. In this study, we investigated these issues by focusing on dendritic arbor and spine development of layer 5 pyramidal neurons in the rat medial prefrontal cortex (mPFC).
Methods: Whistar rats received propofol anesthesia (single dose or 6-hour-long exposure) at defined developmental stages between postnatal days (PND) 5 and 90. The acute as well as the long-term effects of these treatments on neuronal cytoarchitecture were evaluated using iontophoretic injections of Lucifer Yellow into layer 5 pyramidal neurons in the mPFC. Tracing of dendritic tree was carried out using the Neurolucida station, whereas dendritic spines were analyzed by confocal microscopy. Quantitative electron microscopy was applied to investigate synapse density.
Results: Layer 5 pyramidal neurons of the mPFC displayed intense dendritic growth and spinogenesis during the first postnatal month. Propofol administration did not affect dendritic arbor development at any time points examined during this period. Exposure of rat pups to either a single dose or a 6-hour-long propofol regimen at PND5 or at PND10 significantly decreased dendritic spine density, while these same treatment paradigms induced a significant increase in the number of dendritic spines at PND15 and 20. At PND30, only the 6-hour-long propofol regimen increased spine density, and no effect of this treatment modality was found at PND60 and 90. Quantitative electron microscopy revealed that the propofol-induced increase in spine density was accompanied by a significant increase in the number of dendritic spine but not shaft synapses. Finally, we found that the propofol-induced modifications in dendritic spine densities persisted up to PND90.
Conclusion: These new results demonstrate that propofol anesthesia can (i) rapidly induce significant changes in dendritic spine density; (ii) these effects are developmental stage-dependent; (iii) persist into adulthood; (iv) and are accompanied with alterations in synapse number. The ensemble of these data suggest that anesthesia in the early postnatal period might permanently impair appropriate circuit assembly in the developing cerebral cortex.

Disclosures: L. Vutskits, None; **A. Briner**, None; **I. Nikonenko**, None; **A. Dayer**, None.

Nanosymposium

525. Development: Activity-Dependent Modulation of Connectivity II

Location: Room 25A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 525.3

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH NINDS Grant 5R01NS060896

March of Dimes Research Grant 6-FY2008

Title: Cortical circuit development and plasticity after neonatal hypoxia-ischemia

Authors: A. IEVINS, V. NGUYEN, M. EVANS, *P. S. MCQUILLEN;
Pediatrics and Neurol., Univ. California, San Francisco, San Francisco, CA

Abstract: Neonatal cerebral hypoxia-ischemia (HI) impairs plasticity of both somatosensory and visual cortex. Impaired plasticity is accompanied by disrupted parvalbumin inhibitory neuron development, yet no evidence is found for weakened functional inhibition. On the contrary, neonatal HI results in reduced cortical response to an optimal visual stimulus. Despite diminished cortical activation, the expression of many activity regulated proteins remains intact at later ages. Here we focus on the effects of neonatal HI on early development of neocortical excitatory and inhibitory circuits and signaling during a period characterized by spontaneous, synchonized cortical activity.

Chloride transporters undergo a developmental shift in expression, with decreased NKCC1 and increased KCC2 expression, that influences the reversal potential for chloride and determines the outcome of GABA-mediated signaling (excitation vs. inhibition). Following neonatal HI at P2, we find decreased expression of KCC2 in HI animals compared with normal controls at P3 (mean±SEM 0.44±0.01au control vs. 0.32±0.031au HI, p=0.015) and at P7 (1.045±0.073 control vs. 0.49±0.053 HI, p=0.0035), with a trend to increased expression of NKCC1 (0.07±0.01 control vs. 0.21±0.23 HI), predicting delayed switch to inhibitory GABA signaling.

Impaired functional development and lesion-induced plasticity of whisker-barrels has been reported in mice lacking glial glutamate transporters GLT-1 and GLAST. Following neonatal HI, we find decreased expression of GLT-1 in HI animals compared with normal controls at 24 hours (mean±SEM 58.09±7.78 control vs. 40.6±7.16 HI, p=0.08). Together, these observations suggest that neonatal HI impairs early maturation of both excitatory and inhibitory signaling.

To determine whether these changes specifically affect excitatory or inhibitory neuron subpopulations, we are characterizing a neonatal mouse HI model using mice with green fluorescent protein labeling inhibitory neurons (GAD67-GFP). Inhibitory neuron number, total neuron number and infarct volume are quantified with optical fractionator and Cavalieri estimator stereological techniques three weeks following neonatal HI. Understanding early circuit development following neonatal brain injury will identify specific interventions to optimize recovery.

Disclosures: A. Ievins, None; **V. Nguyen**, None; **M. Evans**, None; **P.S. McQuillen**, None.

Nanosymposium

525. Development: Activity-Dependent Modulation of Connectivity II

Location: Room 25A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 525.4

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH grant RO1 AG027233

Title: MPP family members as new cytoplasmic partners of nectins

Authors: *S. T. LIM[1], A. DUDAK[2], J. KIM[2], H. J. FEDEROFF[3];
[1]Georgetown Univ., WASHINGTON, DC; [2]Neurosci., [3]Neurosci. and Neurol., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Nectins are cell-cell adhesion molecules involved in the formation of various intercellular junctions and the establishment of apical-basal polarity at cell-cell adhesion sites. To have a better understanding of the roles of nectins in the formation of cell-cell junctions, we searched for new cytoplasmic binding partners for nectin. We report that nectin-1alpha associates with MPP3, one of the human homologues of a Drosophila tumor suppressor gene, Disc large. Two major forms of MPP3 at 66 and 98 kDa were detected, in conjunction with nectin-1alpha, suggesting that an association between the two may occur in various cell types. Nectin-1alpha recruits MPP3 to cell-cell contact sites, mediated by a PDZ-binding motif at the carboxyl terminus of nectin-1alpha. Association with MPP3 increases cell surface expression of nectin-1 and enhances nectin-1alpha ectodomain shedding, indicating that MPP3 regulates trafficking and processing of nectin-1alpha. Further study showed that MPP3 interacts with nectin-3 alpha, but not with nectin-2alpha, showing that the association of nectins with MPP3 is isoform specific. MPP5, another MPP family member, interacts with nectins with varying affinity, suggesting that wide interactions between nectins and MPP family members may occur in various cell-cell junctions and these associations may regulate trafficking and processing of nectins.

Disclosures: S.T. Lim, None; **A. Dudak**, None; **J. Kim**, None; **H.J. Federoff**, None.

Nanosymposium

525. Development: Activity-Dependent Modulation of Connectivity II

Location: Room 25A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 525.5

Topic: B.07. Synaptic Transmission

Support: NIH Grant R01 NS31224-17

NSF Grant IOS 0920672

Title: The NMDA receptor potentiates electrotonic coupling between inferior olive neurons

Authors: *J. P. WELSH[1,2], V. Z. HAN[1];
[1]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA; [2]Dept. of Pediatrics, Univ. of Washington, Seattle, WA

Abstract: A role for the NMDA receptor in modulating electrotonic coupling between inferior olive (IO) neurons was addressed. Previous work demonstrated that subthreshold oscillations in membrane potential (STOs) in IO neurons require electrical synapses mediated by connexin36 (Placantonakis et al 2006) and that NMDA receptor activation potentiates STOs (Placantonakis & Welsh 2001). Here, we directly measured the effect of NMDA receptor activation on electrotonic coupling between IO neurons. Paired, whole-cell, patch-clamp recordings (n=59 pairs) were obtained from acutely prepared brainstem slices taken from young (P23-28) rats. Coupling coefficients were calculated from the voltage responses of pre- and post-junctional cells to negative current pulses and were defined as the ratio between the two. The majority of neurons (78%) showed sinusoidal oscillations (8-15 mV, 3-5 Hz) without spontaneous discharges under control conditions. Of the total sample, the somata of 21pairs were separated by less than 35 μm, and showed a mean coupling coefficient of 0.026 ± 0.008, replicating the findings of Devor & Yarom (2002). In those pairs, 50 μM NMDA depolarized IO neurons by 9-21 mV and increased coupling coefficients by 35%, to 0.035 ± 0.011. The effect was specific to NMDA receptor activation because neither 10 μM AMPA (7 pairs) nor 20 μM kainate (8 pairs) enhanced coupling despite producing an equivalent depolarization. In fact, both AMPA and kainate suppressed electrotonic coupling to near zero, from 0.0088 ± 0.0025 to 0.0017 ± 0.0006, and from 0.0075 ± 0.0022 to 0.0007 ± 0.0002, respectively. In 3 examples in which only one cell in the pair exhibited STOs, NMDA receptor activation triggered STOs in the second cell that were synchronized with the first, and entrained synchronous firing of action potentials. In contrast, AMPA and/or kainate abolished synchronous STOs between IO neurons in 6 examples. The data indicate that glutamatergic neurotransmission within the IO plays an important role in regulating its coupling state, and imply that functional states of the brain which potentiate NMDA receptor sensitivity and/or expression will potentiate synchronous firing within the olivocerebellar system by enhancing neurotransmission through electrical synapses.

Disclosures: J.P. Welsh, None; **V.Z. Han**, None.

Nanosymposium

525. Development: Activity-Dependent Modulation of Connectivity II

Location: Room 25A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 525.6

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NSF SBE-0354379

DARPA HR0011-09-3-0001

Title: Spontaneous retinal waves and development of laminar retinogeniculate connections

Authors: *S. GROSSBERG, J. MARKOWITZ, Y. CAO;
Boston Univ., BOSTON, MA

Abstract: How do spontaneous retinal waves drive activity-dependent retinogeniculate development? A neural model is used to describe the spontaneous behavior of starburst amacrine cells and retinal ganglion cells during the production of waves in the first few weeks of mammalian postnatal development (Feller et al., 1996). The model proposes how excitatory and inhibitory mechanisms within individual cells can modulate the spatiotemporal dynamics of waves. The model simulates observed wave properties as emergent properties of interacting mechanisms in starburst amacrine cells early in development: cell-autonomous spontaneous activity, after-hyperpolarizations caused by Ca^{2+}-activated K^{+} channels (Zheng et al., 2004), and regulation via cAMP (Stellwagen et al., 1999). A phase plane analysis complements these simulations to explore the processes underlying the phenomenological properties observed with multi-electrode array and Ca^{2+} imaging experiments. These realistic waves are used to the drive the development of retinogeniculate synapses in order to form a model dLGN with observed topography and layer segregation of inputs from the two eyes (Shatz, 1983; Stafford et al., 2009; Stellwagen and Shatz, 2002).
Supported in part by CELEST, an NSF Science of Learning, and by the SyNAPSE program of the Defense Advanced Research Projects Agency.

Disclosures: S. Grossberg, None; **J. Markowitz**, None; **Y. Cao**, None.

Nanosymposium

525. Development: Activity-Dependent Modulation of Connectivity II

Location: Room 25A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 525.7

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH APA DPN T32 MH18882-22 to JLH

Autism Speaks to PEW

Title: Neuroligin1 and the development of learning and memory

Authors: *J. L. HOY[1], J. R. L. CONSTABLE[1], R. J. ARIAS[1], S. BROWN[1], K. J. BEADLE[1], R. CHEBAC[1], E. SCHNELL[2], M. WEHR[1], P. E. WASHBOURNE[1];
[1]Univ. Oregon, EUGENE, OR; [2]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Failing to process the appropriate sensory experiences that shape the brain during critical periods of development has dire and potentially irreparable consequences on sensory perception and behavior in the adult organism. While there is evidence that critical periods are key to the successful expression of explicit forms of learning and memory in the adult brain, it is less clear what mechanisms may underlie these epochs of enhanced sensitivity. Interestingly, Neuroligin1 (Nlg1) is a molecule known to impact learning and memory related behaviors. Moreover, prior studies of its function support the idea that it plays a dominant role in the activity dependent synaptic specification that underlies critical periods for primary sensory system development. We therefore tested the hypothesis that Nlg1 plays a significant role in regulating appropriate synaptic specification during a critical period for the development of explicit forms of memory. Until recently most perturbations of such targets have been difficult to spatially and temporally restrict precluding a definitive conclusion regarding their roles as regulators of a critical period. In order to circumvent this limitation, we generated and employed a transgenic line of mice in which a mutant form of Nlg1 could be conditionally expressed during distinct periods in development within targeted regions of the forebrain. Our data support that Nlg1 function is specifically disrupted in this novel line of mice. Further, spatial learning and memory behavior as well as glutamate receptor levels at excitatory synapses are significantly altered in a manner consistent with previously identified roles for Nlg1. Surprisingly, disruption of Nlg1 function exclusively during early development lead to a specific and significant change in behavioral performance in learning and memory tasks in the adult. Our data are the first to support the idea that Nlg1 performs a vital role in regulating a critical period relative to the development of specific mnemonic systems. Overall this work furthers our understanding of the mechanisms that underlie the development of higher order cognitive systems and suggests that they are akin to those that underlie the development and refinement of primary sensory systems. Such studies facilitate our ability to understand and address developmentally relevant neurological disorders that impact both cognitive function and sensory perception.

Disclosures: J.L. Hoy, None; **J.R.L. Constable**, None; **R.J. Arias**, None; **S. Brown**, None; **K.J. Beadle**, None; **R. Chebac**, None; **E. Schnell**, None; **M. Wehr**, None; **P.E.**

Washbourne, None.

Nanosymposium

525. Development: Activity-Dependent Modulation of Connectivity II

Location: Room 25A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 525.8

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: CIHR

MSFHR

Title: NRX-NLG stabilize dendrite growth through activity-independent and dependent mechanisms in vivo

Authors: *S. CHEN[1], K. HAAS[2];
[1]Neurosci., [2]Cell. and Physiological Sci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Precise neuronal growth and synapse formation is crucial for development of functional neural circuits. The synaptotropic model of neuronal growth posits that these events are linked through activity-dependent synaptogenesis-mediated stabilization of labile neuronal processes. While multiple synaptogenic mechanisms have been found to substantiate synaptotropic dendritogenesis, lacking from this model is the role of cell adhesion molecules in dendrite growth. Cell adhesion molecules, including neuroligin (NLG) and neuroexin (NRX), have been well characterized in mediating synapse formation and specification by providing trans-synaptic recognition and nucleation of other synaptic proteins, yet their contribution to dendritic arbor development has not yet been investigated. Here, we use *in vivo* 2-photon time-lapse imaging to study the effects of NRX-NLG interactions on dendritic arbor development of growing neurons within the intact and awake *Xenopus laevis* tadpole brain. Using brain infusion of soluble β-neurexin and postsynaptic expression of wild-type or dominant negative neuroligin-1 constructs in single newly-formed brain neurons, we find distinct functions of activity dependence for cell adhesion and synaptogenic actions of NLG-NRX on filopodia dynamics and dendritic stabilization. Results demonstrate that in addition to a role in synapse formation and differentiation, trans-synaptic NRX-NLG interactions also play a critical role in directing dendritic arborization by conferring stabilization to labile filopodia which are precursors to longer and persistent branches

Disclosures: S. Chen, None; **K. Haas**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.1

Topic: B.08. Synaptic Plasticity

Support: Gordon and Betty Moore Foundation

NIH grant NS44306 to MBK

Title: Kinetic models of CaMKII activation

Authors: *T. L. KINZER-URSEM[1], S. L. PEPKE[2], S. MIHALAS[4], M. B. KENNEDY[3]; [1]California Inst. Technol., Pasadena, CA; [2]Ctr. for Advanced Computing Res., [3]Biol., Caltech, Pasadena, CA; [4]The Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Changes in synaptic strength (plasticity) in the brain underlie learning and formation of memories. One form of experimentally induced synaptic plasticity, long term potentiation (LTP), depends on activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a serine/threonine protein kinase that constitutes 1-2% of forebrain protein by weight. Mice homozygous for deletion of the alpha subunit of CaMKII cannot perform spatial learning tasks; heterozygotes have behavioral endophenotypes associated with schizophrenia in humans. CaMKII is activated upon binding of Ca^{2+}/calmodulin (CaM), itself a Ca^{2+}-activated protein that binds four Ca^{2+} ions, two at its carboxyl (C) and two at its amino (N) terminus.
We present two kinetic models of activation of monomeric catalytic subunits of CaMKII (mCaMKII) that include binding of Ca^{2+} to free CaM and to CaM bound to individual CaMKII subunits. Both models include the distinct kinetics of binding of Ca^{2+} to CaM at its N and C termini, and thermodynamic stabilization of Ca^{2+}-binding when CaM is bound to mCaMKII. The models allow us to consider the complex dynamics of association among Ca^{2+}, CaM, and mCaMKII separately from the issue of cooperativity of binding of CaM to subunits within its dodecameric holoenzyme. The first model is a complete model of binding of Ca^{2+} to the two CaM termini, including 9 Ca^{2+}/CaM states and their interactions with mCaMKII leading to autophosphorylation of mCaMKII. The second model is a coarse-grained one in which binding of the second Ca^{2+} to each terminus of CaM is assumed to be rapid, and thus occurs at the same time as binding of the first Ca^{2+}. This model includes just 4 Ca^{2+}/CaM states.
We used the models to compare predictions about the time course of autophosphorylation of CaMKII under conditions commonly used in test tube experiments and under conditions that are

believed to exist in synaptic spines. We determined a range of physiological concentrations under which the results of the coarse-grained model deviate significantly from those of the complete model. We present three major predictions of the models: 1. Ca^{2+}/CaM species with fewer than four bound Ca^{2+} play a highly significant role in determining the level of autophosphorylation of CaMKII in spines. 2. Competition for binding of Ca^{2+}/CaM among the various targets of CaM is an important determinant of activation of CaMKII during the non-equilibrium conditions that often prevail during synaptic activity *in vivo*. 3. The kinetics of Ca^{2+} flux and binding of Ca^{2+} to CaM lead to frequency-dependence of autophosphorylation of mCaMKII. Thus, kinetic analyses can reveal complex and interesting behavior of synaptic signaling pathways.

Disclosures: T.L. Kinzer-Ursem, None; **S.L. Pepke**, None; **M.B. Kennedy**, None; **S. Mihalas**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.2

Topic: B.08. Synaptic Plasticity

Support: KAKENHI 20670002 (HB)

Research fellowship for young scientists 05J11967 (HF)

Title: Dual FRET-based analysis of biochemical computation performed by neuronal Ca^{2+} signaling processes

Authors: *H. FUJII, M. INOUE, H. OKUNO, S. TAKEMOTO-KIMURA, H. BITO;
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Abstract: At synapses, information that is digitally encoded by neurotransmitter release events is transformed into 2 distinct kinds of analog information, electrical and biochemical, in the postsynaptic neurons. Biochemically, the initial input information is decoded by multiple layers of signaling components that are organized in distinct molecular cascades, each of which computes and generates different final outputs that are critical to many neuronal functions. Despite this significance, we know little about the complex signaling landscape of a stimulated neuron and how a critical biological decision is obtained as a consequence of a biochemical computation derived from co-existing signaling pathways. This bottleneck is in part because

there are few practical generic methods to quantitatively measure two separate signaling components (in a hierarchical or parallel configuration) in an individually interrogated living cell, with high spatial and temporal resolution.
To address this current limitation, we developed a novel imaging approach named dFOMA (dual FRET with Optical Manipulation). This novel dual FRET system has a relatively simple optical layout, and allows it to be combined with photo-manipulation such as UV uncaging. To carry out a proof-of concept experiment, we first designed a new CaMKIIα activation FRET indicator, K2α, whose FRET response tightly correlated with its kinase activity. We then applied the dFOMA imaging to examine the real-time decoding of NMDA-dependent synaptic Ca^{2+} influx by CaMKII, a proposed biochemical switch for memory. Ca^{2+} rises and CaMKII activation were measured simultaneously by dual FRET imaging using modified TN-XL and K2α probes, while introducing high-frequency inputs to individual neurons through UV uncaging of caged glutamate. By altering glutamate pulse frequency, we found that input frequency information is encoded into two separable parameters of CaMKIIα activation, amplitude and integral amount of CaMKIIα activation in a living neuron, suggesting that CaMKII is not a switch, but rather close to a complex tunable gear. Comparison of strictly tuned input-output function curves obtained from distinct Ca^{2+} signaling components start to indicate unique bandwidths for individual Ca^{2+} signaling pathways. Taken together, these findings define some of the unique features of neuronal Ca^{2+} signaling landscape during precise and reliable multiplex biochemical information processing at a single neuron level.

Disclosures: H. Fujii, None; **M. Inoue**, None; **H. Okuno**, None; **S. Takemoto-Kimura**, None; **H. Bito**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.3

Topic: B.08. Synaptic Plasticity

Support: CHIR

E.W.R. Steacie Foundation

NIH

Title: Stabilization of postsynaptic GluR2/AMPA receptors maintains long-term memories

Authors: *P. V. MIGUES[1], O. HARDT[1], D. C. WU[2], K. GAMACHE[1], T. C. SACKTOR[3], Y. T. WANG[2], K. NADER[1];
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Abstract: The recent discovery that PKMζ activity is required for the maintenance of many type of memories opened the possibility for investigating the synaptic processes required for the stability of stored memories. We set out to investigate what are the synaptic alterations sustained by the activity of PKMζ that are responsible for the maintenance of memories. We found that inactivating PKMζ in fear conditioned rats leads to a decrease of GluR2 subunit in the PSDs in the amygdala. The extent of the GluR2 removal positively correlates with the memory impairment. Blocking GluR2 removal from synapses abolished the memory and LTP impairment caused by inactivating PKMζ as well as the decrease in postsynaptic GluR2 expression, again correlating with memory performance. These findings imply that the perpetuation of long-term memories relies on the active prevention of the synaptic removal of GluR2-AMPA receptors that would lead to memory loss. We are currently examining the effects of destabilizing synaptic AMPA receptors by directly interfering with their trafficking pathways.

Disclosures: P.V. Migues, None; **D.C. Wu**, None; **K. Gamache**, None; **T.C. Sacktor**, None; **Y.T. Wang**, None; **K. Nader**, None; **O. Hardt**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.4

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS20686

Title: Rapid destabilization of place cell representations by hippocampal injections of the PKMzeta inhibitor ZIP

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Abstract: PKMzeta, an atypical PKC isoform, is both necessary and sufficient for the maintenance of LTP. Injections of the PKMzeta pseudosubstrate inhibitor ZIP reverse

established late-phase LTP at hippocampal synapses and also disrupt several forms of hippocampus-dependent spatial memory. Because hippocampal place cells are believed to play a crucial role in spatial memory, we asked if ZIP infusions would interfere with an existing place cell representation of a familiar environment.
To examine if injecting a volume of 1.0 microliter disturbs single cell waveforms recorded from tetrodes, we used Tris-buffered saline and muscimol as test agents. Saline injections do not affect unit activity. In contrast, 4.4 mM muscimol leads to a gradual silencing of both pyramidal cells and interneurons. Units closer to the injection site are inactivated earlier than units further from the injection site. Simultaneously recorded, putative axon fibers remain active, convincingly demonstrating tetrode stability.
Bilateral injections of 10 nmol ZIP in 1 microliter saline severely perturb the characteristic stability of place cell firing fields in a familiar environment; even 5 hours after the injection there is no sign of reversal to the pre-injection firing pattern. Many pyramidal cells that had discharged as place cells lose their spatial firing specificity. The firing fields of cells that continue to show location-specific firing are no longer stable across recording sessions.
The results indicate that the persistent activity of PKMzeta is necessary for the stability of hippocampal place cell firing fields and may explain how inhibition of the kinase causes the loss of stored spatial information in the hippocampus. Additional work is needed to ask whether firing fields recover after longer intervals or, more likely, if new fields develop normally in the familiar environment after drug washout. It is also important to investigate network-level effects of local LTP reversal by comparing the activity of cells closer to the injection site to those that are more distant.

Disclosures: J.M. Barry, None; **B. Rivard**, None; **T.C. Sacktor**, None; **A.A. Fenton**, None; **R.U. Muller**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.5

Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH53576

NIH Grant MH57068

Title: New synthesis of PKMζ is critical for late-LTP

Authors: *P. TSOKAS[1], C. HSIEH[2], R. D. BLITZER[5], J. E. COTTRELL[3], T. C. SACKTOR[4]; [1]Furchgott Ctr. for Neural & Behav. Sci., Physiol. & Pharmacol., Anesthesiol., SUNY Downstate Med. Center, Brooklyn, NY, BROOKLYN, NY; [2]Furchgott Ctr. for Neural & Behav. Sci., Physiol. & Pharmacol., [3]Anesthesiol., [4]Furchgott Ctr. for Neural and Behav. Sci., Physiol. & Pharmacol., Neurol., SUNY Downstate Med. Ctr., Brooklyn, NY; [5]Pharmacol. & Systems Therapeutics, Psychiatry, Mount Sinai Sch. of Med., New York, NY

Abstract: Protein kinase Mζ (PKMζ) plays a key role in the maintenance of long-term memory. PKMζ is a brain-specific, constitutively active isoform of PKC that is synthesized by an mRNA encoding a PKCζ catalytic domain without a regulatory domain, which is transcribed from an internal promoter within the PKCζ gene. Using the selective ζ pseudosubstrate inhibitory peptide ZIP, previous studies have shown that the enzymatic activity of PKMζ is required for long-term memory and for the maintenance of the protein synthesis-dependent form of long-term potentiation (late-LTP). Upon induction of late-LTP, PKMζ levels increase in the CA1 region of hippocampal slices. The blockade of PKMζ activity by ZIP, however, cannot differentiate between the roles of pre-existing and newly synthesized PKMζ in the maintenance of late-LTP. We have therefore devised an antisense oligodeoxynucleotide (ODN) approach to address this question by specifically blocking new PKMζ synthesis from the PKMζ mRNA.
Rats received single intrahippocampal injections of either an antisense ODN construct targeted against the translation start site of PKMζ, or a scrambled ODN sequence. Acute slices were prepared an hour later, and extracellular field EPSPs were recorded from CA3-CA1 synapses beginning 2 hr after dissection. Late-LTP was induced by two 100 Hz tetani delivered 20 sec apart or via a cross-capture protocol (late-LTD induced by strong low frequency stimulation, followed by one 100 Hz tetanus delivered to an independent pathway). Slices were either followed for 2 hours, or frozen 30 min post-tetanus. Lysates from excised CA1 regions from the frozen slices were probed by immunoblotting with antibodies against PKMζ and actin, as control.
Compared to sham-stimulated controls, slices that had received late-LTP inducing stimulation showed increases in PKMζ by immunoblot. These increases were unaffected by scrambled ODN treatment, but were blocked in slices that were derived from hippocampi injected with antisense ODN. Thus the antisense blocks new, activity-dependent synthesis of PKMζ during LTP, but does not affect basal levels, presumably because of the short duration of ODN treatment and long half life of PKMζ (see van de Nes et al., Soc Neuro Abstr 2010).
The antisense treatment blocking PKMζ synthesis had no effect on late-LTD, but the antisense ODN blocked late-LTP, the tetanization inducing only early-LTP with potentiation returning to basal levels within 2 hours post-tetanus. Scrambled ODN treatment had no effect on late-LTP or late-LTD.
Taken together, these results demonstrate that in response to late-LTP induction, PKMζ is synthesized de novo, and this pool of PKMζ is required for late-LTP.

Disclosures: P. Tsokas, None; **C. Hsieh**, None; **R.D. Blitzer**, None; **J.E. Cottrell**, None; **T.C. Sacktor**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.6

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R37NS029563 (D.L.G)

Title: Evidence that PKM maintains long-term facilitation in Aplysia

Authors: *D. CAI[1], D. L. GLANZMAN[2,3,4];
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Abstract: Recent evidence from studies on rats and mice indicate that long-term memories can be effectively erased by disrupting the ongoing activity of a specific isoform of protein kinase C (PKC), PKMzeta. However, at present little is known about the cellular mechanisms underlying the phenomenon of memory erasure. We (Pearce et al., 2010) have shown that maintenance of long-term behavioral sensitization in Aplysia depends on an Aplysia PKM isoform, PKM Apl III (Bougie et al., 2009). Long-term sensitization is due, in part, to long-term facilitation (LTF) of the monosynaptic connection between the sensory and motor neurons that mediate the withdrawal reflex (Frost et al., 1985). We therefore wished to know whether PKM Apl III plays a role in maintaining LTF, as well as long-term sensitization. Accordingly, we tested whether inhibiting the activity of PKM Apl III would disrupt established LTF. Either the pseudosubstrate peptide inhibitor ZIP or the PKC inhibitor chelerythrine was applied to sensorimotor cocultures for 1 hr at 24 hr after treatment with five 5-min applications of 5-HT (5 X 5-HT treatment). Sensorimotor synapses that received the 5-HT treatment without subsequent exposure to ZIP or chelerythrine exhibited significant LTF 24 hr and 48 hr later compared to control synapses that were not treated with 5-HT. By contrast, synapses exposed to ZIP or chelerythrine at 24 hr after 5-HT training did not exhibit facilitation at 48 hr. The ZIP/chelerythrine treatment by itself did not appear to have any deleterious effect on the cocultures, as indicated by the comparison between the untreated control synapses and synapses treated with either ZIP or chelerythrine alone. We attempted to reinstate LTF after its disruption by inhibition of PKM Apl III. For this attempt cocultures received a single 5-min application of 5-HT at 6 hr after chelerythrine treatment (here, 18 hr after the 5 X 5-HT training). The additional exposure to 5-HT failed to reinstate LTF after its apparent erasure by chelerythrine. Our in vitro results indicate that PKM Apl III plays a key role in maintaining long-term synaptic plasticity in Aplysia, and set the stage for a rigorous mechanistic analysis of memory erasure by inhibition of PKM.

Disclosures: D. Cai, None; **D.L. Glanzman**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.7

Topic: B.08. Synaptic Plasticity

Support: CIHR MOP12046

FRSQ doctoral fellowship

Conrad Harrington postdoctoral fellowship

Title: Mechanisms of protein kinase m (pkm) formation in Aplysia: Cleavage of the atypical protein kinase c (pkc) zeta, Apl III

Authors: *J. K. BOUGIE[1], C. ABI FARAH[2], W. S. SOSSIN[3];
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[3]Montreal Neurolog. Inst., McGIll Univ., Montreal, QC, Canada

Abstract: Protein kinase Cs (PKCs) are important regulators of synaptic plasticity and learning and memory. In vertebrates, the atypical PKC zeta has a brain-specific transcript that produces a constitutively active truncated kinase known as PKM zeta and inhibition of this kinase erases memory. We are examining a nervous system isoform of the atypical PKC zeta in Aplysia, PKC Apl III. We do not find a transcript in Aplysia that forms a PKM zeta, and evolutionary analysis of atypical PKCs suggests formation of this transcript is restricted to vertebrates. However, inhibitors of PKM zeta block the maintenance of synaptic plasticity in Aplysia. We suggest that PKM forms of atypical PKCs play a conserved role in memory formation, but the mechanism of formation of these kinases has changed over evolution. Over-expression of PKC Apl III in Aplysia sensory and motor neurons leads to production of a PKM fragment of PKC Apl III, formed by calpain cleavage (Bougie et al, 2009). To further examine the cleavage of PKCs to PKMs, we have developed Forster Resonance Energy Transfer (FRET) constructs where both PKC Apl I (a classical isoform of PKC) and PKC Apl III are tagged with cyan fluorescent protein (CFP) on the N-terminal and a yellow fluorescent protein (YFP) at the Carboxy-terminal. These constructs show basal FRET that should decrease after protein cleavage, as the fluorescent chromophores dissociate from each other. Indeed, overexpression of eCFP-PKC Apl III-eYFP led to a concentration dependent loss of FRET that was not see with eCFP-PKC-Apl I-eYFP. In contrast, it was found that addition of a calcium ionophore, ionomycin, was able to significantly

decrease FRET for eCFP-PKC-Apl I-eYFP but not eCFP-PKC Apl III-eYFP. These results suggest that these two isoforms of PKC are cleaved under different conditions. In motor neuron processes, serotonin (5-HT) was able to produce a significant decrease in the FRET ratio for PKC Apl III, indicating that 5-HT is able to induce cleavage of PKC Apl III into PKM Apl III under physiological conditions. Using this FRET assay, we will determine the location and requirements for PKM formation in Aplysia.

Disclosures: J.K. Bougie, None; **C. Abi Farah**, None; **W.S. Sossin**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.8

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R37NS029563 (D.L.G.)

Title: PKM maintains long-term sensitization in Aplysia

Authors: K. C. PEARCE[1], D. CAI[1], S. CHEN[1], T. PHAN[1], R. BARAKAT[1], D. LI[1], L. NGUYEN[1], *D. L. GLANZMAN[1,2,3];
[1]Integrative Biol. and Physiol., [2]Neurobio., [3]Brain Res. Inst., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Recent studies in mammals have shown that inhibiting the activity of the zeta isoform of PKM disrupts established long-term synaptic plasticity and long-term memory. PKM is the independent catalytic fragment of the atypical PKC isoform. A homolog of atypical PKC has recently been cloned and been shown to form a PKM (PKM Apl III) in the marine snail *Aplysia* (Bougie et al., 2009). Here, we asked whether PKM Apl III mediates the persistence of long-term sensitization (LTS) of the siphon-withdrawal reflex (SWR). To address this question, we tested whether injecting the pseudosubstrate peptide inhibitor ZIP or the PKC inhibitor chelerythrine into *Aplysia* after the induction of LTS disrupted the long-term memory for sensitization. The sensitization training consisted of five bouts of electrical shocks, spaced 20 min apart, which were delivered to the tail via implanted electrodes. The drug or vehicle solution was injected into an animal's hemocoel at various time points after sensitization training. In initial experiments we found that an injection of either ZIP or chelerythrine disrupted the memory for LTS when made 24 hr after training. In subsequent experiments we observed that an injection of chelerythrine could disrupt established LTS when made as late as 7 d after training.

Furthermore, one bout of tail shocks delivered at 24 hr after chelerythrine treatment failed to reinstate the disrupted LTS. Finally, no spontaneous recovery of sensitization was observed for a period of 72 hr after chelerythrine was injected into trained animals. Our results indicate that PKM Apl III plays a key role in the maintenance of LTS. However, according to an alternative hypothesis, long-term memory in *Aplysia* is maintained by local protein synthesis regulated by *Aplysia* cytoplasmic polyadenylation element binding protein (ApCPEB). To compare PKM Apl III and ApCPEB as potential molecular mechanisms for maintaining LTS in *Aplysia*, we tested whether inhibiting protein synthesis would disrupt the week-old memory for LTS. We first confirmed that inhibition of protein synthesis with anisomycin prior to sensitization training blocks the induction of LTS. We then treated animals with anisomycin via intrahemocoel injection 7 d after training. In striking contrast to chelerythrine's disruptive effect, anisomycin had no effect on the maintenance of LTS. These results argue against the idea that ApCPEB activity is critical for maintaining long-term memory in the intact animal, at least after one week. Our data provide support for the idea that PKM is a general mechanism underlying the persistence of memory in invertebrate and vertebrate nervous systems.

Disclosures: K.C. Pearce, None; **D. Cai**, None; **S. Chen**, None; **T. Phan**, None; **R. Barakat**, None; **D. Li**, None; **L. Nguyen**, None; **D.L. Glanzman**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.9

Topic: B.08. Synaptic Plasticity

Support: Alzheimer's Research Trust

Title: Involvement of the inhibitory serine-phosphorylation of glycogen synthase kinase-3 in synaptic plasticity and memory formation

Authors: L. SHAHAB[1], E. E. IRVINE[1], M. A. SMITH[2], F. A. EDWARDS[1], D. R. ALESSI[3], D. J. WITHERS[2], *F. PLATTNER[1];
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Abstract: Glycogen synthase kinase-3 (GSK3) is a multifunctional serine/threonine kinase that regulates diverse cellular processes including metabolism, apoptosis and transcription. Despite

evidence that dysregulation of GSK3 is associated with disorders such as Alzheimer's disease and bipolar disorder, there is limited information on the mechanisms by which GSK3 might contribute to these pathologies. We have recently demonstrated that elevated GSK3 activity impairs long-term potentiation (LTP) in mice over-expressing GSK3β and that LTP induction leads to increased inhibitory phosphorylation of GSK3β at serine 9 and GSK3α at serine 21 (Hopper *et al.* 2007, *EJN* 25, 81-86).
In this study, we have examined a double GSK3 knock-in mouse line, in which the inhibitory serine phosphorylation sites of both GSK3 isoforms have been substituted with alanine (GSK3α Ser21Ala and GSK3β Ser9Ala). In acute hippocampal slices, CA1 LTP is attenuated in the GSK3 knock-in mice when compared to wild-type controls. However, there are no differences in paired pulse facilitation. Consistent with the impairment in LTP, we observe a deficit in hippocampus-dependent fear memory in the knock-in mice.
Together, these results suggest that inhibition of GSK3 by serine phosphorylation is important for synaptic plasticity and memory formation. Thus, it is possible that dysregulation of GSK3 may induce synaptic dysfunction and thereby contribute to memory impairment in various human neuronal disorders.

Disclosures: L. Shahab, None; **F. Plattner**, None; **E.E. Irvine**, None; **M.A. Smith**, None; **F.A. Edwards**, None; **D.J. Withers**, None; **D.R. Alessi**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.10

Topic: B.08. Synaptic Plasticity

Support: MRC Grant G0601812

NIH MH060252

Danish Medical Research Council Grant 271-05-0712

Title: Induction of STP and LTP at CA1 synapses in the hippocampus is mediated by different NMDAR subtypes

Authors: A. VOLIANSKIS[1], *N. BANNISTER[2], V. J. COLLETT[2], M. W. IRVINE[1], D. T. MONAGHAN[3], S. M. FITZJOHN[2], M. S. JENSEN[4], D. E. JANE[1], G. L. COLLINGRIDGE[2]; [1]Dept. of Pharmacol. and Physiol., [2]Dept of Anat., Univ. of Bristol, Bristol, United Kingdom;

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Abstract: Synaptic potentiation at CA3-CA1 synapses in adult rat hippocampus slices contains a stimulation-labile phase of short-term potentiation (STP) that is followed by stable long-term potentiation (LTP). It is well established that induction of both phases depends on the synaptic activation of NMDA receptors (NMDARs) and, although it has not been shown that STP and LTP reflect an expression of a unitary phenomenon, STP is generally regarded as a non-stabilized form of LTP. A possibility exists that the cellular mechanisms that underlie STP and LTP are different and that these two phases of potentiation are induced through NMDARs expressing different subunits. GluN2A, 2B and 2D containing NMDARs are expressed in adult hippocampus and we investigated their roles in the induction of STP and LTP by using four subtype-selective NMDAR antagonists.
In addition to the prototypical NMDAR antagonist D-AP5, which is more potent at GluN2A/2B containing receptors than at GluN2D, we chose to study NVP-AAM077 (NVP), Ro 25-6981 (Ro) and UBP 145 (UBP). In HEK293 cells expressing rat recombinant NMDARs we confirmed that the selectivity of these antagonists is sufficient to establish roles of the NMDAR subtypes in the induction of STP and LTP. NVP was more potent at GluN2A/2D subunits than at GluN2B. Ro was most potent at GluN2B containing receptors, blocked GluN2A at high concentrations and had no effects on GluN2D. UBP was more potent at GluN2D containing receptors than at other subunits.
We induced potentiation of f-EPSPs either in the presence or in the absence of antagonists and constructed concentration response curves for the inhibition of STP and LTP. The concentration response relationships for the inhibition of STP by AP5, Ro and UBP were best fitted by biphasic sigmoidal functions suggesting the involvement of more than one receptor subunit in the induction of STP. In contrast, single sigmoidal functions were sufficient to describe effects of all antagonists on LTP. Both Ro and UBP, at selective concentrations, were able to inhibit the induction of STP whilst having no effects on LTP, suggesting the involvement of GluN2B/2D and not 2A receptors in the induction of STP. AP5 and NVP, at selective concentrations, blocked LTP whilst sparing STP. Ro blocked LTP too but only at concentrations that were ≈ 10 fold higher than its effect on GluN2B-containing receptors. These data suggest the involvement of both diheteromeric (2A/2A) and triheteromeric (2A/2B) receptor assemblies in the induction of LTP.
We conclude that STP and LTP are induced through NMDARs expressing different subunits and that the mechanisms underlying these two forms of plasticity are unlikely to be the same.

Disclosures: A. Volianskis, None; **N. Bannister**, None; **V.J. Collett**, None; **M.W. Irvine**, None; **S.M. Fitzjohn**, None; **D.E. Jane**, None; **G.L. Collingridge**, None; **M.S. Jensen**, None; **D.T. Monaghan**, None.

Nanosymposium

526. Postsynaptic Signaling Mechanisms

Location: Room 24A

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 526.11

Topic: B.07. Synaptic Transmission

Support: NINDS Intramural Research program

Title: A new perspective of the postsynaptic density

Authors: *X. CHEN, C. WINTERS, T. S. REESE;
Lab. Neurobiol, NINDS-NIH, BETHESDA, MD

Abstract: The classical view from electron microscopy is that the postsynaptic density is a band of continuous electron dense material at the postsynaptic membrane 20-30 thick and ~ 300 nm long, with its edges sharply demarcated and well aligned with the electron dense active zone material at the presynaptic terminal. It is often assumed that alignment of the pre and postsynaptic active zones defines the fixed boundary of the postsynaptic density. EM tomographic reconstructions of freeze substituted intact hippocampal spine synapses allow individual protein components of the PSD to be revealed in molecular detail. Much of the electron density that defines the PSD largely depends on the large cytoplasmic domains (~ 20 nm diameter) of the NMDAR type of structures as well as on the dense population of 20 nm long vertical filaments containing PSD-95 and presumably other MAGUK proteins. Vertical filaments are uniformly distributed throughout the PSD, while the NMDAR type of structures form a regular spaced cluster, typically near the center of the PSD. The AMPA receptor structures surrounding the NMDA receptors have much thinner and relatively flat cytoplasmic domains that contribute very little to the electron density of the PSD. After shRNA knockdown of PSD-95, which led to losses or thinning of patches of vertical filaments, the edges of the PSDs became blurred due to losses of PSD-95 vertical filaments around NMDA receptor cluster, so the only sharp edges remaining are at the edges of the NMDAR clusters. AMPAR type structures are also reduced in the thinned regions, so the peripheral region of the PSD, including its edge, is potentially more dynamic than the NMDAR regions. Thus, only the NMDAR clusters present a stable edge, and the idea that the PSD has of a stable outer edge is better replaced by the idea of a dynamic interface between the PSD and the spine that can be readily obscured by conditions inducing rapid turnover of scaffolding molecules and AMPA receptors.

Disclosures: X. Chen: Employment; Bethesda, MD, 20892, Lab. of Neurobiology, NINDS-NIH. Other Research Support; Supported by NINDS intramural research program. **C. Winters:** None. **T.S. Reese:** None.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.1

Topic: C.02. Alzheimer's disease and other dementias

Support: IUAP

Title: Analysis of signaling cascades involved in APP-induced Tau-phosphorylation using viral vectors and transgenic mice

Authors: *I. DEWACHTER[1], N. PIERROT[1], V. LAPORTE[1], B. TASIAUX[1], K. LEROY[2], P. KIENLEN-CAMPARD[1], J.-P. BRION[2], J.-N. OCTAVE[1];
[1]UCL St.Luc, Woluwe-saint-Lambert, Belgium; [2]ULB-Université Libre de Bruxelles, Brussels, Belgium

Abstract: Transgenic mice co-expressing APP and Tau clearly identified APP as a Tau modifier in vivo, alleviating Tau pathology in the brain stem, correlating with decreased mortality, and aggravating Tau-pathology in limbic and cortical brain regions. Importantly, a dramatic similarity between the modulatory role of APP and GSK3β on Tau induced neuronal dysfunction was obvious in Tau transgenic mice (Terwel et al. 2008). Furthermore phosphorylation of Tau was significantly increased in hippocampus of APP transgenic mice, with concomitant increase in phosphorylation of GSK3β at Tyr-216 (Terwel et al. 2008), very similar as in AD brain (Leroy et al. 2007). We are currently further dissecting the signaling pathways involved in the communication between APP and Tau in vitro and in vivo by combination of viral vectors and transgenic mice. The data of this analysis will be presented.

Disclosures: I. Dewachter, None; **J. Octave**, None; **B. Tasiaux**, None; **P. Kienlen-Campard**, None; **N. Pierrot**, None; **V. Laporte**, None; **K. Leroy**, None; **J. Brion**, None.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.2

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH grant R21NS067127

H. Lundbeck A/S

Title: Impacts of Abeta peptide on the progression of tauopathy

Authors: *N. SAHARA[1], D. KANG[1], J. KNIGHT[1], J. HOWARD[1], E. ECKMAN[1], C. CEBALLOS DIAZ[1], K. JANSEN-WEST[1], Y. LEVITES[1,2], J. EGEBJERG[3], T. GOLDE[2], J. LEWIS[1];
[1]Dept. of Neurosci., Mayo Clin., Jacksonville, FL; [2]Ctr. for Translational Res. in Neurodegenerative Dis., Univ. of Florida, Gainsville, FL; [3]H. Lundbeck A/S, Copenhagen, Denmark

Abstract: The 'amyloid cascade hypothesis', posits that Aβ aggregates can accelerate neurofibrillary tangle (NFT) formation. Animal modeling studies support the idea that Aβ aggregates can trigger NFT formation. Studies demonstrating that Aβ40 has an anti-amyloidogenic effect *in vivo* suggest that therapies non-selectively targeting Aβ may be less effective then therapies targeting the more aggregation prone Aβ42 species. Using the BRI-Aβ fusion technology to deliver the Aβ peptides, we are evaluating the effect of individual secreted Aβ peptides on the cognitive function in the rTg4510 mouse model which develops NFTs, neuronal loss and memory impairment in an age-dependent manner. BRI-Aβ constructs were expressed in the brain using somatic brain transgenic technology. The rTg4510 mice with or without Aβ40 or Aβ42 peptide expression have been analyzed for cognitive function, brain biochemistry, and neuropathology. The results of these ongoing studies will be presented and may provide important insights into the putative link between Aβ peptides, tau pathology, and cognitive dysfunction in mice.

Disclosures: N. Sahara: Research Grant; NIH/NINDS R21NS067127. **D. Kang:** None. **J. Knight:** None. **J. Howard:** None. **E. Eckman:** None. **C. Ceballos Diaz:** None. **K. Jansen-West:** None. **Y. Levites:** None. **J. Egebjerg:** Employment; H. Lundbeck A/S. **T. Golde:** None. **J. Lewis:** Research Grant; NIH/NINDS R01NS046355, Alzheimer's Association IIRG-06-27277, Johnnie B. Byrd, Sr. Alzheimer's Center & Research Institute Grant Program. Other Research Support; Mayo Foundation, H. Lundbeck A/S.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.3

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH K08NS049237

NIH R01NS065069

Burroughs Wellcome Career Award in the Biomedical Sciences

P30 NS057105

Title: Controlled cortical impact traumatic brain injury in 3xTg-AD mice causes acute intra-axonal amyloid-beta accumulation and independently accelerates the development of tau abnormalities

Authors: *H. T. TRAN[1], F. M. LAFERLA[3], D. L. BRODY[2];
[1]Neurol., Washington Univ. St Lo, SAINT LOUIS, MO; [2]Neurol., Washington Univ. St Lo, Saint louis, MO; [3]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Traumatic brain injury (TBI) can increase the risk of subsequent development of dementia of the Alzheimer's type. Accumulations of amyloid-beta (Aβ) and tau proteins, pathological hallmarks of Alzheimer's disease (AD), have been observed acutely in human TBI patients. It is therefore hypothesized that TBI may play a causal role in acceleration of these AD-related pathologies. To test this hypothesis, we performed controlled cortical impact TBI on 5-7-month old 3xTg-AD mice, which produce both human Aβ and tau, but do not normally have extensive pathology at this age. At 24 hours after TBI, we found acute intra-axonal Aβ accumulation in pericontusional white matter, particularly the fimbria, by immunohistochemistry and an approximately 2-fold increase in guanidine soluble Aβ in the ipsilateral hippocampus by ELISA. Similarly, we found tau accumulation in pericontusional white matter. Additionally, there was increased tau immunoreactivity ipsilateral amydgala and contralateral hippocampal CA1 region of injured 3xTg-AD mice. Furthermore, tau appeared to be hyperphosphorylated following TBI in these mice, as evident by immunohistochemical and western blotting studies using the antibody PHF1. Aβ accumulation increased monotonically over 24 hours following TBI. Tau immunoreactivity in the fimbria and amydgala had a biphasic time course with peaks at 1 hour and 24 hours, and tau in CA1 had a delayed monotonic rise starting at 12 hours after TBI. Aβ pathology has been reported to be upstream of tau pathology in mouse models of Alzheimer's disease. To probe this relationship in the setting of TBI, we inhibited Aβ production using compound E, a small molecule inhibitor of γ-secretase. Γ-secretase-mediated proteolytic cleavage of APP is required for Aβ production. Systemic treatment with Compound E reduced the post-traumatic intra-axonal Aβ accumulation by 90%. However, tau pathology was unaltered after Compound E treatment.
Overall, these findings support a causal role of TBI in acceleration of AD-related pathologies, and suggest TBI may affect Aβ and tau independently. This small animal model may be useful for many additional mechanistic and preclinical therapeutic investigations.

Disclosures: H.T. Tran, None; **F.M. LaFerla**, None; **D.L. Brody**, None.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.4

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH/NIA Grant R01AG029802-01

NIH/Fogarty Grant R21AG024024063

COLCIENCIAS Grant 11150418078

COLCIENCIAS Grant 111545921503

Title: Interaction between Beta-secretase 1 and Hsc70 are implicated in the paired helicoidal filaments formation

Authors: *D. PIEDRAHITA[1], I. HERNÁNDEZ[3], J. CASTRO[2], R. BODREAU[4], B. DAVIDSON[4], F. LAFERLA[5], J. GALLEGO-GÓMEZ[2], K. KOSIK[3], G. CARDONA-GÓMEZ[2]; [1]Cell. and Mol. Neurobio. Area, Viral Vector Core and Gene Therapy. G, Medellin, Colombia; [2]Cell. and Mol. Neurobio. Area, Viral Vector Core and Gene Therapy. G, MEDELLIN, Colombia; [3]Neurosci. Res. Institute, Univ. of California, Santa Barbara, CA; [4]Viral Vector Core and Davidson Laboratory, Univ. of Iowa, Iowa City, IA; [5]Inst. for Brain Aging and Dementia, Univ. of California, Irvine, CA

Abstract: Alzheimer´s disease (AD) is the most common cause of senile dementia. AD is associated with Beta-amyloid (AB) plaques, neurofibrillary tangles (NTF) and large-scale of neuronal loss. BACE1 is a Beta-secretase, which initiates de formation of Beta-amyloid. <Beta-amyloid was reported as an inductor of tau pathology through alterations directly on the proteosome function, however, is not very clear, which are the mechanisms that links Beta-amyloid and NTF. In this study, we evaluated the relationship between BACE1 and Hsc70 in the NTF formation in a triple transgenic Alzheimer mice model (3xTgAD), in Alzheimer´s human tissue and in neuronal primary cultures. Our data shown, that BACE1 protein level is up-regulated and co-immunoprecipite with Hsc70 in Alzheimer human brains; and these were associated in lipid rafts, when Hsc70 was decreased in the cytoplasm of the hippocampus of 3xTg-AD mice. We design a shRNAmiR against BACE1 (BACE1miR), and realized intra-

hippocampal injections of AAVmiBACE1 and AAV.GFP in 3xtg AD mice. We detected a specific silencing of BACE1 that produced a decrease of Beta-amyloid, a significant down-regulation of the PHF-1, as well as a strong increase of Hsc70 compared to the AAV.GFP injected mice. In addition, inhibitors of proteosome (lactacystin) and Hsp (KNK437) did not reverse the effect of BACE1miR, when significantly decreased PHF, Hsp90 and pAkt in neuronal primary cultures. Our data suggest that BACE1miR promotes the degradation of PHF, but it is not dependent of proteosomal pathway and probably involves autophagy-lysosomal degradation pathway. In summary, our findings show that gene silencing of BACE1 reduces the formation of PHFs in a Beta-amyloid toxicity-independent mode, becoming a new and promising therapeutic alternative for Alzheimer´s disease and other taupathies.

Disclosures: D. Piedrahita, None; **I. Hernández**, None; **J. Castro**, None; **R. Bodreau**, None; **B. Davidson**, None; **F. LaFerla**, None; **J. Gallego-Gómez**, None; **K. Kosik**, None; **G. Cardona-Gómez**, None.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.5

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH AT003008 (G.M.C.)

NIA AG16570 (G.M.C.)

NIA AG13471 (G.M.C.)

NIH U0128583 (S.A.F.)

NIH AG021975 (S.A.F.)

Alzheimer’s Association NIRG-07-59659 (QL.M.)

Title: Treatment of abeta oligomer-induced increases in total and hyperphosphorylated tau, soluble tau oligomers and synaptic deficits

Authors: *Q.-L. MA[1,2], O. J. UBEDA[1,2], W. BEECH[1,2], B. BEECH[1,2], Y. ZHAO[1,2], S. A. FRAUTSCHY[1,2], G. M. COLE[1,2];

[1]UCLA, North Hills, CA; [2]Geriatric Res. and Clin. Center, GLAVAHS, VA Med. Ctr., North Hills, CA

Abstract: The important central role of amyloid beta-protein (Aβ) in Alzheimer's disease (AD) pathogenesis is represented in the revised amyloid cascade hypothesis that the gradual cerebral accumulation of soluble and insoluble assemblies of the amyloid Aβ in limbic and association cortices triggers a cascade of biochemical and cellular alterations that produce the clinical phenotype of AD. Soluble Aβ oligomers are currently proposed as the major factor that directly induces accumulation of hyperphosphorylated aggregates of microtubule-associated protein tau, most notably neurofibrillary tangles, a prominent feature in AD pathology progress regionally with neurodegeneration, synapse loss and cognitive decline. While recent transgenic mouse and other data argue that soluble tau oligomers may be more closely related to synaptic and cognitive deficits than tangles, there is limited evidence that directly links the Aβ oligomer-induced tau hyperphosphorylation with the appearance of soluble tau oligomers or selective synaptic deficits. Here we report that low dose soluble Aβ oligomer treatment of cultured primary hippocampal neurons significantly induces tau hyperphosphorylation at multiple epitopes including Ser214, Thr212 (AT100), Ser202 (AT8), Thr205 (AT8), Ser396 (PHF1), Ser404 (PHF1), Ser42 without inducing acute neuron death. Low dose Aβ oligomers increased levels of total tau protein monomer and high MW TBS soluble phospho-tau immunoreactive oligomers, but reduced excitatory synaptic proteins NR2B, PSD-95 and drebrin. Pre-administration of tau kinase inhibitors or the omega-3 fatty acid DHA to neuron cultures suppressed the hyperphosphorylated and total tau and tau oligomers and prevented excitatory synaptic protein loss. These results imply Aβ oligomer-induced soluble tau oligomers and synaptic deficits can be treated with safe interventions.

Disclosures: Q. Ma: Research Grant; AT003008 (G.M.C.), NIA AG16570 (G.M.C.), AG13471 (G.M.C.), U0128583 (S.A.F.), AG021975 (S.A.F.), Alzheimer’s Association NIRG-07-59659 (QL.M.).. **O.J. Ubeda:** None. **W. Beech:** None. **B. Beech:** None. **Y. Zhao:** None. **S.A. Frautschy:** None. **G.M. Cole:** None.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.6

Topic: C.02. Alzheimer's disease and other dementias

Support: Mitchell Foundation

Title: Characterization of tau oligomers in Alzheimer`s disease

Authors: *R. KAYED[1], C. LASAGNA-REEVES[1], M. GUERRERO-MUNOZ[2], U. SENGUPTA[2], J. TRONCOSO[3], G. JACKSON[2];
[1]Neurol., Univ. Texas Med. Br., GALVESTON, TX; [2]UTMB, Galveston, TX; [3]Johns Hopkins, Baltimore, MD

Abstract: Neurofibrillary tangles (NFTs) are a key hallmark of Alzheimer`s disease (AD); nevertheless, the correlation between NFTs and disease progression remains controversial. Some studies show that neuronal loss actually precedes NFTs formation in humans with AD. This has sparked a considerable interest in the pathological role of the newly discovered tau oligomeric intermediates. In part due to methodological challenges, there are limited insights about the biochemical properties and the cellular distribution of or tau oligomers formed in vivo during the progression of the disease. We used newly developed tau oligomers specific antibody in combinations with other commercially available antibodies and reagents to characterize tau oligomers in AD brains. We performed detailed immunohistochemical (IHC) analysis of tau oligomer burden in the large population of AD brains and studied the biochemical characteristics of tau oligomers formed in vivo. These studies revealed new and surprising information about tau oligomers, their formation, phosphorylation state, stability and biochemical properties. Moreover, we used the immunereactivity of the novel tau oligomer specific antibody to determine the presence of the oligomeric toxic conformation in the different tau structures formed in AD such as, pre-tangles, neuritic plaques and neuropil threads.

Disclosures: R. Kayed, None; **C. Lasagna-Reeves**, None; **M. Guerrero-Munoz**, None; **U. Sengupta**, None; **G. Jackson**, None; **J. Troncoso**, None.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.7

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH PO1AT004511

CurePSP

Title: Grape derived polyphenols attenuate tau neuropathology in the tmht mouse model of Alzheimer’s disease

Authors: *J. N. WANG[1], I. SANTA-MARIA[2,4], L. HO[2,5], H. KSIEZAK-REDING[2,5], K. ONO[6], D. B. TEPLOW[7], G. M. PASINETTI[3,5];
[1]Mt Sinai Sch. Med., NEW YORK, NY; [2]Neurol., [3]Mount Sinai Sch. of Med., New York, NY; [4]Alzheimer Dis. Res. Unit, CIEN Foundation-Queen Sofia Fndn., Madrid, Spain; [5]Geriatric Res., James J. Peters Veterans Affairs Med. Ctr., Bronx, NY; [6]Med., Kanazawa Univ., Kanazawa, Japan; [7]UCLA, Los Angeles, CA

Abstract: Objective: This study was designed to test whether a select grape seed polyphenolic extract (GSPE) could benefit tau-mediated neuropathology in a mouse model of Alzheimer's disease.
Methods: In this study, we used in vitro circular dichroism (CD) spectroscopy and electron microscopy (EM) to examine the effect of GSPE on tau peptide aggregation in vitro. Using TMHT mouse model of AD-type tauopathy, we evaluated the in vivo efficacy of GSPE on tau-mediated neuropathology in the brain.
Results: We found that GSPE potently interfered with the assembly of tau peptides into neurotoxic aggregates. Moreover, oral administration of GSPE significantly attenuated the development of tau-mediated neuropathology in the brains of TMHT mice through mechanisms associated with the reduction of extracellular signal-receptor kinase (ERK) 1/2 signaling in the brain
Interpretation: Our study provides impetus for the continued development of GSPE for the prevention/treatment of tau-mediated neurological diseases.

Disclosures: J.N. Wang, None; **I. Santa-Maria**, None; **L. Ho**, None; **H. Ksiezak-Reding**, None; **K. Ono**, None; **D.B. Teplow**, None; **G.M. Pasinetti**, None.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.8

Topic: C.02. Alzheimer's disease and other dementias

Support: ADDF

Title: Validation of extracellular tau oligomer target for drug discovery in a novel animal model

Authors: *J. G. MOE[1], I. CHATTERJEE[1,2], D. PUZZO[4], A. STANISZEWSKI[3], M. FA[3], E. DAVIDOWITZ[1], O. ARANCIO[3];

[1]OLIGOMERIX, Inc., NEW YORK, NY; [3]Taub Inst., [2]Columbia Univ. Med. Ctr., New York, NY; [4]Città Universitaria, Catania, Italy

Abstract: Tau protein is found primarily associated with axons in differentiated neurons where it functions to stabilize microtubule structure and regulate transport. However, during Alzheimer's disease (AD) and other tauopathies tau loses its normal function and gains toxic activity. Tau protein aggregates and is sequestered into filaments and higher order neurofibrillary tangles (NFT), a pathological hallmark of AD, and is modified by multiple mechanisms (Ballatore C et al. Nat Rev Neurosci. 2007 8:663-72). Studies using mouse models of AD and tauopathies show a strong correlation between the accumulation of soluble oligomeric species of tau and neuronal loss and memory impairment (Berger Z et al. J Neurosci. 2007 27:3650-62; Brunden KR et al. J Alzheimers Dis. 2008 14:393-9), and have challenged the assumption that NFT are the neurotoxic structures of tau.
As AD progresses, tau pathology reproducibly spreads through the hippocampal structure to the cortex in a contiguous, highly selective and orderly fashion (Braak, H. and E. Braak. J Neural Transm Suppl, 1998. 53:127-40; Schönheit B et al. Neurobiol Aging. 2004 25:697-711) suggesting that aberrant tau protein may be involved in transmitting pathology to neighboring neurons during disease progression. Tau pathology may be transmitted to neighboring healthy neurons through muscarinic receptors I and III (Gómez-Ramos A et al. Eur Neuropsychopharmacol. 2009 19:708-17) or by directly entering cells and functioning as a template for intracellular tau to misfold, aggregate and cause neurodegeneration (Clavaguera F et al. Nat Cell Biol. 2009 11:909-13; Frost B et al. J Biol Chem. 2009 284:12845-52). In AD the levels of extracellular tau increase in cerebrospinal fluid, presumably due to release of intracellular proteins during cell death; hence its use as a biomarker for AD (Trojanowski JQ et al. Alzheimers Dement. 2010 6:230-8). Tau secretion to the extracellular space and to postsynaptic neurons was shown to be dependent on the N-terminus of tau and tauopathy mutations facilitating tau aggregation (Kim W et al. J Alzheimers Dis. 2010 19:647-64).
Here, we show that extracellular tau oligomers have a causative effect on disrupting memory in studies of synaptic function in hippocampal slices and behavior in mice. Extracellular tau oligomers, but not monomeric tau, reduced long-term potentiation (LTP) (IC50 5 nM) and impaired associative fear memory in normal mice. These results strongly support extracellular tau oligomers as a target for drug discovery for AD and related tauopathies.

Disclosures: J.G. Moe: Employment; OLIGOMERIX, Inc.. Research Grant; ADDF. **I. Chatterjee:** ; • Alzheimer's Drug Discovery Foundation. **D. Puzzo:** Employment; Città Universitaria. Research Grant; ADDF. **A. Staniszewski:** Employment; Columbia University Medical Center. **M. Fa:** ; Columbia University Medical Center. Research Grant; ADDF. **E. Davidowitz:** Employment; OLIGOMERIX, Inc.. Research Grant; ADDF. **O. Arancio:** Employment; Columbia University Medical Center. Research Grant; ADDF.

Nanosymposium

527. Tau: Abeta and Tau Dependent and Independent Pathways

Location: Room 32B

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 527.9

Topic: C.02. Alzheimer's disease and other dementias

Support: VA Merit Review, B.K.

Title: Proteasome inhibition drives tau into aggresomes in a cellular model for pathological tau

Authors: *C. R. GUTHRIE[1,2], B. C. KRAEMER[1,2];
[1]VA Puget Sound Hlth. Care Syst., SEATTLE, WA; [2]Medicine, Gerontology Div., Univ. of Washington, Seattle, WA

Abstract: Aggregated tau protein is a hallmark of a number of neurodegenerative diseases. We have developed a model by overexpressing WT human tau in HEK 293 cells to promote formation of pathological tau species in the absence of tau mutations. To dissect the cellular mechanisms at work in clearing pathological tau species, we have explored the effects of proteasome inhibition on tau aggregation and clearance. When exposed to proteasome inhibitor overnight, HEK293/Tau cells exhibit hallmarks of aggresome formation. These include clustering of tau into a spherical body at the microtubule organizing center (MTOC), localization of the retrograde motor protein dynein to the centrosome, formation of a vimentin cage, and clustering of mitochondria around the MTOC localized tau. We have followed this process in live cells over time and have found aggresomes to be formed after 9 hours. We have also found many abnormal tau epitopes seen in disease states are recruited to aggresomes. Furthermore, tau containing aggresomes are shown to accumulate detergent insoluble aggregated tau species. The relevance of this cellular model to authentic tau pathology and its clearance will be discussed.

Disclosures: C.R. Guthrie, None; **B.C. Kraemer**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.1

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant R01 NS047973

NIH Grant R01 EY09024

NIH Grant P01 ES016738

NIH Grant P30 NS057096

NIH Grant MH62512

NIH Grant MH62962

Title: Erythropoietin plus insulin-like growth factor-I protect against neuronal damage in a murine model of HIV-associated neurocognitive disorders

Authors: *Y.-J. KANG[1], M. MURAT DIGICAYLIOGLU[3], R. RUSSO[3], M. KAUL[2], C. L. ACHIM[4], L. FLETCHER[2], E. MASLIAH[5], S. A. LIPTON[3,5];
[1]Ctr. for Neuroscience, Aging and Stem Cell Res., [2]Infectious and Inflammatory Dis. Ctr., Sanford-Burnham Med. Res. Inst., La Jolla, CA; [3]Ctr. for Neuroscience, Aging and Stem Cell Res., Sanford-Burnham Res. Inst., La Jolla, CA; [4]Dept. of Psychiatry and Pathology, [5]Dept. of Neurosciences, UCSD, La Jolla, CA

Abstract: Prolonged human immunodeficiency virus-1 (HIV-1) infection leads to neurological debilitation including motor dysfunction and frank dementia. Although pharmacological control of HIV infection is now possible, HIV-associated neurocognitive disorders (HAND) remains intractable. Here, we report that chronic treatment with erythropoietin (EPO) and insulin-like growth factor-I (IGF-I) protects against HIV/gp120-mediated neuronal damage in culture and *in vivo*. Initially, we tested the neuroprotective effects of various concentrations of EPO, IGF-I, or EPO+IGF-I from gp120-induced damage *in vitro*. To assess the chronic effects of EPO+IGF-I administration *in vivo*, we treated HIV/gp120-transgenic or wild-type (WT) mice transnasally once a week for 4 months and subsequently conducted immunohistochemical analyses. Low concentrations of EPO+IGF-I provided neuroprotection from gp120 *in vitro* in a synergistic fashion. *In vivo*, EPO+IGF-I treatment prevented gp120-mediated neuronal and synaptic damage, but did not alter microgliosis or astrocytosis. Strikingly, in the brains of both humans with HAND and gp120-transgenic mice, we found evidence for hyperphosphorylated tau protein (PHF-I tau), which has been associated with neuronal damage and loss. In the mouse brain following transnasal treatment with EPO+IGF-I, in addition to neuroprotection we observed increased phosphorylation/activation of Akt (PKB) and increased phosphorylation/inhibition of glycogen synthase kinase (GSK)-3β, thus dramatically decreasing downstream hyperphosphorylation of tau. These results indicate that the peptides affected their cognate signaling pathways within the brain parenchyma. Our findings suggest that chronic combination therapy with EPO+IGF-I provides neuroprotection in a mouse model of HAND, in part, through cooperative activation of phosphatidylinositol 3-kinase (PI3K)/Akt/GSK-3β signaling. This combination peptide therapy should therefore be tested in humans with HAND.

Disclosures: Y. Kang: Employment; Sanford-Burnham Medical Research Institute. Research

Grant; NIH R01 NS047973, R01 EY09024. **M. Murat Digicaylioglu:** Employment; University of Texas Health Science Center. Ownership Interest; MD is named inventor on patents for the use of EPO and IGF-I for neurodegenerative disorders. These patents are assigned to the Sanford-Burnham Medical Research Institute.. **R. Russo:** None. **M. Kaul:** None. **C.L. Achim:** None. **L. Fletcher:** None. **E. Masliah:** Research Grant; NIH grant MH62512, MH62962. **S.A. Lipton:** Ownership Interest; SAL is named inventor on patents for the use of EPO and IGF-I for neurodegenerative disorders. These patents are assigned to the Sanford-Burnham Medical Research Institute.. Research Grant; NIH grant R01 NS047973, R01 EY09024, P01 ES016738, P30 NS057096.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.2

Topic: C.02. Alzheimer's disease and other dementias

Support: Department of Veteran Affairs

GRECC and Basic and Biomedical Training Program at the James J. Peters Veteran Affairs Medical Center (Bronx, NY)

Title: Molecular topology as novel approach in Alzheimer's disease drug discovery

Authors: *G. M. PASINETTI[1,3], H. FIVECOAT[2], J. WANG[2];
[2]Neurol., [1]Mount Sinai Sch. of Med., New York, NY; [3]Grecc, James J. Peters Veteran Affairs Med. Ctr., Bronx, NY

Abstract: Background: Alzheimer's disease (AD) is a progressive and ultimately fatal degenerative brain disorder that primarily affects the elderly in the parts of the brain that control thought, memory and language. As beta-amyloid (Abeta) accumulation in the brain has been demonstrated to be critical to AD pathogenesis, therapies aimed at inhibiting its production, blocking its oligomerization/aggregation, or enhancing its degradation are actively being investigated.
Objectives: The present study was designed using a molecular topology approach, which uses the power of mathematical chemistry to advance bioactive molecule design and discovery, to identify novel therapeutic compounds in new chemical classes and to improve *in vivo* translatability for treatments of Alzheimer’s disease (AD).
Methods: Molecular topology was used to identify novel compounds in new chemical classes.

Primary neuron culture was used to evaluate Abeta-lowering activity, and *in vitro* aggregation assay and photo-induced cross-linking of unmodified proteins (PICUP) techniques were used to evaluate anti-Abeta oligomerization activity. Eight-week old TgCRND8 transgenic mice, which model AD, were used to evaluate the short-term *in vivo* efficacy of identified compounds.
Results: Eight compounds were identified as having dual anti-amyloid and anti-aggregation activity. Two of the eight compounds demonstrated significant *in vivo* reductions of (1) total Abeta1-40 and Abeta1-42 (~10-30%) and (2) high molecular weight oligomers (~20-25%) after administration at 2 mg/kg/day in TgCRND8 mice for two weeks.
Implications: These studies support the continued development of the two identified compounds as a treatment in AD, in addition to the utilization of molecular topology as a novel approach for AD drug discovery.
Supported by the Department of Veteran Affairs, and the GRECC and Basic and Biomedical Training Program at the James J. Peters Veteran Affairs Medical Center (Bronx, NY) to GMP.

Disclosures: G.M. Pasinetti, None; **H. Fivecoat**, None; **J. Wang**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.3

Topic: C.02. Alzheimer's disease and other dementias

Support: discretionary funding to GMP

Title: Transplantation of human embryonic stem cell-derived cholinergic neurons in a mouse model of Alzheimer’s disease

Authors: *W. ZHAO[1], G. M. PASINETTI[1,2];
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Abstract: Objective: Cholinergic neuronal dysfunction of the basal forebrain is observed in patients with Alzheimer’s disease and dementia, and it has been linked to decreased neurogenesis in the hippocampus, a region involved in learning and memory. The goal of this study is to elucidate the effect of cholinergic neuron on dementia in a mouse model of nucleus basalis of Meynert (NBM) lesion.
Methods: Human embryonic stem cells (hES, H9 line) were differentiated into a population of neuronal cells that express the cholinergic enzyme choline acetyltransferase (ChAT) and

homeobox proteins specifying neuronal progenitors of ventral telencephalic lineage. hES-derived cholinergic neurons were transplanted into the frontal cortex and barrel field and barrel field of S1 cortex of C57BL/6 mice four weeks after NBM lesion. All animals (vehicle and cell groups) received cyclosporin at 10mg/kg in saline to minimize possible immune rejection from one day before surgery and throughout the course of the experiment. Behavioral test by Morris water maze were conducted 2 weeks after transplantation and tissues were collected after the last behavioral test. Brain slices were stained with human specific anti-neural cell adhesion molecule, ChAT, serotonin and amyloid-beta-protein.
Conclusion: These data provide evidence for in vivo survival of hESC-derived cholinergic neurons, a key requirement in the development of hESC-based cell therapy in neurodegenerative diseases.

Disclosures: W. Zhao, None; **G.M. Pasinetti**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.4

Topic: C.02. Alzheimer's disease and other dementias

Support: 1PO1AT004511-01 Proj.-1 (LH)

1PO1AT004511-01, Proj.-3 (GMP)

J.J. Peters VA GRECC Program (GMP)

Title: Identification and characterization of brain-targeting grape-derived polyphenolics: Implications in Alzheimer’s disease prevention and therapy

Authors: *L. HO[1], M. FERRUZZI[2], E. JANLE[3], J. LOBO[2], T.-Y. CHEN[3], S. S. TALCOTT[4], J. SIMON[5], Q. L. WU[5], J. WANG[1], C. WEAVER[3], S. S. PERCIVAL[6], G. M. PASINETTI[1,7]; [1]Dept Neurol., Mt. Sinai Sch. Med., NEW YORK, NY; [2]Food Sci., Perdue Univ., West Lafayette, IN; [3]Foods and Nutrition,, Perdue Universithy, West Lafayette, IN; [4]Nutr. and Food Sci., Texas A&M Univ., College Station, TX; [5]Plant Biol. and Plant Pathol., Rutgers Univ., New Brunswick, NJ; [6]Food Sci. and Human Nutr., Univ. Florida, Gainesville, FL; [7]Grecc, James J {eters VA Med. Ctr., Bronx, NY

Abstract: Accumulating evidence suggests that selective bioactive grape-derived polyphenols

may protect against the Alzheimer's disease (AD)-type cognitive deterioration, in part, by interfering with β-amyloid (Abeta)-mediated neuropathologic mechanisms. Our previous studies revealed that polyphenolics from red wines and Concord grape juice may inhibit the generation of Abeta peptides and/or the assembly of Abeta peptides into neurotoxic oligomeric aggregated Abeta species. To gather insights on specific bioactive polyphenolics that might exert beneficial disease-modifying activity in vivo, we explored the pharmacokinetic and bioavailability of red wine and grape juice polyphenolics. We identified a number of polyphenolic derivatives that are bioavailable in the blood and/or in the brain following oral administrations of total polyphenolic extract from red wine and grape juice. Evidence from our ongoing studies exploring the potential bioactivities of these brain-targeting polyphenols revealed specific polyphenolic that significantly reduced the generation of Aβ peptides, in vitro, in primary cortico-hippocampal neuron cultures. Moreover, we have tentatively identified another brain-targeting polyphenolic that may promote activation of cAMP responsive element binding protein (CREB), a transcription factor that is implicated in neuronal synaptic plasticity. Results from our studies suggest that dietary polyphenolics may benefit AD by modulating multiple disease-modifying modalities, including Abeta-dependent and independent mechanisms in the brain, and provides the impetus to develop selective polyphenolic compounds for AD prevention and/or therapy.

Disclosures: L. Ho, None; **M. Ferruzzi**, None; **E. Janle**, None; **J. Lobo**, None; **T. Chen**, None; **S.S. Talcott**, None; **J. Simon**, None; **Q.L. Wu**, None; **J. Wang**, None; **C. Weaver**, None; **S.S. Percival**, None; **G.M. Pasinetti**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.5

Topic: C.02. Alzheimer's disease and other dementias

Title: Green coffee may benefit Alzheimer's disease through epigenetic gene modifications and promotion in mitochondrial energy metabolism in the brain

Authors: *F. CHEN[1], J. WANG[2], M. VARGHESE[1], W. ZHAO[1], L. HO[1], G. PASINETTI[1,3]; [1]neurology, MOUNT SINAI MEDICAL SCHOOL, NEW YORK, NY; [2]neuology, Mount Sinai Sch. of Med., NYC, NY; [3]Geriatric Research, Educ. and Clin. Ctr., James J. Peters Veteran Affairs Med. Ctr., Bronx, NY

Abstract: Background: Alzheimer Disease (AD) is a progressive neurodegenerative disorder in which clinical manifestations appear in old age. The sporadic nature of 90% of AD cases,

varying susceptibility to the course of the illness, as well as the late age of onset suggest that the epigenetic and environmental factors play an important role in the etiology of late-onset AD. Till now, the cause of most of late-onset AD cases still remain unexplained, and current biomedical science is still a long way from the ultimate goal of revealing clear risk factors that could help in the prevention and treatment of the disease. Coffee is one of the world's most popular beverages. The numerous beneficial health effects of coffee consumption have received significant scientific attention. Studies suggest that drinking coffee regularly helps prevent several chronic diseases. Extensive investigations have revealed that most of these effects are attributed to chlorogenic acids (CGAs), which are found most potently in green coffee.
Experimental Approach: To investigate the beneficial effects of green coffee on both metabolic and epigenetic changes of Alzheimer's disease, we used both the CRND8 mouse model and wild type mice (n=10) on high-fat and low-fat diets treated with Green coffee (Svetol, gift from NATUREX company) for 6 months. Body weight and food and drink consumption were monitored weekly, and glucose tolerance assays were carried on monthly. The frontal cortex and muscles were isolated after 6 months of treatment; mRNA of the frontal cortex was extracted for microarray assay and further qPCR confirmation, followed by ChIP-Chip. Mitochondria were also isolated to detect the oxygen consumption rate (OCR) .
Results: We found that glucose tolerance was significantly improved in green-coffee treated mice compared to control, as was mitochondrial function (as measured by OCR). Further analysis using microarray and qPCR suggested functional expression level changes in certain epigenetic genes, including Histone deactylase 1 (HDAC1) and DNA methyltrasferase 3a (Dnmt3a). ChIP-Chip of these two genes is currently under investigation, and will elucidate the mechanism(s) of green coffee's effect in this mouse model of AD.
Conclusions: Our data suggest that the green coffee (CGAs) benefits AD by improving mitochondrial functions and through epigenetic alterations in certain genes.
Supported by discretionary funding to GMP.

Disclosures: **F. Chen**, Mount Sinai School of Medicine, Employment; **J. Wang**, None; **M. Varghese**, None; **W. Zhao**, None; **L. Ho**, None; **G. Pasinetti**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.6

Topic: C.02. Alzheimer's disease and other dementias

Support: The Department of Veterans Affairs

NIH grant AG27505

NIH grant AG30144

The Larry L. Hillblom Foundation

Title: Reduced beclin 1 in microglia impairs phagocytosis and clearance of amyloid beta

Authors: *K. LUCIN[1], E. CZIRR[1], B. SPENCER[2], T. WYSS-CORAY[1];
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Abstract: Beclin 1 is a protein that plays a critical role in the generation of mature autophagic vesicles and is subsequently involved in the clearance of protein aggregates. Beclin 1 is reduced in affected brain regions of Alzheimer's disease (AD) patients and is associated with an accumulation of amyloid aggregates. Recent studies suggest reduced Beclin 1 levels in neurons impairs autophagy and promotes amyloid accumulation. However, microglia are also capable of regulating the clearance of these aggregates through phagocytosis. Despite emerging links between autophagy and phagosome maturation, the role of Beclin 1 in microglia remains unclear. Here we use lentivirus encoding Beclin 1 shRNA to reduce Beclin 1 levels in microglial cells (i.e., BV-2 cells) and assess whether reduced Beclin 1 impairs phagocytosis or amyloid beta (AB) clearance. To measure phagocytosis we utilize latex beads, which are rapidly phagocytosed when added to microglial cultures. Using this model we find that reduced Beclin 1 levels in microglia decreases both the number of cells that phagocytose beads and the number of internalized beads per cell. Impaired internalization of beads in Beclin 1 deficient microglia appears to specifically result from defects in phagocytosis since endocytosis of transferrin receptors is not affected. Importantly, phagocytic defects in Beclin 1 deficient microglia can be reversed by rescuing Beclin 1 levels via a Beclin 1 overexpressing lentivirus. Using an ex-vivo amyloid precursor protein (APP) brain slice model, we also find that Beclin 1 deficient microglia are less efficient at degrading cortical and hippocampal AB. Impaired AB clearance by Beclin 1 deficient microglia cannot be explained by deficits in lysosomal function or cellular migration, both of which are comparable with control cells, suggesting that Beclin 1 regulates intrinsic cellular machinery required for phagocytosis. Together these data suggest that reduced Beclin 1 levels in AD brains may allow for enhanced disease progression by impairing microglial phagocytosis. Therefore, strategies that enhance Beclin 1 levels may provide a novel approach for the treatment of AD.

Disclosures: K. Lucin, None; **E. Czirr**, None; **B. Spencer**, None; **T. Wyss-Coray**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.7

Topic: C.02. Alzheimer's disease and other dementias

Support: Grant from the Promotion and Mutual Aid Corporation for Private Schools of Japan

Title: Improved learning parallels normalization of electrophysiological properties after transcranial magnetic stimulation in a mouse Alzheimer disease model

Authors: *N. KATO[1], F. WANG[1,2], Y. ZHANG[1,3], L. WANG[1,4], P. SUN[1,2], R. YAMAMOTO[1], T. SUGAI[1], Z. WANG[1];
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Abstract: Beneficial effects of transcranial magnetic stimulation (TMS) on cognitive functions have been reported not just in normal subjects but also in Alzheimer disease (AD) patients or elderly people with memory dysfunction. However, molecular and cellular underpinnings of such TMS effects have not been well explored. We thus examined behavioral, electrophysiological and molecular biological effects of chronic TMS on 3xTg AD model mice (AD mice; Oddo et al, 2003), in which genes for amyloid precursor protein, tau, and presenilin-1 are modified to produce excessive extracellular deposits as well as intracellular accumulation of amyloid beta (Aβ). In 3-6 months of age, the cortex and hippocampus in this model mouse bear intracellular accumulation of Aβ without major extracellular deposits. The present experiments were done in mice of this age range. One session of TMS, consisting of 10 Hz volleys for 5s, was delivered daily for 4 weeks at suprathreshold intensities for muscle contraction in the lower extremity. AD mice subjected to TMS exhibited better Morris maze performance than no-TMS-control AD mice. Resting membrane potential in cingulate cortex pyramidal cells was more depolarized in slices obtained from AD mice than in non-transgenic control mice, but became significantly hyperpolarized after TMS. Reduction of the amplitude of long-term potentiation (LTP) at hippocampal Schaffer collateral-CA1 synapses was observed in slices from AD mice, but was recovered partly after TMS. Quantitative real time PCR analysis revealed upregulated expression of large conductance calcium-activated potassium (BK) channel and Homer 1a. These results were consistent with our previous report that membrane hyperpolarization and BK channel facilitation were observed after electroconvulsive shock, which turned out to depend on Homer 1a (Sakagami et al, 2005). Conclusions: (1) chronic high frequency TMS ameliorates cognitive learning of AD mice; (2) this amelioration seems to be in parallel with normalization of altered electrophysiological properties in the cortex and hippocampus; (3) toxicity of intracellular Aβ manifested in terms of membrane potential and LTP induction may be linked causatively to cognitive impairment in AD mice.

Disclosures: N. Kato, None; **F. Wang**, None; **Y. Zhang**, None; **L. Wang**, None; **P. Sun**, None; **R. Yamamoto**, None; **T. Sugai**, None; **Z. Wang**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.8

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH AG02219

Department of Veteran Affairs to GMP

Title: Brain inflammation promotes memory formation through pCREB -C/EBP pathway

Authors: *Y. PAN[1], L. HO[1], W. ZHAO[1], J. WANG[1], H. FIVECOAT[1], S. BARNUM[2], G. M. PASINETTI[1,3];
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Abstract: Factors associated with aging and dementia in the young-old (YO, age < 86) elderly are not necessarily central to the health and well-being of the oldest-old (OO, age > 87) elderly. Since the numbers of the OO elderly are growing more rapidly than any other demographic in the US and in the world, understanding the neurobiological substrates of successful aging and of dementia among this population are of paramount significance. Anti-inflammatory strategies have been a cornerstone of therapeutic developments for Alzheimer's disease (AD) and other neurodegenerative disorders. However, recent evidence from our group and from others suggests that certain immune/inflammatory modulators in the brain, such as the complement-derived components C5a and C3a, anaphylatoxins, may benefit cognitive functions. Our evidence has also revealed that C5a might promote memory consolidation processes through mechanisms involving the transcription factors cAMP/Ca2+ response element-binding protein (CREB) and CCAAT/enhancer-binding protein (C/EBP). We hypothesized that C5a-mediated responses in the brain contribute to successful aging and that insufficient C5a responses in the brain may result in increased sensitivity of OO subjects for developing AD. To test this hypothesis, we treated primary neuron cells from wild type mice with human recombinant C5a and performed a Luminex assay, which resulted in significantly elevated phosphorylated CREB (p-CREB) levels. To validate our in vitro findings, we generated transgenic mice overexpressing C5a and found, using Luminex assay, that C5a mouse brains have significantly higher levels of p-CREB compared to age- and gender-matched wild type mice. Since C/EBP is an immediate CREB-

downstream gene required for synaptic plasticity and memory formation, we further investigated whether C/EBPβ levels are increased in the brains of C5a mice; analysis by ELISA revealed that indeed C/EBPβ levels were significantly elevated in the brains of C5a transgenic mice compared to age- and gender-matched wild type mice. These findings collectively suggest that C5a may benefit cognition by promoting memory consolidation through mechanisms involving the CREB, C/EBP, and their upstream regulatory pathways, which may be utilized as therapeutic target in AD to modulate specific inflammatory mechanisms in the brain, instead of non-specific suppression of overall inflammatory processes.

Disclosures: Y. Pan, None; **L. Ho**, None; **W. Zhao**, None; **J. Wang**, None; **H. Fivecoat**, None; **S. Barnum**, None; **G.M. Pasinetti**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.9

Topic: C.02. Alzheimer's disease and other dementias

Support: Alz Assoc IIRG-06-27532 (CAL)

Title: Long-term oral administration of L-3-n-butylphthalide (L-NBP) reduces cerebral Aβ levels in 3xTg-AD mice by interfering amyloid precursor protein processing

Authors: *B. LIU[1], Y. PENG[2], H. J. FU[1], J. L. FROST[1], X. L. WANG[2], C. A. LEMERE[1]; [1]Dept. of Neurol., Ctr. for Neurologic Diseases, Brigham & Women's Hosp. and Harvard Med. Sch., Boston, MA; [2]Dept. of Pharmacol., Inst. of Materia Medica, Chinese Acad. of Med. Sci. & Peking Union Med. Col., Beijing, China

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder resulting in memory loss. Cerebral amyloid-β peptide (Aβ) deposition and abnormal tau hyperphosphorylation (tau-p) are hallmarks of AD. L-NBP was first extracted from Chinese Celery seeds and then chemically synthesized and shown to partially improve Aβ-induced cognitive deficits in AD-like mouse models. In a long-term treatment study, we examined the potential of L-NBP to prevent, treat, and/or reverse cognitive impairment and AD-associated pathogenesis in triple-transgenic (3xTg-AD) mice. Mice were fed L-NBP-containing or control chow from 1.9-19 months of age (prevention trial), 9.3-19.3 months of age (therapeutic trial), and 19.1-25.1 month of age (reversal trial). In SFN Meeting last year, we reported the robust and consistent effects of L-NBP in lowering plaque deposition (R1282 immunoreactivity and

Thioflavin S staining) and cerebral Aβ levels (Aβx-40, x-42 and 1-total ELISAs) in all three trials. Furthermore, earlier treatment of L-NBP (prevention and therapeutic) showed a significant attenuation of tau hyperphosphorylation at specific sites (PHF-1, AT8 and AT180). More recently, we further explored the underlying mechanisms including the capability of L-NBP to interfere with Aβ production and/or clearance. We found that L-NBP significantly reduced the βAPPs/APP ratio by 22.8% ($p<0.05$) and inhibited BACE-1 (β-secretase) activity by 22.4% ($p<0.01$) in the prevention trial, decreased βAPPs/APP ratio by 19.4% ($p<0.05$) and BACE-1 activity by 52.8% ($p<0.001$), respectively, in the therapeutic trial, and increased αAPPs/APP ratio by 24.0% ($p<0.05$) and ADAM17 (α-secretase) protein expression by 26.4% ($p<0.01$) in the prevention trial. The inhibitory effect of L-NBP on BACE-1 activity was further confirmed in a cell free assay in vitro. In addition, L-NBP showed no effect on microglia-mediated Aβ phagocytosis or Aβ degrading enzymes (IDE-1 and neprilysin) in any of the 3 trials, indicating that the L-NBP's main effect appears to be regulation of Aβ generation through direct suppression of the cleavage of the Aβ N-terminus from the amyloid precursor protein, APP.

Disclosures: B. Liu, None; **Y. Peng**, None; **H.J. Fu**, None; **J.L. Frost**, None; **X.L. Wang**, None; **C.A. Lemere**, None.

Nanosymposium

528. Alzheimer's Disease and Neurodegeneration: Therapies in Animal Models

Location: Room 31C

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 528.10

Topic: C.02. Alzheimer's disease and other dementias

Support: Illinois Department of Public Health

Title: Environmental enrichment as a key modulator of brain plasticity and neuropathology in Alzheimer's disease

Authors: *Y.-S. HU[1], P. XU[2], G. PIGINO[1], S. BRADY[1], J. LARSON[2], O. LAZAROV[1]; [1]Anat. and cell biology, [2]Psychiatry, Univ. of Illinois At Chicago, CHICAGO, IL

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by memory impairments and cognitive deterioration that are associated with a massive and progressive loss of neurons in specific brain areas, such as the hippocampal formation and cortex. The hallmarks of the disease are amyloid deposition and neurofibrillary tangles. The familial form of the disease (FAD) is caused by mutations in amyloid precursor protein (APP), presenilin-1 (PS1) and presenilin-2 (PS2). Our previous work suggests that in addition to genetic

cause, environmental factors play a role in the formation of the disease. We showed that experience of transgenic harboring FAD-linked APPswe/PS1ΔE9 in a complex environment dramatically reduced extent of amyloid deposition. To examine whether experience in a complex environment would rescue additional deficits in brain plasticity in these mice, FAD-linked APPswe/PS1ΔE9 were exposed to a complex environment for one month right after weaning. Here we show that experience of APPswe/PS1ΔE9 mice in enriched environmental conditions enhanced neural progenitor cell (NPC) proliferation, early differentiation, as well as the number of mature neurons incorporated in the granule layer of the dentate gyrus. Functionally, environmental enrichment significantly enhanced hippocampal long-term potentiation (LTP), without notable alternation in basal synaptic transmission. In addition, exposure of these mice to an enriched environment attenuated levels of oligomeric Aβ, the neurotoxic precursor of amyloid deposition, as well as tau hyperphosphorylation. We further observed upregulation of the main anterograde motor protein, kinesin-1, in the brains of transgenic mice that experienced environmental enrichment, suggesting an enhancement of axonal transport. Taken together, this study suggests that environmental experience can modulate neuroplasticity, attenuates neuropathology and enhances synaptic plasticity in FAD-linked mouse model. This study provides evidence for the critical significance of environmental enrichment as a potential therapeutic approach.

Disclosures: Y. Hu: None. **P. Xu:** None. **G. Pigino:** None. **S. Brady:** None. **J. Larson:** None. **O. Lazarov:** None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: National Ataxia Foundation fellowship

Title: Ataxin-3 mediated regulation of parkin stability

Authors: *T. DURCAN[1], M. KONTOGIANNEA[1], A. DJARMATI[2], A. J. WILLIAMS[2], H. L. PAULSON[2], E. A. FON[1];
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Abstract: The ubiquitin proteasome system (UPS) involves ubiquitin (Ub), a protein that is

conjugated to lysine residues within a target protein, with ubiquitination involved in processes from cell signalling to proteasomal degradation. Moreover, the Ub system has been heavily implicated in the pathogenesis of neurodegenerative diseases. One such disease is Machado-Joseph's disease (MJD), the most common dominantly inherited ataxia worldwide, caused by a polyglutamine expansion in the enzyme ataxin-3, an enzyme that functions to remove Ub moieties from substrate proteins in a process termed deubiquitination. In this study, we identify the Parkinson's disease (PD) associated E3 ligase parkin as a novel substrate for ataxin-3, with ataxin-3 binding to and deubiquitinating parkin as it self-ubiquitinates. Studies with other E3 ligases demonstrate a clear role for self-ubiquitination in forming K48-linked Ub conjugates that promote targeting of the E3 ligase for degradation, with deubiquitination of such linkages by its DUB partner, preventing targeting of the E3 ligase for degradation. Remarkably, ataxin-3-mediated deubiquitination of parkin failed to affect parkin stability, with no changes in parkin levels observed in brain lysates from mice in which ataxin-3 was absent or over-expressed. Moreover, pulse-chase assays demonstrated parkin to be inherently stable with little decrease in parkin levels after 24h in the presence of cycloheximide, a protein synthesis inhibitor. Surprisingly, a decrease in parkin levels was observed in both cells and brain lysates only in the presence of the MJD-associated mutant ataxin-3. In understanding how mutant ataxin-3 regulates parkin stability, we demonstrate that expanded ataxin-3 is more active at deubiquitinating parkin compared to wild-type ataxin-3 and such activity promotes targeting of parkin for degradation through the autophagy pathway. Taken together, we speculate that self-ubiquitination is protecting parkin from degradation as a result of non-K48 linked Ub conjugates forming on parkin. The mutant ataxin-3 can efficiently cleave these Ub conjugates on parkin compared to wild-type ataxin-3 and promote clearance of parkin through the autophagy pathway. Moreover, decreased parkin levels may be a novel contributing factor in the pathogenesis of MJD.

Disclosures: T. Durcan, None; **M. Kontogiannea**, None; **A. Djarmati**, None; **A.J. Williams**, None; **H.L. Paulson**, None; **E.A. Fon**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01NS27699-17

NIH Grant F32NS055545-01

Title: Haploinsufficiency of Capicua rescues spinocerebellar ataxia phenotypes by relieving the hyper-repressive transcriptional activity of polyglutamine-expanded Ataxin-1

Authors: *J. D. FRYER[1], J. CRESPO-BARRETO[2], C. MANDEL-BREHM[2], H. KANG[2], P. YU[3], C. A. SHAW[2], W. LI[4], H. Y. ZOGHBI[3];
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Abstract: The fatal neurodegenerative disease spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited disease caused by the expansion of a translated CAG trinucleotide repeat encoding glutamine in Ataxin-1. We have previously shown that the transcriptional repressor Capicua binds to Ataxin-1 and modulates pathogenesis in a Drosophila model of SCA1. Here we used knock-in mouse model of SCA1 (Ataxin-1154Q mice) and studied the effect of haploinsufficiency of Capicua on several disease parameters at the behavioral and molecular level. Surprisingly, and contrary to the results in the Drosophila SCA1 model, we found that loss of one copy of Capicua was enough to substantially rescue several motor problems with SCA1 mice measured by behavioral assays such as open field, rotarod, dowel, and wire hang. Additionally, loss of one copy of Capicua also partially rescued the weight loss phenotype as well as prolonged the reduced lifespan of the SCA1 mice. To determine the mechanism of this rescue, we focused on the Capicua-dependent transcription in wild-type vs. Ataxin-1154Q vs. Ataxin-1154Q;Cic+/- mice. There were many transcripts that were upregulated in Ataxin-1154Q cerebellum that were even further upregulated in Ataxin-1154Q cerebellum, consistent with the fact that some component of the disease process occurs through some loss of Capicua function. Surprisingly, we found a substantial number of transcripts that were repressed in Ataxin-1154Q cerebellum but this repression returned to near wild-type levels in Ataxin-1154Q;Cic+/- cerebellum. Further, we found that Capicua was strongly bound to these promoters by chromatin immunoprecipitation analysis. These data are best explained by a model in which the polyglutamine-expanded Ataxin-1 protein alters Capicua such that it functions as a hyper-repressor at certain genomic loci. When the level of Capicua is reduced, this hyper-repression is partially relieved and results in rescue of the disease phenotypes. These results suggest that modestly reducing the level of Capicua or reducing the association between Capicua and polyglutamine expanded Ataxin-1 could rescue several disease phenotypes in SCA1.

Disclosures: J.D. Fryer, None; **W. Li**, None; **H.Y. Zoghbi**, None; **J. Crespo-Barreto**, None; **C. Mandel-Brehm**, None; **H. Kang**, None; **P. Yu**, None; **C.A. Shaw**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant K99/R00 NS064146

Title: Role of Nemo-like kinase in the pathogenesis of spinocerebellar ataxia type 1

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Abstract: Spinocerebellar ataxia type 1 (SCA1) is a dominantly inherited neurodegenerative disease caused by an expansion of CAG repeats encoding a glutamine tract in the protein Ataxin1. Key questions in SCA1 are to understand the pathogenic mechanism(s) by which glutamine expanded Ataxin1 mediates neurodegeneration and to identify modulator(s) of SCA1 neurotoxicity, which may lead to the development of therapeutic interventions. We identified the Nemo-like kinase (NLK) as an interactor of Ataxin1. Several lines of evidence demonstrate that NLK is a key protein contributing to the pathogenesis of SCA1 and a potential target for the development of therapeutics. First, NLK physically interacts with Ataxin1 and phosphorylates it at Serine 239. Second, expansion of the polyglutamine tract in Ataxin1 strongly increased the phosphorylation level of Serine 239. Third, NLK significantly enhanced the formation of Ataxin1 nuclear aggregates in HeLa cells. Fourth, overexpression of wild-type, but not kinase-inactive, form of NLK enhanced SCA1-induced neurodegenerative phenotypes in Drosophila. Last, we generated Nlk knockout mice and tested whether varying the level of NLK could modify phenotypes in SCA1 knock-in mice. We found that loss of one allele of Nlk suppressed Sca1154Q/+ knock-in phenotypes on the dowel-walking test. Also, we evaluated SCA1 neuropathology in the Nlk+/- heterozygote background and found that SCA1 cerebellar pathology was suppressed when one allele of Nlk was deleted. Overall, these data indicate that decreased activity of NLK has beneficial effects against the neurotoxicity induced by polyglutamine-expanded Ataxin1.

Disclosures: J. Lim, None; **J. Kahle**, None; **S. Kim**, None; **R. Richman**, None; **K. Chirala**, None; **H.T. Orr**, None; **H.Y. Zoghbi**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant 1R01EY018815-01A2

NIH Grant 1RC1NS068099-01

Batten Disease Support & Research Association

Title: A reversal learning task detects early cognitive deficiencies in a Dachshund model of late-infantile neuronal ceroid lipofuscinosis

Authors: *D. N. SANDERS[1], J. COATES[2], S. KANAZONO[2], M. KATZ[3], D. O'BRIEN[2]; [1]Ophthalmology, Univ. of Missouri - Columbia, Columbia, MO; [2]Vet. Med. and Surgery, [3]Ophthalmology, Univ. of Missouri, Columbia, MO

Abstract: Neuronal ceroid lipofuscinosis (NCL) is a heritable neurodegenerative disease characterized by accumulation of autofluorescent lysosomal storage granules in neural tissues accompanied by cognitive decline, seizures, and locomotor deficits. Our laboratory identified a mutation, occurring in long-haired Dachshunds, of tripeptidyl peptidase I (TPP1), the canine ortholog of human *CLN2*. [Awano, 2006*] In order to establish biomarkers for evaluating experimental therapies in this model, we characterized phenotypic changes in 13 age-matched dogs over an 8 month period.

Genotyping indicated that 4 puppies were homozygous for the mutant allele, 4 were heterozygous carriers and 5 were homozygous normal. All dogs had regular physical and neurologic examinations. Funduscopy, electroretinography (ERG) and cognitive function testing were performed at regular intervals. Cognitive function testing was assessed using a reversal learning task in a CanCog T-maze apparatus.

Physical examination remained normal for all dogs. In affected dogs, changes in funduscopic analyses, ERG and pupillary light reflexes presented between 5.5 and 6 months of age. By 7 months mild intention tremor and cerebellar ataxia were observed. Affected dogs were still visual at 8 months. MRI revealed diffuse cerebral and cerebellar atrophy and brain weights were reduced by 15% when compared to the normal dogs of similar size.

There were pronounced differences in performance between affected and normal dogs in the reversal learning task. A cognitive deficit in the affected dogs was clearly present at 4 months of age and progressively worsened at each subsequent time point. We conclude that the reversal learning task is a sensitive measure of disease progression which could serve as a useful biomarker for evaluation of clinical treatment strategies.

*Awano, T., Katz, M.L., O'Brien, D.P., Sohar, I., Lobel, P., Coates, J.R., Khan, S., Johnson, G.C., Giger, U., Johnson, G.S., 2006. A frame shift mutation in canine *TPP1* (the ortholog of human *CLN2*) in a juvenile Dachshund with neuronal ceroid lipofuscinosis. Mol. Genet. Metabol. 89, 254-260.

Disclosures: D.N. Sanders, None; **J. Coates**, None; **S. Kanazono**, None; **M. Katz**, None; **D. O'Brien**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant GM023562

NIH Grant U54RR025204

Title: Charcot-Marie-Tooth disease-causing mutations in GARS induce Daxx-mediated neuronal cell apoptosis

Authors: *X.-L. YANG[1], W. ZHANG[1], W. HE[1], H. NAWAZ[1], T. XU[1], M. PARK[2], Q. ZHOU[1], S. KIM[2], P. SCHIMMEL[1];
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Abstract: Charcot-Marie-Tooth (CMT) diseases are the most common heritable peripheral neuropathies. At least 11 different mutant alleles of GARS (the gene for glycyl-tRNA synthetase (GlyRS)) have been reported to cause a dominant axonal form of CMT. GlyRS catalyzes the conjugation of glycine to the cognate tRNA(Gly) to be used for protein synthesis. As about half of the 11 mutant human GlyRSs have wild type-like aminoacylation activity, CMT disease associated with GARS mutations is not simply caused by defect in aminoacylation and protein synthesis. Possibly, mutant GlyRSs have gained a pathological function. Or, a new, undefined function of GlyRS is affected by the CMT-causing mutations. Here we report that neuroblastoma cells transfected by transgenes encoding mutant GlyRSs undergo different degree of apoptosis, while cells transfected by a gene encoding wild type GlyRS do not. We found that transfections with genes encoding CMT mutant GlyRSs, but not with those encoding wild type GlyRS, induce translocation of Daxx from the nucleus to the cytoplasm, where Daxx bridges Fas ligand receptor and Apoptosis Signaling Kinase (ASK1) to transmit apoptosis. Interestingly, the severity of apoptosis associated with an individual GlyRS mutation correlates with the age of onset of CMT, and does not correlate with the defect in aminoacylation activity. The results suggest a connection between CMT and neuronal cell apoptosis via mutations on GlyRS, and raise the possibility of a role for GlyRS in neuronal cell homeostasis.

Disclosures: **X. Yang**, aTyr Pharma, Research Grant; **W. Zhang**, aTyr Pharma, Employment; **H. Nawaz**, None; **T. Xu**, None; **M. Park**, None; **W. He**, None; **Q. Zhou**, None; **S. Kim**, None; **P. Schimmel**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: 5T32NS007459-09

5T32NS007492-08

1R01AG036871-01

5R01NS041777-08

Forest Graduate Student Center of Aging Award

Lois Pope LIFE Fellowship

Title: Mitochondrial oxidative phosphorylation dysfunction causes preferential neurodegeneration of the striatum

Authors: *A. M. PICKRELL[1], H. FUKUI[2], C. T. MORAES[1];
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Abstract: Neuronal oxidative phosphorylation (OXPHOS) deficiency has been associated with normal aging as well as with age-related neurodegenerative diseases. Different neuroanatomical regions have been reported to contain cytochrome *c* oxidase (COX) negative neuronal populations. However, it is not known whether different neurons have different susceptibilities to mitochondrial respiratory chain deficiencies. We compared different types of neuronal populations with the same OXPHOS deficiency to determine their susceptibility to the defect and the impact of this susceptibility to neurodegenerative mechanisms.
We generated a mouse model with a temporally controlled induction of mitochondria-targeted

restriction enzyme, PstI or mito-PstI. Mito-PstI is transcriptionally activated by the absence of doxcycline using the "Tet-Off" system. The tetracycline transactivator (tTA) gene targeted to cortical, hippocampal, and striatal neurons under the CamKIIα promoter mediates the spatial regulation of mito-PstI. Mito-PstI expression causes double strand breakage of the mitochondrial DNA (mtDNA) creating an OXPHOS-deficiency.
Animals expressing mito-PstI over the course of their lifespan had no obvious phenotype until age 4 months. At age 4 months and on, they started to show behavioral impairments in motor coordination, abnormal limb grasping, and memory loss indicative of neurodegeneration. These animals showed neuronal mitochondrial dysfunction after 2 months-of-age up until 6 months-of-age when measuring enzymatic activities of COX and citric acid cycle's citrate synthase. At 6 months and on, *in vivo* imaging and histology showed that mito-PstI mice experienced massive degeneration of the striatum. Cortical atrophy was also observed, though to a lesser extent. These results showed that the striatum is particular sensitive to defects in OXPHOS. These results may help explain the neuropathological features associated with Huntington's disease, which have been associated with not only huntingtin, but also OXPHOS defects.

Disclosures: A.M. Pickrell, None; **H. Fukui**, None; **C.T. Moraes**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: AG026467-01

AG17490

Title: Deletorious effect of hyperglycemia on Munc13-1 expression in the diabetic peripheral nerve

Authors: J. JURANEK[1], T. BRANNAGAN[1], A. HAYS[1], N. REINIGER[1], L. JOHNSON[1], E. RATTIGAN[1], R. ROSARIO[1], A. SCHMIDT[1], *M. S. GEDDIS[2];
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Abstract: The aim of this project was to conduct a comparative study on the immunoexpression of Munc13-1, a diacylglycerol (DAG)/phorbol ester receptor and active zone protein in peripheral nerve of human diabetic patients and animal models with diabetes. Munc13-1

deficiency in mice inhibits neurotransmitter vesicle maturation at glutamergic excitatory synapses leading to severe neuronal dysfunction and early postnatal death. In the peripheral nervous system, Munc13-1 has been found in autonomic en passant synapses of vas deferens and in sciatic nerve fibers of healthy rodents. For the purpose of the study, different models of diabetic animals were used. Sciatic nerves from Strepotozotocin (STZ)-injected C57 B6 mice, STZ-injected laboratory swine, type 1 diabetic Ove26 mice (induced by transgenic expression in islet cells of calmodulin), and from human subjects (biopsies) with long term diabetes were collected and processed for immunofluorescence studies. Our results reveal abundant presence of Munc13-1 in control sciatic nerve, which was highly reduced in the respective diabetic nerve samples. We postulate that the observed reduction in Munc13-1 immunofluorescence likely results in reduced number of release-ready neurotransmitter vesicles aggravating already present hyperglycemia-induced changes in neuronal cells, thereby leading to impaired neurotransmission. Taken together, we conclude that the reduction of Munc13-1 likely contributes to nerve dysfunction in diabetes. Quantitative studies assessing the difference in number of immunopositive Munc13-1 fibers and reduction of immunofluorescent signal between control and diabetic nerves from all studied specimens will be examined.

Disclosures: J. Juranek, None; **T. Brannagan**, None; **N. Reiniger**, None; **L. Johnson**, None; **E. Rattigan**, None; **R. Rosario**, None; **A. Schmidt**, None; **M.S. Geddis**, None; **A. Hays**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01 NS047973

NIH R01 EY09024

NIH P01 ES016738

NIH P30 NS057096

Title: Isobaric tagging-based quantification by mass spectrometry of differentially regulated proteins in synaptosomes of hiv/gp120 transgenic mice: Implications for hiv-1_associated neurodegeneration

Authors: S. BANERJEE[1], L. LIAO[3], R. RUSSO[4], A. EROSHKIN[2], J. R. YATES, III[3], *S. A. LIPTON[2];
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Abstract: Aim: To identify neuronal markers associated with HIV-associated neurocognitive disorders (HAND) by proteomic screening of synaptosomes from HIV/gp120 transgenic mice.
Methods: Synaptosomes from cortex of 13 month-old gp120 transgenic mice were subjected to tandem mass tag (TMT2-127) labeling. Synaptosomes from age-matched, wild-type (WT) animals labeled with TMT2-126 served as a control. The labeled peptides were trypsinized overnight at 37ºC and subjected to MS/MS analysis using an LTQ-Orbitrap mass spectrometer in collision induced dissociation (CID) mode. Identified proteins were validated using western blot analysis. To elucidate putative pathways associated with the proteomic profile, we employed a bioinformatics tool (ingenuity pathway analysis (IPA)).
Results: A total of 1040 proteins were identified in both WT and gp120 transgenic mice. Several of the differentially regulated proteins were validated using western blot analysis. To understand putative pathways associated with the proteomic profile, proteins manifesting a differential change in expression of ≥1.5 fold were analyzed using the bioinformatics pathway analysis tool. This analysis revealed direct or indirect involvement of the PI3K/AKT pathway in the proteomic profile of the gp120 transgenic mice. Western blot analysis revealed a significantly lower phospho (p)AKT/AKT ratio in synaptosomes from gp120 transgenic animals compared to WT, suggesting that this neuroprotective pathway was inactivated in the gp120 transgenic brain. Using this information, we then compared western blots of pAKT/AKT in the forebrains of these mice and in human brains obtained at autopsy. We observed a significant decrease ($p < 0.05$) in the pAKT/AKT ratio in the forebrain gp120 transgenic compared to WT mice, and a similar decrease in western blots of human forebrain from HAND patients compared to controls without CNS disease ($p < 0.006$).
Conclusion: Mass spec proteomic profiling followed by pathway analysis revealed dysregulation of the PI3K/AKT pathway in gp120 transgenic mice. Western blot analysis confirmed that AKT activity was decreased in human brains with HAND, suggesting that this pathway may be associated with HAND neuropathogenesis.

Disclosures: S. Banerjee, None; **S.A. Lipton**, None; **A. Eroshkin**, None; **L. Liao**, None; **J.R. Yates**, None; **R. Russo**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Six month MRI-volumetry study in mouse model of neurodegenerative Unverricht-Lundborg (EPM1) disease

Authors: *O. H. MANNINEN[1], O. KOPRA[1], O. H. GRÖHN[2], A.-E. LEHESJOKI[1]; [1]Folkhälsan Inst. of Genet. and Neurosci. Center, Univ. of Helsinki, Helsinki, Finland; [2]Dept. of Neurobiology, A. I. Virtanen Inst. for Mol. Sci., Univ. of Eastern Finland, Kuopio, Finland

Abstract: Unverricht-Lundborg disease is an autosomal recessive disorder (EPM1, OMIM 254800), characterized by onset at the age of 6 to16 years, severe incapacitating stimulus-sensitive myoclonus and tonic-clonic epileptic seizures. Mutations in the gene encoding Cystatin B (CSTB) underlie EPM1. We carried out magnetic resonance imaging (MRI) of the brains of the *Cstb* gene -targeted mouse model of the EPM1 disease (*1*) to evaluate spatiotemporal relations of volumetric changes characteristic for this neurodegenerative disease. MRI has not been previously performed in this animal model and we propose novel findings.
MRI was done in five *Cstb* -deficient (-/-) and eight wild-type (*Cstb +/+)* mice at 1, 2, 4 and 6 months of age. Mice were anesthetized with 1 % isoflurane in 70 % N_2O / 30 % O_2, and the body temperature was maintained at 37°C during imaging. MRI was performed using a 4.7 T Varian ^{UNITY}*INOVA* MRI system with an actively-decoupled volume coil-quadrature surface coil pair. An adiabatic multi-slice (NS=17) spin echo sequence was used to acquire anatomical images for volumetric analysis with following parameters; FOV 20x20 mm^2, Matrix 256x256, slice thickness 0.7 mm, TE 20,40 and 60 ms, TR 1.8 s, NT 4. A mean image was averaged out of 3 TE:s. Regions of interest (ROIs) were drawn according to Paxinos atlas for whole brain (excluding medulla), cerebellum, cortices, cerebellum, striatum and hippocampus for each time point.
We detected a significant decrease in volume of the whole brain, cerebellum, cortices, hippocampus and striatum (Student's T-test $p<0.05$) in the *Cstb* -/- mice. We also observed that the rate of volume loss is neither spatially nor temporally uniform over the brain, as some areas are more severely affected than others (cerebellum), and some brain areas are also affected at much earlier time points than others (striatum). The volumetric results are consistent with the previous histological findings in the mouse model (*1*), as well as with the MRI findings in EPM1 patients (*2,3*). Our data directs further studies to the areas of most drastic changes during the periods of most severe volume loss. It also provides a novel longitudinal look into progression of the disease and the volumetric changes present in EPM1, facilitating a more detailed understanding of the disease progression.
References:
1. Pennacchio LA et al. 1998 *Nature Genet* 20:251-258
2. Mascalchi M et al. 2002 *Neurology* 58:1686-1689
3. Koskenkorva P et al. *Neurology* 2009 73:606-611

Disclosures: O.H. Manninen, None; **O. Kopra**, None; **A. Lehesjoki**, None; **O.H. Gröhn**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MRC Project Grant G-0700949

Title: Opa1 deficiency in a mouse model of dominant optic atrophy leads to retinal ganglion cell dendropathy

Authors: *P. A. WILLIAMS[1], J. E. MORGAN[1,2], M. VOTRUBA[1,2];
[1]Sch. of Optometry and Vision Sci., Cardiff, United Kingdom; [2]Univ. Hosp. of Wales, Cardiff Eye Unit, Cardiff, United Kingdom

Abstract: Purpose:
The heterozygous mutation, B6;C3-*Opa1*^{Q285STOP} , which models autosomal dominant optic atrophy (ADOA), results in a 50% reduction in Opa1 transcript and protein in the mouse retina and neural tissues and is associated with visual dysfunction and structural changes in the retina and optic nerve. This study aims to quantify the dendritic morphology of retinal ganglion cells (RGCs) in this model.
Methods:
Retinas of Opa1+/- mutant mice (*Opa1+/-*) (n=16) and their accompanying age and sex matched controls (*wt*) (n=11) of various age groups (<10 months, 10-15 months, >20 months) were flat-mounted and stained using the DiOlistic method (DiI, DiO) to label RGCs. Retinas were fixed with 4% PFA and imaged at 20x using a Zeiss LSM510 confocal microscope. Image stacks of RGCs were collected allowing analysis of their dendritic architecture using ImageJ. Sholl analyses were undertaken using a custom Matlab macro. A further 5 *Opa1+/-* and 5 *wt* retinas were labelled using haematoxylin and eosin or Hoechst 33258 for cell counts.
Results:
The mean dendritic field area was reduced in ON-centre RGCs of 10-15 month mice (-24.24%; C_V=0.68; $p < 0.05$) and >20 month mice (-43.22%; C_V=0.75; $p < 0.05$) compared with age matched *wt* controls. Similar changes were seen in average total dendritic length in ON-centre RGCs of 10-15 month mice (-31.66%; C_V=0.67; $p < 0.05$) and >20 month mice (-49.55%; C_V=0.63; $p < 0.05$). Sholl analysis showed a marked difference in the dendritic arborisation of ON-centre RGCs in the 10-15 month group (area under the curve (AUC) -21.67%; $p > 0.05$) and of the >20 month group (AUC -42.12%; $p < 0.05$) compared to the control group. There was no

detectable change in dendritic morphology in <10 month *Opa1*+/- mice compared to *wt* ($p > 0.05$). No significant changes ($p > 0.05$) were seen in OFF-centre RGCs. Finally, there was also no significant change ($p > 0.05$) in RGC count across all age groups.

Conclusion:

Selective dendritic pruning of ON-centre RGCs can be seen in the Opa1+/- mouse model of ADOA from as early as 10 months. These findings show, for the first time, evidence that *Opa1* mutation leads to RGC dendritic pruning and morphological changes that precede clinical visual loss and structural changes in the optic nerve in the absence of widespread RGC loss. Our data highlight the importance of normal mitochondrial fusion balance, as influenced by the OPA1 protein in maintaining the dendritic morphology of RGCs. Therapeutic interventions targeting dysfunctional RGCs may provide a means of preventing vision loss in ADOA.

Disclosures: P.A. Williams, None; **J.E. Morgan**, None; **M. Votruba**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Pug Dog Club of America

Title: Genome-wide association study of necrotizing meningoencephalitis in pug dogs using high density snp arrays

Authors: J. J. CORNEVEAUX[1], S. J. SCHATZBERG[2], A. N. ALLEN[1], J. J. PRUZIN[1], R. M. BARBER[2], *M. J. HUENTELMAN[1];
[1]Translational Genomics Res. Ins, PHOENIX, AZ; [2]Col. of Vet. Med., Univ. of Georgia, Athens, GA

Abstract: Necrotizing meningoencephalitis (NME) is an inflammatory disorder of the canine central nervous system that shares some characteristics with multiple sclerosis (MS). NME occurs in several of the small breed dogs including the Maltese and Chihuahua but is found at higher frequency in the Pug. The etiology of NME is unknown, although studies have suggested a genetic role in development of the disease, including a recent study that utilized single tandem repeat markers (STR) to identify a risk association within the dog leukocyte antigen (DLA) complex on chromosome 12. We conducted a genome-wide association study of NME utilizing the Illumina CanineHD SNP array in 98 Pug dogs (30 cases and 68 controls). Genotyping of

these samples on the CanineHD array yielded study-wide call rates greater than 95%. Of the 159,252 SNP assayed on the CanineHD array, 85,014 were considered for further analysis based on the criterion of minor allele frequencies exceeding 5%. Genome-wide association analysis was performed in PLINK. Several SNPs on chromosome 12 within the DLA class II region remained significant following Bonferroni correction (p=0.001 for 85014 SNPs). The most significant SNP in this region (rs9125534) demonstrated an odds ratio of 8.35 [95% CI: 3.80-18.36] and resides in a common 4.1 Mb haplotype also associated with increased risk for NME (p=7.96x10-7, 85.1% frequency in cases and 38.4% in controls). Based on this finding, the previous STR study which identified the same locus, and the similarities to human MS, we propose that one aspect of NME risk is likely linked to an autoimmune response in affected dogs. We found an additional region of significance on chromosome 8 spanning the genes the STYX and GNPNAT1. STYX is a proposed pseudophosphatase that has been shown to bind the calcineurin substrate, CARHSP1 GNPNAT1 is an acetyltransferase and is involved with amino sugar and glutamate metabolism. The top SNP in the chromosome 8 locus (BICF2S23516667) is found within STYX and nearly surpassed Bonferroni correction (p=1.17x10-6, uncorrected) with an odds ratio of 6.01 [95% CI: 2.83-12.75]. This single SNP was the only SNP outside of the DLA locus that remained significant after 100K Max(T) permutations. These associations provide evidence that NME risk is likely polygenic in nature and additional fine mapping in other small breed dogs susceptible to NME may help refine the risk loci. Lastly this provides further evidence of the utility of the canine model in mapping of disease risk loci and we hypothesize that the loci identified in this study may be important risk factors for MS in humans particularly for the severe nonprototypic forms that exhibit disease progression similar to NME.

Disclosures: J.J. Corneveaux, None; **S.J. Schatzberg**, None; **A.N. Allen**, None; **J.J. Pruzin**, None; **R.M. Barber**, None; **M.J. Huentelman**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS32214

NRSA T32-DK07705

Title: Aberrant proteolysis of polyglutamine-expanded androgen receptor and dynamics of aggregation in SBMA

Authors: *E. HEINE[1], C. ORR[2], D. E. MERRY[2];
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Abstract: The aggregation of mutant and misfolded proteins is a common feature of many polyglutamine repeat disorders. Evidence from models of SBMA showing that nuclear inclusions lack C-terminal androgen receptor (AR) epitopes supports the hypothesis that aberrant proteolysis of the expanded polyQ AR leads to accumulation of N-terminal AR fragments, a process central to SBMA pathogenesis. The protease and the circumstances of cleavage are currently unknown.
In order to identify the AR proteolytic cleavage site, we immunoprecipitated AR aggregates from a PC12 cell model exhibiting DHT- and polyQ length-dependent nuclear cleavage and aggregation. Dissolution of NI with organic solvents resulted in the release of a 45 kDa N-terminal AR fragment that was analyzed by mass spectrometry for C-terminal residue identification. Preliminary results suggest that the cleavage point is L163, and genetic alterations are being used to determine the role cleavage at this residue has in aggregation. These data also suggest candidate proteases that may be involved in AR cleavage and aggregation. One protease implicated in this process is the proteasome; upon pharmacological inhibition of the proteasome, both aberrant cleavage and aggregation of the mutant protein are reduced. Investigations into the ubiquitin proteasome system by more targeted means, to elucidate its involvement in the aggregation process, are underway.
To examine the aberrant cleavage and aggregation event, we created a PC12 cell line with expanded AR fused to CFP and YFP at the N- and C- termini, respectively. A proportion of cells contain AR aggregates that maintain the C-termini, leading to the hypothesis that AR initially aggregates in its full-length form and is subsequently cleaved. Live-cell imaging confirmed that the observed distinct phenotypic aggregation stages represent a continuum of aggregate formation/maturation, and inclusions mature in a defined and consistent time frame. Moreover, these studies show that full-length AR aggregates prior to cleavage. These studies redefine the role of AR proteolysis in SBMA pathogenesis and suggest the identity of the protease (the 26S proteasome) responsible for AR cleavage. Additional experiments designed to determine the role of AR cleavage in toxicity are underway. We expect for these studies to reveal a novel pathway by which the AR is processed in SBMA. Identification of the protease responsible for AR cleavage and characterization of the process of inclusion formation will not only lead us to a better understanding of the role of AR cleavage and aggregation is disease but may lead to improved strategies for therapy development in SBMA.

Disclosures: E. Heine, None; **D.E. Merry**, None; **C. Orr**, None.

Nanosymposium

529. Other Neurodegenerative Disorders I

Location: Room 10

Time: Tuesday, November 16, 2010, 8:00 am - 11:15 am

Program Number: 529.13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01 NS32214

NIH Grant R01 NS047381

Title: The 11S proteasomal activator REGγ enhances expanded AR aggregation through a non-proteasome-activating mechanism

Authors: *J. YERSAK[1], Y. LIU[2], M. RECHSTEINER[3], D. E. MERRY[2];
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Abstract: Recent studies of proteotoxicity in neurodegenerative disease have illuminated the importance of both proteasomal and autophagic degradation pathways in clearing mutant proteins from affected neurons. In SBMA, inefficient nuclear proteasomal degradation of expanded polyglutamine androgen receptor (ARQ112) is implicated in the production of a toxic amino-terminal ARQ112 fragment, which is present in nuclear inclusions. REGγ, a nuclear-restricted 11S proteasomal activator, has limited proteasome activation capabilities compared to REG cytoplasmic isoforms α and β. We sought to determine the role of REGγ in SBMA pathogenesis.

To elucidate whether REGγ impacts nuclear aggregation, we overexpressed REGγ and a mutant REGγK188E that has REGα/β proteasomal activation capability, in our inducible PC12 SBMA cell model. We show that both REGγ isoforms resulted in a significant increase in expanded AR aggregation; the similar effect of both isoforms suggests that this effect is independent of REGγ capacity to activate the 20S proteasome core. In addition, knockdown of REGγ significantly decreased AR aggregation, further implicating a role for REGγ in expanded AR nuclear aggregation.

Mutation analysis of REGγ in our SBMA PC12 cell model revealed that REGγ increases ARQ112 aggregation and toxicity independently of its 20S proteasome core binding. A role for REGγ in promoting polyubiquitylation of another protein, p53, has been recently described; whether this function mediates the effects seen here is under investigation. In support of this idea, we found that REGγ interacts with expanded ARQ112. It does not, however, interact with unexpanded ARQ10, suggesting that it does not have a role in normal AR metabolism. Overall, these results define a role for REGγ in promoting ARQ112 aggregation. The findings that REGγ binding to the proteasome core is not required for its effects, coupled with the similar effects of both REGγ and REGγK188E on aggregation, indicates that REGγ enhances expanded AR aggregation through a non-proteasome-activating (11S) mechanism.

Disclosures: J. Yersak: None. **Y. Liu:** None. **M. Rechsteiner:** None. **D.E. Merry:** None.

Nanosymposium

530. Stem Cell and Glia

Location: Room 23A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 530.1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Foundation Fighting Blindness Grant TA-NP-0507-0388-GSF

ProRetina Foundation Grant Pro/Re/KP/02

BMBF(HOPE)

RETICS Grant RD07/0062

Title: The neuroprotective effect of retinal müller glial cell-derived neurotrophic factors in retinal neurons

Authors: *P. DEL RIO[1], S. M. HAUCK[1], M. IRMLER[2], P. DE LA VILLA[4], M. GÖTZ[3], J. BECKERS[5], U. BARTSCH[6], E. VECINO[7], M. UEFFING[1,8];
[1]Protein Sci., [2]Exptl. Genet., [3]Stem Cell Res., Helmholtz Zentrum München|910007330|0, Munich, Germany; [4]Dept. of Physiol., Univ. of Alcalá, Alcalá de Henares (Madrid), Spain; [5]Ctr. of Life and Food Sci., Technise Univ. München, Weihenstephan, Germany; [6]Dept. of Ophthalmology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [7]Dept. of Biol. and Histology, Univ. of the Basque Country, Bilbao, Spain; [8]Ctr. of Ophthalmology, Univ. Med. Ctr., Tübingen, Germany

Abstract: Introduction: Neuroprotection is a promising strategy to develop therapies against neurodegenerative diseases. Therefore it is crucial to investigate the neuroprotective mechanisms through glia-neuron interactions in the nervous system. Glial-Cell-Line-Derived Neurotrophic Factor (GDNF) is a neuroprotective factor which enhances the survival of neurons. In the retina, the protection of photoreceptors (PR) is transmitted indirectly via Retinal Mueller Glial cells (RMG) (Hauck et al., 2006). We aimed at identifying GDNF-induced factors from RMG and at validating their neuroprotective potential in mouse models of PR degeneration.
Materials and methods: Three strategies for identification neuroprotective factors were followed: I) FACS-isolated RMG from hGFAPeGFP transgenic mice were treated with GDNF for 24 hours their transcriptome changes studied by microarray analysis. II) Similar experiments were performed with explanted total retinas from 20 mice. III) As a third strategy, isolated RMG were taken into short term culture and after GDNF treatment, the secretion of molecules was

monitored directly from the cell culture medium by an antibody-based array approach. From these approaches, several molecules were selected to test their functional neuroprotective effect in primary PR cultures. The positively tested factors for PR survival *in vitro* were then applied in rd1 mouse organotypic cultures as well as injected intravitreally into rd1 and rd10 mouse models of retinal degeneration to analyze the effect of these factors for the maintenance of PR function *in vivo*.
Results: GDNF treatment induces distinct changes of RMG transcriptome and proteome. Combining those transcripts with the secreted proteins directly detected on culture medium with the candidate array approach, we accumulated 35 proteins increasedly secreted from RMG after GDNF stimulation. Osteopontin (OPN), a well known secreted glycoprotein, was one of the candidates expressed in RMG and upregulated after GDNF induction, both on the transcript level as well as on the protein level in lysates and supernatants of primary mouse RMGs. OPN and as well as additional candidate factors were confirmed to promote PR survival *in vitro* trough the activation of PI3-K/Akt survival pathway. In organotypic retinal cultures from rd1 mice, OPN significantly increased survival of PR.
Conclusions: Among the GDNF-induced molecules secreted from RMG, we have discovered novel candidate factors for neuroprotection. The survival effect of these factors in animal models for inherited retinal degeneration, encourages further studies on their therapeutic properties towards future clinical application.

Disclosures: P. Del Rio, None; **S.M. Hauck**, None; **M. Irmler**, None; **P. de la Villa**, None; **M. Götz**, None; **J. Beckers**, None; **U. Bartsch**, None; **E. Vecino**, None; **M. Ueffing**, None.

Nanosymposium

530. Stem Cell and Glia

Location: Room 23A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 530.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Fondazione Neurothon Onlus

Fondazione Cellule Staminali di Terni

Associazione pro-Roberto

Fondazione Borgonovo Onlus

Title: Establishment of a human neural stem cell factory for clinical stem cell-mediated therapy

of neurodegenerative diseases

Authors: *A. L. VESCOVI[1,2], L. DE FILIPPIS[1], D. FERRARI[1], L. ROTA NODARI[1], D. PROFICO[2], M. PROJETTI PENSI[2], G. MUZI[2], M. GELATI[2];
[1]Univ. of Milan Bicocca, Milan, Italy; [2]Azienda Ospedaliera di Terni, Terni, Italy

Abstract: Cell therapy in the CNS is reaching the stage of clinical application, with the first few clinical trials already underway in some post-traumatic, post-ischemic or neurodegenerative disorders. A critical element in this endeavor is represented by the cells to be used in neural trasplantation. A continuous and standardized, clinical grade source of normal human CNS cells (hNSCs), combining the plasticity of fetal tissue with extensive proliferative capacity and functional stability would be of paramount importance in this field. Here, we describe the establishment of continuous stem cell lines from the fetal human CNS, with emphasis on cells from spontaneous miscarriages, which provide a plentiful and consistent source of non-transformed human neural cells. These hNSCs are now generated in a good manufacturing practice (GMP) cell factory and will be compatible with the development of standardized clinical trials in neurodegenerative disorders. Our hNSCs have now been serially expanded under chemically defined conditions, resulting in a 107-108 fold increase in cell number, and are being cryopreserved, establishing a GMP-grade, hNSCs bank. In order to certify these cells by the GMP standard, a panel of cellular, functional and biochemical criteria must be met prior to cell release, which include but are not limited to, karyotype analysis, stable differentiation and growth capacity, lack of biological contamination by adventitious agents. We will present the results of this process of certification for a sample of these GMP-grade hNSCs lines, together with some evidence of their efficacy and immunogenic tolerance upon transplantation into animal model of neurological disorders, in particular into the adult ischemic rat brain.. While a request for using these cells in a phase I clinical trial in ALS patients has been filed with the Italian NIH (Istituto Superiore di Sanità), in a second phase, it is our goal to make GMP-grade hNSCs accessible to investigators worldwide, whom have plans to develop similar trials but find an insurmountable obstacle in gaining access to clinical grade hNSC. The distribution model adopted by our hNSCs bank will be strictly non-profit, since this initiative has been funded by the non-profit organizations Neurothon Onlus and Fondazione Cellule Staminali of Italy. We feel that this initiative may significantly expand the breadth of cell therapy clinical trials in neurological disorders.

Disclosures: A.L. Vescovi, None; **L. De Filippis**, None; **D. Ferrari**, None; **L. Rota Nodari**, None; **M. Gelati**, None; **M. Projetti Pensi**, None; **G. Muzi**, None; **D. Profico**, None.

Nanosymposium

530. Stem Cell and Glia

Location: Room 23A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 530.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Glaucoma research foundation

Title: Infiltrating monocyte-derived macrophages support survival of retinal ganglion cells and enhance progenitor cell renewal in the adult retina following an insult

Authors: *A. LONDON, E. ITSKOVICH, I. BENHAR, M. SCHWARTZ;
The Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Injury to the inner neural retina results in the degeneration of retinal ganglion cells (RGC) which are part of the central nervous system (CNS). Similar to other CNS tissues, regeneration, cell renewal and healing capacities in the inner retina are limited. In this study we wished to test whether infiltrating monocyte-derived macrophages affect the survival of RGCs and are involved in progenitor cell renewal following retinal insult in a model of glutamate intoxication. Proliferation of retinal progenitor cells (RPC), survival of RGCs and the recruitment of distinct myeloid populations were analyzed by immunohistochemistry and flow-cytometry. Here we demonstrate that glutamate intoxication stimulated proliferation of $Pax6^+$ RPCs in the adult ciliary body and changed the relative contribution of distinct myeloid populations in the retina. Specifically, retinal insult resulted in the infiltration of CX_3CR1^+ blood-borne monocyte-derived macrophages to the damaged retinal ganglion cell layer. Augmentation of the monocytic population increased survival of RGCs and RPC proliferation. Accordingly, RGC survival and RPC renewal were reduced by ablation of monocytes in the peripheral blood. Moreover, these monocyte-derived macrophages determined the anti-inflammatory and neuroprotective retinal environment following the insult. Thus, we evidently attributed monocyte-derived macrophages an essential immunoregulatory neuroprotective role in both RGC survival and progenitor cell renewal. Boosting the recruitment of these cells at the right time is an appealing potential therapeutic target.

Disclosures: A. London, None; **E. Itskovich**, None; **I. Benhar**, None; **M. Schwartz**, None.

Nanosymposium

530. Stem Cell and Glia

Location: Room 23A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 530.4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Neurothon Onlus Foundation

Stemgen S.p.a.

Italian Ministery of Health

AIRC Association

Title: Differential tropism of immortalized and non-immortalized human neural stem cell lines in a focal demyelination model

Authors: *L. DE FILIPPIS[1,2], D. FERRARI[1], C. ZALFA[1], L. ROTA NODARI[1], M. GELATI[1], A. L. VESCOVI[1,2];
[1]Univ. Bicocca Milano, Milan, Italy; [2]Stemgen S.p.a., University Milano Bicocca, Italy

Abstract: Human neural stem cells (hNSC) represent an optimal tool for the therapy of neurodegenerative diseases, since their ability to differentiate into neurons, astrocytes and oligodendrocytes. In experimental settings the slow proliferation rate of hNSC represent a limit that can be overcome by the use of immortalized hNSC lines, such as v-myc (v-IhNSC) or c-myc T58A (T-IhNSC) transduced hNSC. We previously showed that, compared with hNSC and v-IhNSC, T-IhNSC rise high percentages of oligodendrocytes soon after removal of mitogens and are prone to a precocious differentiation. Given the differential in vitro oligodendrogenic potential we analyzed the progeny of hNSC, T-IhNSC and v-IhNSC in an animal model of focal demyelination induced by LPC, to verify if local environmental cues inducing endogenous remyelination could address their integration and differentiation. The three lines were unilaterally transplanted in the subventricular zone (SVZ) of adult Sprague Dawley rats at 5 days from the lesion (subacute phase) induced by injection of lysolecithine. By immunohistochemical analysis and confocal microscopy we showed that non immortalized hNSC as well as immortalized T-IhNSC and v-IhNSC were able to survive with a differential rate along the antero-posterior or medio-lateral axis and to migrate along the ventricular wall. In particular, after 15 days from transplantation, hNSC and T-IhNSC were able to reach the striatum and the corpus callosum differentiating into O4+ and MBP+ oligodendrocytes, with T-IhNSC colonizing the lesioned area, whereas v-IhNSC remained mainly confined in the SVZ. The three hNSC lines displayed differential survival rates and migration patterns, possibly depending on their intrinsic proliferative potential and differentiation ability. To note, a significant reduction of Iba1+ microglia activation together with a shift of the morphology of Iba1+ cells from the amoeboid macrophagic to the stellate resident phenotype was also observed in transplanted animals with respect to controls. This finding suggests an immunomodulatory effect of hNSC on the acute inflammatory reaction. These results support T-IhNSC line as a reliable cell model to study therapeutic applications of hNSC for demyelination disorders and show a differential tropism in vivo of hNSC depending on their intrinsic proliferation potential.

Disclosures: L. De Filippis, None; **D. Ferrari**, None; **L. Rota Nodari**, None; **C. Zalfa**, None; **A.L. Vescovi**, None; **M. Gelati**, None.

Nanosymposium

530. Stem Cell and Glia

Location: Room 23A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 530.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01NS/HL036124

Title: Systemic treatment with secreted factors from undifferentiated rat umbilical cord matrix stem cells reduces CA1 neuron death after cardiac arrest

Authors: *Y. XU, N. R. BRANDON;
Anesthesiol., Univ. Pittsburgh Sch. Med., Pittsburgh, PA

Abstract: Introduction: Cardiac arrest causes severe damage to the CA1 region of the hippocampus. Previous results in our laboratory show that intercranial transplantation of undifferentiated rat umbilical cord matrix (RUCM) stem cells significantly improves survival and recovery of the CA1 neurons. Transplantation in one hemisphere led to protection in both hemispheres, suggesting protection through an extracellular signaling mechanism.
Methods: To determine whether such extracellular signaling process plays a significant role in global ischemia, undifferentiated RUCM stem cells were cultured in 75-cm2 flasks using 7 mL of defined media. Media from confluent cells was concentrated approximately 3x via evaporation in a speed-vac. One 75-cm2 flask of confluent RUCM or fibroblast cells was collected and resuspended in 1 mL of fresh media for cell lysate treatments. The cells were lysed via sonication, and cellular debris was centrifuged at 14k for 5min. All treatments were stored at -20°C until use. 12 male Sprague-Dawley rats (150-250 grams) underwent 12-min cardiac arrest as described previously. Rats were resuscitated with rapid infusion (< 1 min) of 2-2.5 cc of their own oxygenated arterial blood mixed with epinephrine, sodium bicarbonate, and heparin. Over the next 30-60 minutes, 600-900 μL of one of three treatments were slowly infused retrograde into abdominothoracic aorta: concentrated RUCM stem cell conditioned media, RUCM stem cell lysate, or fibroblast cell lysate. After 10 days of recovery, the rats were sacrificed, and their brains collected for histological study.
Results: The rats who received conditioned media had 50% more healthy neurons in their CA1 regions compared to the other two groups (who were not statistically different). 54% ± 10% survival for the RUC media group, compared to 36%±11% for the RUCM lysate group and 36± 12% for the fibroblast group.
Conclusions: Secreted molecules from the rat umbilical cord matrix stem cells are

neuroprotective against global ischemic injuries. Our results also show that systemic treatment with stem cell factors can be potentially used for treating cardiac arrest without the need for transcranial stem cell transplantation.

Disclosures: Y. Xu: Employment; University of Pittsburgh School of Medicine. Research Grant; NIH. **N.R. Brandon:** Employment; University of Pittsburgh School of Medicine.

Nanosymposium

530. Stem Cell and Glia

Location: Room 23A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 530.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: VR K2010-54X-14185-09-3

Title: Detrimental effects of LPS on dividing stem cells in the newborn brain

Authors: A. S. NAYLOR[1], K. JÄRLESTEDT[1], J. DEAN[1], H. HAGBERG[2,3], *C. MALLARD[4]; [1]Dept Neurosci. and Physiol., [2]Dept Obstetrics and Gynecology, Sahlgrenska Academy, Univ. of Gothenburg, Gothenburg, Sweden; [3]Inst. of Reproductive and Developmental Biol., Imperial Col., London, United Kingdom; [4]Dept Neurosci. and Physiology, Sahlgrenska Academy, Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Inflammation has been identified as an independent risk factor for perinatal brain injury. Experimental studies show that inflammation reduces the regenerative capacity in the adult brain. However, the effect of inflammation on cell proliferation in the newborn brain is unclear. In this study we examined if an early life inflammatory challenge alters cell proliferation and survival.
To investigate the effect of LPS on hippocampal cell survival in the young postnatal mouse, pups (n = 10 in each group) were injected with a single dose of LPS (1mg/kg) or saline i.p. at P9. Cells undergoing division were labelled with BrdU (50mg/kg/injection) at either P8 (24h prior to LPS injection), to investigate the effect of LPS on cells undergoing division prior to inflammation, or at P11 (48h post LPS injection) to investigate cells undergoing cell division post injection of LPS.
There was no effect on the survival of total number of cells that were born before the LPS injection or on the total number of newborn neurons and astrocytes. However, there was a decrease in the total number of cells born during the inflammatory period after LPS injection, with concomitant decreases in the total number of newborn neurons and astrocytes after 30 days.

There was no effect on progenitor proliferation during the inflammatory period in the dentate gyrus. We also found no long-term effects on the number of immature neurons and proliferating cells 30 days after inflammation.
Together, these results demonstrate that inflammation has strong effects on the survival of cells born during an inflammatory response but no effect on the survival of cells born prior to inflammation. These data highlight that inflammation has differential effects on survival and proliferation in the immature hippocampus.

Disclosures: A.S. Naylor, None; **C. Mallard**, None; **K. Järlestedt**, None; **J. Dean**, None; **H. Hagberg**, None.

Nanosymposium

530. Stem Cell and Glia

Location: Room 23A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 530.7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS NS056422-01A1

NINDS NS064191-01A1

NINDS NS062180

NINDS NS042269-05A2

Title: Direct and astrocyte-mediated toxicity of organophosphates for motor neurons: a potential in vitro model for sporadic ALS

Authors: M. PRISSETTE[1], D. B. RE[1], D. OAKLEY[1], S. PRZEDBORSKI[1], *C. E. HENDERSON[2];
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Abstract: Sporadic cases of amyotrophic lateral sclerosis (ALS) are hypothesized to result from the interaction of environmental factors with elements of intrinsic genetic susceptibility. Organophosphate (OP) exposure is implicated through epidemiological studies showing an increased risk of sALS in Gulf War veterans and farm workers. Moreover, genetic studies suggest that polymorphisms in the genes encoding paraoxonases - the enzymes which detoxify organophosphates - may confer risk for sALS on specific populations. We are studying potential

mechanisms of toxicity using two cell types known to play an important role in ALS pathogenesis: motor neurons and astrocytes. First, using cultures of primary and ES cell-derived motor neurons, we tested the direct toxicity of two different OPs (chlorpyrifos, paraoxon). Using 10 μM OP, a concentration similar to that in brains of patients with acute intoxication, 40% of motor neurons died within 6 days, whereas other spinal neurons, cortical neurons and non-neuronal cells were strikingly resistant. To determine whether astrocytes can mediate indirect toxicity for motor neurons, dialyzed conditioned media from astrocytes cultures exposed to OPs for 3 to 7 days were assayed for their effects on motor neuron survival. Media from OP-treated astrocytes were highly toxic, whereas OP-treated fibroblasts produced no toxic factors. We are using microarray profiling of OP-treated astrocytes to identify candidate toxic factors. Overall, direct and indirect mechanisms of organophosphate toxicity provide *in vitro* models of one potential trigger of the ALS disease process.

Disclosures: M. Prissette, None; **D.B. Re**, None; **S. Przedborski**, None; **C.E. Henderson**, None; **D. Oakley**, None.

Nanosymposium

530. Stem Cell and Glia

Location: Room 23A

Time: Tuesday, November 16, 2010, 8:00 am - 10:00 am

Program Number: 530.8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NARSAD Young Investigator Award

NIH Grant MH38752

Title: Glycogen synthase kinase-3 regulates inflammatory tolerance in astrocytes

Authors: *E. BEUREL, R. S. JOPE;
Psychiatry & Behavioral Neurobiol, UAB, BIRMINGHAM, AL

Abstract: Inflammatory tolerance is the down-regulation of inflammation upon repeated stimuli, which is well-established to occur in peripheral immune cells. However, less is known about inflammatory tolerance in the brain although it may provide an important protective mechanism from detrimental consequences of prolonged inflammation, which appears to occur in many psychiatric and neurodegenerative conditions. Focusing on lipopolysaccharide (LPS)-tolerance in interleukin-6 (IL-6) production, we found that microglia exhibited a strong tolerance response that matched that of macrophages, whereas astrocytes exhibited only partial tolerance. The

astrocyte semi-tolerance was found to be regulated glycogen synthase kinase-3 (GSK3). GSK3 inhibitors or knocking down GSK3 levels promoted LPS-tolerance and astrocytes expressing constitutively active GSK3 did not develop LPS-tolerance. These findings identify the critical role of GSK3 in counteracting IL-6 inflammatory tolerance in cells of the CNS, supporting the therapeutic potential of GSK3 inhibitors to reduce neuroinflammation in part by promoting tolerance.

Disclosures: E. Beurel, None; **R.S. Jope**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.1

Topic: D.04. Vision

Support: Academy of Finland Grant 124698

Title: Decorrelation between input and tuning explains multiple physiological findings in visual cortex

Authors: *S. VANNI;
Low temperature laboratory, Aalto Univ. Sch. of Sci. and Technol., Espoo, Finland

Abstract: Contextual modulation has been linked to efficient coding and efficient information transmission in monkey and cat primary visual cortex [1, 2]). We have recently shown that a simple decorrelation model [3] can explain robust contextual modulation in human functional magnetic resonance imaging (fMRI) data, which suggests that overlapping mean activation in the cortex is actively decorrelated [4]. Here, I explore the model further and simulate responses of a set of arbitrary units, as well as simultaneous changes in tuning functions of the units. This model decorrelates the input and tuning by the same simple subtractive method earlier applied to fMRI data: Response = Input - decorrelation coefficient x OriginalTuning; Tuning = OriginalTuning - decorrelation coefficient x Input. As in our earlier work, model assumes that the decorrelation coefficient, a scalar number, is a monotonous function of correlation between Input and OriginalTuning, i.e. it depends only on input strength. Such coefficient can fully decorrelate any two vectors [4]. In addition to decorrelation, the model assumes a decay in Response as a function of distance from the centre of tuning function. In simulations, this model can explain area summation function [5], far surround facilitation [6] and shifts in tuning function with changes in contextual [1] or background [7] stimulation. Compared to our recent

work[4], this model steps closer to physiological explanation, because it suggest that the only independent parameter determining modulation strength is input strength. This is close to established physiological models explaining contextual modulation [8, 9]. The model is very general, because it is not linked to any particular input or level of hierarchy. Instead, it can operate with any tuning space.

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Disclosures:

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.2

Topic: D.04. Vision

Support: Singapore MOE R-263-000-355-112

NIH NEI EY019965

Title: Natural movies evoke responses in primary visual cortex that exhibit lower variability than drifting gratings

Authors: *S.-C. YEN[1], R. HERIKSTAD[1], J. BAKER[2], J.-P. LACHAUX[3], C. M. GRAY[2]; [1]Natl. Univ. Singapore, Singapore, Singapore; [2]Montana State Univ., Bozeman, MT; [3]U 280, Inserm, Lyon, France

Abstract: Neuronal responses in primary visual cortex have been found to be highly variable. This has led to the widespread belief that neuronal responses have to be averaged over large

numbers of neurons in order to obtain suitably invariant responses that can be used to reliably encode or represent external stimuli. However, some have argued that while most of these studies have used simple, artificial stimuli that contain stationary stimulus properties, the nervous system is perhaps best suited to respond to dynamic stimuli. To investigate this question, we recorded the responses of primary visual cortical neurons in the anesthetized cat evoked with time-varying natural movies, as well as drifting gratings. By comparing the neuronal responses to a time-varying Poisson process with a relative refractory period, we found that cortical neurons on the whole exhibited a high degree of spike-count variability, but a surprisingly low degree of spike-time variability. The spike-time variability was further reduced when subsequent spikes in a burst were removed. We also found that responses exhibiting low spike-time variability also exhibited lower spike-count variability, suggesting that rate coding and temporal coding might not be as incompatible as previously believed. In addition, we found the spike-time variability to be significantly lower when stimulated by natural movies as compared to stimulation using drifting gratings. Our results suggest that response variability in primary visual cortex might exhibit stimulus specificity, and might be much lower than previous measurements have indicated.

Disclosures: S. Yen, None; **R. Herikstad**, None; **J. Baker**, None; **C.M. Gray**, None; **J. Lachaux**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.3

Topic: D.04. Vision

Support: NSF 0713206

NIH F32 EY017770

NIH R90 DA023428

Title: Connecting scene statistics to probabilistic population codes and tuning properties of V1 neurons

Authors: B. POOLE[1], I. LENZ[1], G. LINDSAY[2], J. M. SAMONDS[1], *T. LEE[3];
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Abstract: Populations of V1 neurons recorded from three macaque monkeys were analyzed to evaluate the relationship between the distribution of the disparity tuning properties and the scene statistics of depth distribution relative to fixation depth. We found that there are more neurons preferring disparities with a higher probability of occurrence in the natural environment, and fewer neurons preferring disparities with a lower probability of occurrence. The tuning curves of neurons selective to the more probable disparities tend to be sharper than those for less probable disparities. Both the distribution of preferred disparities and the distribution of relative scene depth can be fit with similar Laplacian distributions, but with an asymmetry favoring near disparities. This finding is consistent with the hypothesis that neurons are performing optimal sampling of the natural environment based on the information maximization principle. These tuning properties manifest in a probabilistic population code at the V1 level to explicitly represent the statistical priors of natural scenes for depth inference.

Disclosures: B. Poole, None; **I. Lenz**, None; **G. Lindsay**, None; **J.M. Samonds**, None; **T. Lee**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.4

Topic: D.04. Vision

Support: EPSRC COLAMN project. Ref. EP/C010841/1

Howard Hughes Medical Institute

HPC-EUROPA Ref. RII3-CT-2003-506079

Title: Neural adaptation reduces energy cost while preserving coding accuracy

Authors: *J. M. CORTES[1], D. MARINAZZO[2], P. SERIES[3], M. W. ORAM[4], T. J. SEJNOWSKI[5], M. C. W. VAN ROSSUM[3];
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Abstract: Neurons in the primary visual cortex initially respond vigorously when a preferred stimulus is presented, but typically adapt as stimulation continues. The functional consequences of adaptation are unclear. Typically a reduction of firing reduces single neuron accuracy, but it has been suggested that, on the population level, adaptation increases coding accuracy. This question requires careful analysis as adaptation affects coding not only through the firing rates of neurons, but also through the neural variability and correlations between neurons. We calculate the coding accuracy using a computational model that implements two forms of adaptation: spike frequency adaptation and synaptic adaptation in the form of short-term synaptic plasticity. We find that the net effect of adaptation is subtle and heterogeneous and can both increase and decrease coding accuracy depending on adaptation mechanism and test stimulus. Yet, for all cases the contribution due to adaptation of the firing rates is almost completely cancelled by changes in the noise correlation. Thus, adaptation reduces firing rates and energy expenditure, while maintaining coding accuracy.

Disclosures: J.M. Cortes, None; **D. Marinazzo**, None; **P. Series**, None; **M.W. Oram**, None; **T.J. Sejnowski**, None; **M.C.W. Van Rossum**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.5

Topic: D.04. Vision

Support: NIH NEI Grant F32 EY017770

NSF CISE Grant 0713206

Title: V1 interactions reduce local uncertainty about binocular disparity over time

Authors: *J. M. SAMONDS[1], B. POOLE[2], T. LEE[3];
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Abstract: Recent studies have proposed that binocular disparity-dependent interactions among V1 neurons reduce stereo mismatches and sharpen disparity tuning over time. The temporal

sharpening of disparity tuning suggests that these interactions are reducing uncertainty about stereo matching. We tested for this possibility by measuring the Fisher information that the mean firing rate provides about disparity over time during the presentation of dynamic random dot stereograms. We found that Fisher information gradually increases shortly after response onset. Information increases mostly near the peak and at the steepest slope of the tuning curve. When we introduced noise to stimuli and increased stereo ambiguity, and therefore increased uncertainty about stereo matching, we found that disparity tuning developed at a slower rate than our original dynamic random dot stereograms. However, the sharpening and increase in Fisher information was more dramatic for these new conditions. These findings suggest that the neuronal interactions were playing a more prominent role during more ambiguous stereo computations.

Disclosures: J.M. Samonds, None; **B. Poole**, None; **T. Lee**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.6

Topic: D.04. Vision

Support: BBSRC Grant BB/G005044/1

Title: Spatiotemporal predictions along the apparent motion trace in V1 relate to feedback from V5 (shown by TMS) and update quickly after saccades

Authors: *L. F. MUCKLI[1,2,3], G. EDWARDS[3], A. ALINK[4], P. VETTER[2,3];
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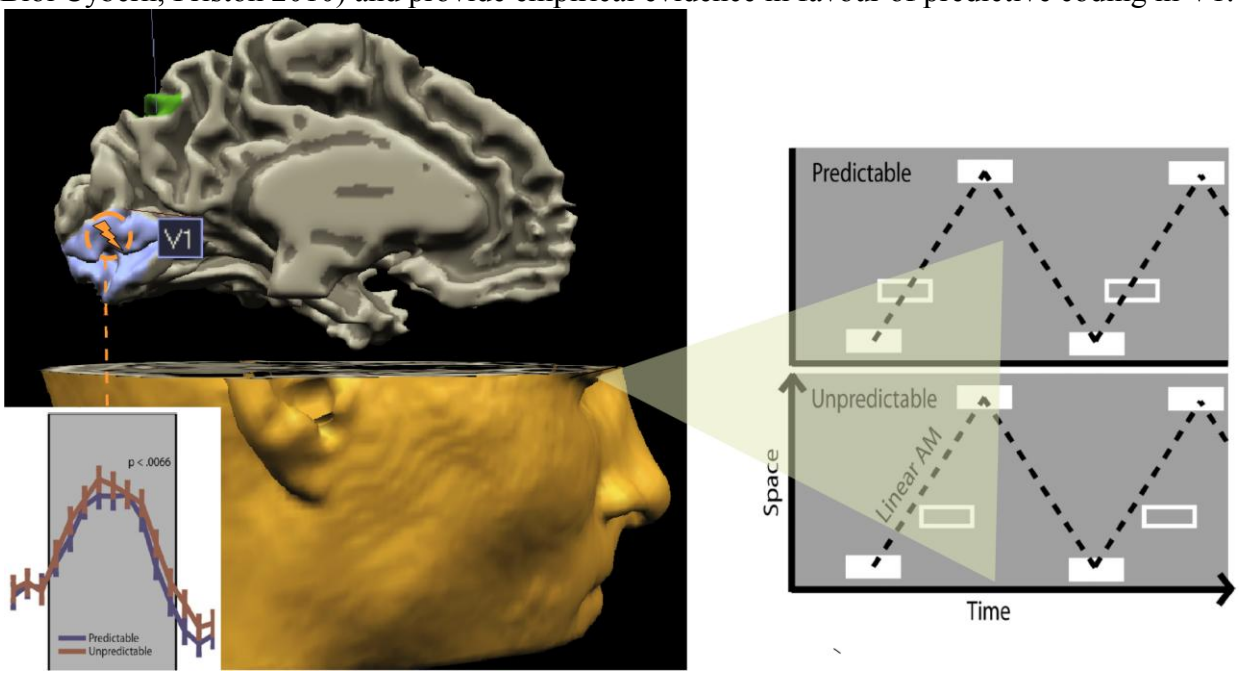
Abstract: One of the core functions of the visual cortical system is to predict upcoming visual stimulation based on regularities from our environment (Friston 2010, NRN). In a recent study, we showed spatial-temporal predictions along the apparent motion path in V1 (Alink et al. 2010, JNS). While activity was reduced for predicted stimuli in V1 detection rates were found to increase (Schwiedrzik et al., 2007, VisRes). We propose that this effect of predictability is mediated by feedback from V5/hMT+. Moreover, we propose that predictability is quickly updated at new retinal positions after saccades. To advance our knowledge of this predictability effect we carried out a TMS experiment. TMS pulses were applied to hMT/V5+ while testing

stimulus predictability along the apparent motion trace. Stimulus predictability was tested by presenting a test stimulus either in time with the apparent motion illusion or temporally slightly displaced out of time. In a second experiment, we tested the predictability effects relative to the execution of eye-movements. Application of TMS pulses 50 to 10 ms prior to the target presentation interfered with the prediction effect. Moreover we found that within the first 200 ms after saccade execution, the prediction effect is updated to the new retinal position. These findings are consistent with the framework of predictive coding (Hawkins 2004, Mumford 1992 Biol Cybern, Friston 2010) and provide empirical evidence in favour of predictive coding in V1.



Disclosures: L.F. Muckli, None; **G. Edwards**, None; **A. Alink**, None; **P. Vetter**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.7

Topic: D.04. Vision

Support: NASA Graduate Student Researchers Program

Title: The dynamic influence of rhythms on early visual cortex during task engagement

Authors: *K. J. MILLER[1], C. HONEY[2], D. HERMES[3], S. MAKEIG[4], M. SHARMA[5], J. G. OJEMANN[6], E. C. LEUTHARDT[5];
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Abstract: We investigated the influence of rhythms on local cortical processing in early visual areas during a visual search task in 6 human subjects. A rectilinear arrangement of colored boxes was shown, with a star in one of the boxes. The subjects were cued with an arrow, and indicated the color of the box adjacent to the star, in the direction of the arrow. We found that theta (4-8 Hz), alpha (8-12 Hz), and low beta (12-20 Hz) dynamically modulated cortical activity during the active active portions of the task, when compared with interstimulus intervals. Furthermore, this modulation was selective for the direction of the arrow in the search task. During active searching, local activity (as revealed by broadband, power-law, changes), was suppressed in some visual regions, and dramatically augmented in others (one such example is seen in the figure below). These findings indicate that a function of rhythms in visual processing is to dynamically suppress and release different cortical areas.
$$graphic_{27A58520-2A45-4D01-94B3-B217FD1A1F10}$$
(A) Visual cortex ECoG. (B) Task. (C) Extracted broadband change reveals local cortical processing during the task (stimuli - yellow bars). (D) The yellow and black electrodes are the measurement sites for the top (yellow) and bottom (black) series of panels in E to I. (E) Dynamic spectra, event-related potentials, and extracted broadband power for the two electrodes. (F) Broadband power as a function of low frequency phase and frequency reveals the full interaction. (G) Broadband power during the different task conditions in units of z-score with respect to the ISI. The yellow electrode shows a broadband suppression, during the task, while the red electrode shows a categorically specific broadband increase. (H) The amplitude of the 12-20 Hz rhythms during different task conditions. (I) Modulation of the broadband power by phase of the 12-20 Hz rhythm during different task conditions.

Disclosures: K.J. Miller, None; **C. Honey**, None; **D. Hermes**, None; **S. Makeig**, None; **M. Sharma**, None; **J.G. Ojemann**, None; **E.C. Leuthardt**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.8

Topic: D.04. Vision

Support: The Gatsby Charitable Foundation

UK Cognitive Science Foresight Grant #GR/E002536/01

Tsinghua University 985 fund.

Title: V1 neuron properties from visual search reaction times: Tuning to single features and conjunctions thereof

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Abstract: A visual feature singleton is a visual item that has one or more unique features in a scene among many identical background items. For example, a red item among many green ones is a color singleton, a left tilted bar among many right tilted ones is an orientation singleton, while a red and left tilted bar among many green and right tilted bars is a color-orientation double feature singleton. A feature singleton attracts attention automatically or in a bottom-up manner. The strength of this attentional attraction by a visual location is termed the saliency of this location. According to a computational theory (Li 1999, 2002) supported by substantial experimental evidence (e.g., Zhaoping 2008), the highest neural responses by the primary visual cortex (V1) to individual visual locations code the relative saliencies of these locations. Therefore, the saliencies of feature singletons reflect the response properties of the V1 neurons or their tuning to the various features. For example, the saliency of a color singleton indicates the response of the color tuned cells to the color singleton relative to the responses to background items, and thus reflects the strength of neural color tuning. Meanwhile, the saliency of a color-orientation singleton indicates the maximum of the responses from three types of cells to this singleton: those tuned to color, those tuned to orientation, and these tuned to color and orientation conjunctively, and thus reflects the feature tunings of these cells. We analyze the reaction times (RTs) in human visual search for feature singletons (Koene & Zhaoping 2007) to probe V1 neurons' feature tuning properties, particularly the physiologically less well-known properties of conjunctive feature tuning. Some singletons are single feature singletons in color (C), orientation (O), or motion direction (M), each having a particular feature contrast in the corresponding feature dimension against the background items. Others are color-orientation (CO), orientation-motion direction (MO), and color-motion direction (CM) double feature singletons, with the same feature contrasts in the respective feature dimensions as those of the single feature singletons. Note that conjunctive tuning should impact the enhanced saliencies of double feature singletons relative to those of the single feature singletons. Using various generative models to fit the RT data parametrically, we infer the feature tuning properties from the model fitting parameters. Our findings suggest stronger tuning to CO and MO than to CM double features, and that the conjunctive tuning to the CO feature is comparable in strength to the corresponding singleton feature tunings.

Disclosures: L. Zhe, None; **L. Zhaoping**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.9

Topic: D.04. Vision

Title: Measuring contour integration mechanisms using fMRI

Authors: *S. O. DUMOULIN[1], R. F. HESS[2], B. M. HARVEY[1], K. A. MAY[3];
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Abstract: Introduction: A crucial role of our visual system is to detect and segregate objects. Primary visual cortex (V1) extracts local, oriented edges from the visual scene. Object representations could be constructed from these local edges using mechanisms such as contour integration, which integrates information across local edges with similar properties (Field, Hayes, Hess, Vision Research, 1993). Here we measure contour integration properties in visual cortex using fMRI and a new data-analysis method that reconstructs population receptive field (pRF) properties.
Methods: We measured fMRI responses to moving bar apertures that revealed contours. The pRF was modeled by a circularly symmetric Gaussian receptive field in visual space. Convolution of the model pRF with the stimulus sequence predicts the fMRI time-series; the pRF parameters (x,y,σ) are estimated for each voxel by minimizing the sum of squared errors between the predicted and observed fMRI time-series (Dumoulin & Wandell, Neuroimage, 2008). We measured the pRF size as a function of the underlying contour orientation relative to the measurement direction. We also compared relatively straight and curved contours.
Results: PRF sizes vary as a function of the underlying contour orientation relative to the pRF measurement direction. For relatively straight contours, larger pRF sizes are seen in V1 when measuring in the direction of the contours as opposed to other directions. In V2 and V3 a similar result was obtained but for curved contours.
Conclusion: Our results indicate that contour integration mechanisms contribute to the overall pRF size and can be experimentally manipulated. Our results suggest that relatively straight contours are processed in V1 and curved contours in V2/V3.

Disclosures: S.O. Dumoulin, None; **R.F. Hess**, None; **B.M. Harvey**, None; **K.A. May**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.10

Topic: D.04. Vision

Support: NIH Grant EY16224

EYEY16371

NIGMS

1P50-GM071558

core grant EY12867

Title: Cortical feedback orchestrates synchrony and dynamics among LGN neurons

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Abstract: Most of the synapses in the lateral geniculate nucleus (LGN) originate in the visual cortex, but the role of this massive feedback pathway in LGN function is poorly understood. Previous studies have shown that the responses of neighboring LGN neurons to a drifting grating can be synchronized by the cortical feedback. Here we examined the relationship between response synchrony and dynamics in a population of LGN neurons in macaque monkeys, and the influence of the corticofugal pathway on that relationship.
We used microwire bundles to record extracellularly from tens of neurons in the LGN of the anesthetized and paralyzed monkeys and cats while the animal viewed full field stimuli modulated by pseudo-random luminance sequences derived from natural scenes, and compared the population discharge before and during inactivation of the cortical feedback by the GABAA agonist muscimol. The multiple spike trains were converted to continuous rate functions and normalized by their firing rate to eliminate biases. The data were fit by model estimations using adaptive multivariate autoregression, and the suitable models were selected using the Akaike Information Criterion. Based on the optimal model, the multiple rate functions were analyzed using parametric spectral analysis, multivariate coherence analysis, and Granger-causality analysis, to quantify the population synchrony and network dynamic among LGN neural population with or without cortical feedbacks.
Our analysis revealed that cortical feedback weakened the overall coherence in the LGN neural population, especially at lower frequencies (0 - 50 Hz), and consistently showed frequency-

specific effects of the cortical feedback on both the synchrony of LGN responses. Together with previous results, our results suggest that the cortical feedback can affect the local functional connectivity of LGN with distinct Spatio-temporal spreads. Although globally the synchrony among LGN neural ensembles is increased without cortical feedback, local neuronal circuits can show decreased synchrony with inactive cortical feedback.

Disclosures: Y. Yu, None; **Y. Xiao**, None; **M. Crumiller**, None; **E. Kaplan**, None.

Nanosymposium

531. Vision: Neural Coding

Location: Room 5B

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 531.11

Topic: D.04. Vision

Support: Graint-in-Aid for Scientific Research (C) (21500226) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Functional spike synchrony has significantly larger onset latency than anatomical spike synchrony in the cat lateral geniculate nucleus

Authors: *H. ITO[1], P. E. MALDONADO[2], C. M. GRAY[3];
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Abstract: Precisely synchronized neuronal activity has been commonly observed in the mammalian visual pathway (retina, thalamus and cortex) under a wide range of stimulus conditions. Analysis of this neural behavior has often assumed that synchronous firing is stationary and maintained throughout the period of visual stimulation. We tested this assumption by applying the method of Unitary Events Analysis to pairs of simultaneously recorded spike trains in the cat lateral geniculate nucleus (LGN) that were stimulated with stationary spots of light. To evaluate the significance of synchronous spike events, we developed and applied a non-parametric bootstrap test. The analysis showed that about half of the single unit pairs (96/195, 49%) displayed significant synchronous activity (unitary events). Some unit pairs displaying highly transient unitary events failed to show significant synchrony when analyzed with conventional cross-correlation analysis. In many unit pairs, the unitary events were optimally characterized at a bin width of 1 ms, indicating that neural synchrony has a high degree of temporal precision. Synchronous firings in some unit pairs changed their characteristics under

different stimulus context (ON/OFF or stationary spots/moving bar). We also examined the temporal modulation of synchronous firing by another novel bootstrap test and found that half of the unit pairs (46/96, 48%) displayed non-stationary changes in synchrony that could not be predicted by the modulation of firing rates. Those dynamics suggest that synchronous firings in the LGN are functional and are not originated from the anatomical transmission time difference between the retinal inputs to the two relay cells. This conclusion is supported also by another observation: the anatomical synchronies between the retinal afferent (S-potential) and the relay cell had significantly smaller onset latency than the adjacent (intra-tetrode) relay cell pairs. Furthermore, onset latencies were significantly longer in the distant (inter-tetrode, separated by 0.5mm) relay cell pairs than the adjacent relay cell pairs. Irrespective of short onset latency of the firing rates, synchrony in some of distant unit pairs showed a transient increase with a very long latency (300-500ms) after the stimulus presentation. These findings demonstrate that stimulus-evoked synchronous activity within the LGN is highly non-stationary and modulated by endogenous processes that are not tightly correlated with firing rate.

Disclosures: H. Ito: None. **P.E. Maldonado:** None. **C.M. Gray:** None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.1

Topic: D.06. Eye Movements

Support: NIH Grant R01NS057814

NIH 1R01NS063399

NIH 2P01NS044393

Chicago Community Trust

Title: Oculomotor adaptation matches natural changes in the body over time

Authors: *M. V. ALBERT[1], N. CATZ[2], P. THIER[3], K. KORDING[1];
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Abstract: Recent models have described motor adaptation as a process by which the nervous system estimates the changing properties of the body. For example, the body can change through muscle fatigue, strengthening, or damage; the expected timescales and amplitudes of these changes affect the adaptation strategy. Adaptation models generally need to make assumptions about how the body changes over time because measured motor errors are affected simultaneously by changes in the body and adaptation in the nervous system. Here we measured how the body alone, specifically the oculomotor system, actually changes over time. Lesions of the posterior cerebellar vermis abolish saccade adaptation, resulting in error fluctuations that depend only on changes in the body. We find that when we supply models of oculomotor adaptation with information about how the body actually changes they are better at describing experimental data than previous models based on assumptions. Our results indicate that adaptation in the nervous system is well matched to the way the body naturally changes over time.

Disclosures: M.V. Albert, None; **N. Catz**, None; **P. Thier**, None; **K. Kording**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.2

Topic: D.06. Eye Movements

Support: FP7-ICT 217077

DFG 2916860

Title: Eye position effects in the adaptation of reactive saccades

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Abstract: Eye movements provide us with the qualia of a distributed visual space. Saccades connect retinal images of different eye positions to a visual scene. Thus, an accurate execution of saccades with respect to their position in visual space is crucial. The saccadic amplitude is continuously adjusted by the control mechanism of saccadic adaptation. The visual error after an

eye movement is used to adapt subsequent eye movements of the same amplitude and direction. Although the corrections of the saccadic amplitude are achieved at one spatial location, according to the current literature the vector specific adaptation of reactive saccades transfers completely over spatial locations. In this case, the adaptation mechanism would be spatially fully unspecific and could, for example, be performed on the motor signal that drives the saccade. However, many parts of the saccadic system, e.g. the parietal and frontal cortices, the superior colliculus, or the cerebellum additionally encode gaze dependent spatial information. If these structures contribute to saccadic adaptation one might expect to find eye position effects in the spatial transfer of saccadic adaptation as well.
In a double step experiment the spatial transfer of gain change achieved for a saccade of a fixed vector was tested for reactive saccades. Horizontal saccades of 7 deg were examined in a set of five equally distributed starting positions both, on a horizontal and vertical orbit. In separate sessions saccades at each of the starting positions were adapted. Subsequently, the gain change was tested without feedback at all five positions on the orbit. The 7 deg saccadic amplitude was decreased by a 2 deg inward intra-saccadic target step. In this manner a gain transfer profile was determined for the central horizontal and vertical orbit.
An eye position dependent modulation of the gain change was found in all conditions. Gain changes were not constant for the different starting positions, but declined with increasing distance from the adaptation position. These results contrast with the current view of a purely retinotopic coding of reactive saccades. The spatial gain transfer profiles of saccadic adaptation reveal insights in the close interconnections of visuomotor and perceptual systems.

Disclosures: K. Havermann, None; **E. Zimmermann**, None; **P. Fattori**, None; **M. Lappe**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.3

Topic: D.06. Eye Movements

Support: T32 DC000023

T32 EB003383

Title: Doing the right thing when the right thing varies with the setting: Context-specific adaptation of sensorimotor responses

Authors: K. BEATON[1], A. WONG[1], *M. J. SHELHAMER[2];
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Abstract: Sensorimotor responses often require different modes (e.g., gains) in different settings. A form of this effect occurs when the setting itself (the context) induces the appropriate change in mode (without an initial erroneous movement being made). We study such "context-specific adaptation" (CSA) by adapting a response to one state (e.g., high gain) in one context, and to a different state (e.g., low gain) in a different context, and determining if the change in context invokes a change in response mode (adapted state).
The main goal of this study was to determine if the room in which adaptation is imposed can serve as a context. A second goal was to see if the effectiveness of CSA could be increased with an "augmented cue" (one not normally effective as a context).
Two saccade experiments were performed using the double-step paradigm for (horizontal) adaptation. First, gain-increase adaptation was presented in Room A and gain-decrease adaptation in Room B, each day for 4 days. Gain state became associated with each experiment room. For example, in one subject gain changed from 0.83±0.04 to 0.96±0.04 in A and from 0.89±0.06 to 0.82±0.05 in B (both $P<0.001$), with retention after 3 days (0.96±0.05 and 0.85±0.07). Second, gain-increase adaptation was imposed with eyes up 10° and gain-decrease adaptation with eyes down 10° - vertical eye position serving as the context. Target color was different in each context (down/blue, up/yellow) as an augmented cue. With the augmented cue, changes were +7.3% ($P=0.01$) and -12% ($P=0.001$). A control test without color yielded +3.9% (ns) and -5.4% ($P=0.008$), showing the enhanced effect due to augmentation.
CSA of the VOR was also studied using room context. Yaw VOR was adaptively increased in one room and decreased in another, for 4 days. Again, gain became associated with experiment room: for example Subject 1 increased from 0.90±0.16 to 0.92±0.11 in A (+2%), and decreased from 0.90±0.08 to 0.79±0.06 in B (-12%, $P=0.002$). As a test of augmented cues, music was present in only one room during adaptation, and post-adaptation testing was performed in a third "neutral" room with this augmented cue. The gain in this room reflected the gain state (increase/decrease) that was imposed in the presence of the augmented cue.
The results show that the room in which a sensorimotor response is adapted can serve as a context for recalling that adapted state, and that an augmented cue can help pull the adaptation out of the adapting room to a new room. This has implications for the design of effective rehabilitation programs, since such contextual adaptation might undesirably inhibit transfer of learning from a clinical setting to the outside world.

Disclosures: K. Beaton, None; **M.J. Shelhamer**, None; **A. Wong**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.4

Topic: D.06. Eye Movements

Support: Vitsakon

Title: Fixation, saccade, and pursuit ocular-motor performance of junior olympic and indiana university athletes

Authors: M. ELROD[1], S. HITZEMAN[1], K. MONROE[1], *N. L. PORT[2];
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Abstract: Little is known about the ocular-motor performance of elite athletes. The principle hypothesis of this series of experiments is that competitive athletes (Division-I Indiana University or Junior Olympic) have exceptional ocular-motor performance in comparison to age-matched controls. Two additional hypotheses are being tested: A) ocular motor performance predicts athletic performance, and B) ocular-motor performance changes with age.
Four tasks were used to test these hypotheses. Task 1 is a fixation task in which subjects fixate a small spot of light for 30 seconds on a computer with and without a whole-field moving background distractor. Task 2 is a simple saccade task where subjects saccade back-and-forth for 30 seconds between two static stimuli that are 12 deg apart on a computer monitor. Task 3 is a 10 second sinusoidal pursuit task at 4 different frequencies. Task 4 is a classical step-ramp Rashbass pursuit task with 6 different step-sizes and ramp-velocities. Each task yields multiple measures of ocular-motor control. All of our experiments were embedded in a clinical vision screening and were collected in the field using a portable laboratory consisting of a table-mounted Eyelink 2K eye tracker (SR Research), computer monitor, and 2 portable computers. Data collection is ongoing, with a current sample size of 285 Indiana University athletes, 96 Junior Olympic athletes and 32 age-matched controls.
All four tasks showed significant (ANOVA $p < 0.01$) differences with the control group and across sports for various measures of ocular-motor control including, for example, saccade frequency and pursuit gain. In addition, there were clear gender differences both within our control group and between various sports, e.g. men's soccer vs. women's soccer (post-hoc test, $p < 0.01$). For Junior Olympic athletes, there were clear age trends. For example, the frequency of saccade increased and fixation control was more accurate among older rather than younger athletes.

Disclosures: M. Elrod, None; **S. Hitzeman**, None; **K. Monroe**, None; **N.L. Port**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.5

Topic: D.06. Eye Movements

Support: CIHR

Title: What's your next move? Directional biases for sequential limb and eye movements

Authors: *C. D. COWPER-SMITH, G. E. ESKES, D. A. WESTWOOD;
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Abstract: There is considerable interest in the neural encoding of movement direction in the central nervous system, in part because of the potential applications to the development of neural prosthetic devices. Most studies in this field focus on single movements, with little attention given to the interactions that may occur between successive movements. The purpose of our study was to determine, independent of the movement effector system, if a prior movement results in a directional reaction time bias for subsequent movements. In our experiments, participants either made two consecutive eye movements or two consecutive arm movements in directions indicated by arrows presented at the point of fixation. In experiment one, only two possible target locations were shown during a trial. Here, movements were faster when offset by 90 or 180 degrees from the first movement (relative to movements back to the original target location). This pattern is consistent with an 'inhibition of return' (IOR) typically found for repeated movements made in the same direction. Unlike previous IOR studies requiring repeated movements, in experiment two we presented four possible target locations. In contrast with the results of experiment one, here we found that arm and eye movements were faster only when offset by 90 degrees from the initial movement; the well-established advantage for movements offset by 180 degrees was eliminated. Our results reveal an effect of set size (i.e. 4 vs. 2 possible movement locations) on the spatial gradient of RTs for consecutive movements. That similar results were found for eye and arm movements suggests the existence of a common motor programming principle that may be useful for computational models attempting to 'decode' neural signals for the implementation of neuroprosthetic devices.

Disclosures: C.D. Cowper-Smith, None; **G.E. Eskes**, None; **D.A. Westwood**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.6

Topic: D.06. Eye Movements

Title: Love at first feature learning: On the importance of salience in subjective informational value

Authors: *J. D. NELSON[1], N. CHENKOV[2];
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Abstract: The visual system directs the eyes to locations that are expected to be informative, via saccadic eye movements, several times a second. Both the anticipated usefulness of possible fixations and the intrinsic salience of different parts of a visual scene influence this overt visual attention. Lateral intraparietal cortex, frontal and supplementary eye fields, and superior colliculus each track the salience (or information value) of possible eye movements.
Where do people attend, and what do they perceive as informative, if one feature is more salient yet another feature is more useful? We addressed this via a probabilistic categorization task, involving classifying artificial plankton specimens, while tracking subjects' eye movements. The species of each specimen depended probabilistically on two features, for instance the eye (dark or light) and claw (dotted or not). The subjects were not told what the features were, but had to learn both the features and the environmental probabilities through their experience on the task. In each trial, a plankton specimen was chosen at random according to unstated, stationary environmental probabilities. The subject viewed the specimen, guessed and was then shown its true species. This continued for several hundred trials, until the subject achieved optimal classification performance (consistently choosing the most probable species). In the subsequent test phase both features were obscured, and the subject could reveal only a single feature to view in each trial. The subject's choice of which feature to view provided evidence as to which feature they perceived as more useful.
To explore the relationship between salience and usefulness, we made one feature objectively more useful, but the other feature more salient. In the test phase, most subjects preferentially viewed the first-learned, more-salient feature, rather than the subsequently-learned, more-informative feature. This suggests that even after apparent mastery of environmental probabilities, a feature's salience can influence its perceived informational value. An additional experiment found that if two features are learned close to simultaneously, subjects more easily perceive the objective relative informativeness of the features.
One possible explanation of these results is that visual perception may make use of an internalized tree-like classifier, in which the first-learned feature is always viewed first, even after learning. People’s perception of a feature’s informational value may depend as much on its relative primacy in an internalized classification tree as on the objective underlying environmental probabilities.

Disclosures: J.D. Nelson, None; **N. Chenkov**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.7

Topic: D.06. Eye Movements

Support: Swartz Foundation

Title: An exact statistical analysis of visual inference by a neural population amid eye movement

Authors: *E. A. MUKAMEL[1], Y. BURAK[1], M. MEISTER[1], H. SOMPOLINSKY[1,2];
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Abstract: Sensory information is generally corrupted by neural noise, but also by confounding signals. For example, research on high-acuity visual perception has focused on the limits imposed by variability of retinal spike responses. However, a second limit results from random eye movements during fixation. We study the effects of both sources of variability on Vernier hyperacuity in the estimation of a gap between two parallel bars presented simultaneously in the visual field. Introducing a simple model of neural variability and the statistics of eye movement, we exactly derive the optimal estimator of the gap and its performance and compare it with psychophysical data. A main assumption of our model is that the brain estimates both the eye position and the gap on the exclusive basis of retinal ganglion cell spike trains. We calculate the exact joint probability distribution for the eye position and the gap in our model and derive the optimal Bayesian estimator. The optimal strategy depends on one dimensionless parameter: the root mean squared displacement of the eyes between subsequent spikes in any two ganglion cells, divided by the width of a ganglion cell's receptive field. For slow eye movements, the optimal decoder uses all the spikes to estimate the position of each bar and their separation. For fast eye movements, the decoder uses only near-synchronous spikes arising from each of the bars. Such spikes provide snapshots of the visual stimulus during brief temporal windows, in which blurring due to eye movements is minimal. Nearly synchronous spikes occur naturally in a population of independent neurons described by Poisson statistics, and they could be enhanced by mechanisms that coordinate spike times among multiple neurons. The optimal estimator bounds the performance of biological neural systems on this task. We also construct simpler estimating schemes that could be implemented by neural circuits of the visual system and analyze their suboptimal performance. By incorporating temporal filtering in the process of spike generation our model explains the psychophysical phenomena of Bloch's law, relating the Vernier threshold to stimulus duration and contrast. Our work provides insight into the

fundamental limitation imposed on the visual system by fixational eye movements and suggests how neuronal circuits downstream of the retina may cope with this challenge.

Disclosures: E.A. Mukamel, None; **Y. Burak**, None; **M. Meister**, None; **H. Sompolinsky**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.8

Topic: D.06. Eye Movements

Support: NIH Grant EY018585

NIH Grant RR-00166

Title: Fastigial inactivation affects early and late saccade components

Authors: *E. BUZUNOV.[1], A. MUELLER[2], F. R. ROBINSON[2];
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Abstract: The caudal fastigial nucleus (CFN) of the cerebellum influences the horizontal component of saccades. Inactivating the CFN unilaterally makes contraversive saccades undershoot and ipsiversive saccades overshoot (Robinson et al. '93; Iwamoto & Yoshida '02; Goffart et al. '04). We determined how much each of 4 phases of a saccade contributes to these size abnormalities (phases 1-4 are the 1st & 2nd halves of eye acceleration & deceleration). We examined 6,500 saccades after inactivating the CFN unilaterally with muscimol injections 11 times in 5 monkeys.

During saccades contraversive to an inactivated CFN, the distance the eye travels decreases most in phases 1-3 when it averages 0.7X normal. For ipsiversive saccades the distance that the eye travels increases most in phase 4 when it averages 4.4X normal. The timing of post-injection size abnormalities is consistent with CFN neurons firing earlier for contra- than ipsiversive saccades. How does activity in CFNs contra- and ipsiversive to saccade direction influence eye rotation at different times? We propose that CFN activity influences saccades by increasing activity in the inhibitory burst neurons (IBNs) on each side at different times. (IBN activity inhibits contralateral abducens neurons.)

The axons of CFN neurons terminate in, among other targets, the region containing contralateral IBNs in the brainstem. (Batton et al. '77; Gonzalo-Ruiz & Leichnetz '90; Langer & Kaneko '84).

Recent evidence indicates that CFN activity influences IBNs because changes in IBN responses during saccade adaptation (Kojima et al. ’08) are consistent with the adaptation-related changes in CFN neurons (Inabe et al. ’03).
In our proposal, for a rightward saccade, the left CFN increases the activity of right IBNs. This inhibits left abducens neurons near the start of the movement, facilitating rightward eye rotation. Contraversive saccades are too small after CFN inactivation because antagonist resistance is abnormally strong. Later in the saccade, activity in the right CFN increases the off-direction activity in left IBNs, inhibiting right abducens neurons. This ends the agonist contraction at the right time. Ipsiversive saccades are too large after CFN inactivation because the agonist contraction lasts too long. We are now testing this proposal with recording experiments.

Disclosures: E. Buzunov., None; **A. Mueller**, None; **F.R. Robinson**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.9

Topic: D.06. Eye Movements

Support: NIH grant EY06717

Arizona Biomedical Research Commission

Title: Distinctive features of saccadic intrusions and microsaccades in progressive supranuclear palsy

Authors: *J. OTERO-MILLAN[1,2], A. SERRA[3,4], R. LEIGH[3], X. G. TRONCOSO[5,6], S. L. MACKNIK[6], S. MARTINEZ-CONDE[6];
[1]Barrow Neurol Inst., PHOENIX, AZ; [2]Univ. of Vigo, Vigo, Spain; [3]Veterans Affairs Med. Center, Case Western Reserve Univ., Cleveland, OH; [4]Univ. of Sassari, Sassari, Italy; [5]Caltech, Pasadena, CA; [6]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: The eyes do not stay perfectly still during visual fixation; along with ocular tremor and drifts, microsaccades and saccadic intrusions (SIs) continuously change the position of gaze. The most common type of SI, square-wave jerks (SWJs), consists of pairs of mainly horizontal saccades: the first moves the eye away from the fixation target and, after a short interval, the second brings it back towards the target. SWJs are increased in size and number in certain neurological disorders, including progressive supranuclear palsy (PSP). We developed an

objective method to automatically identify SWJs in healthy subjects and in patients with PSP so as to compare their properties. Our results confirm that SWJs are more frequent, larger and more markedly horizontal in PSP patients than in control subjects. We determined that the loss of a vertical component from fixational saccades and SWJs is the feature that best distinguishes PSP patients from healthy subjects. We also found that in PSP patients and controls, the larger the saccade the more likely to be part of a SWJ. Further, all saccades produced by PSP patients had equivalent properties whether they were part of a SWJ or not, suggesting that normal fixational saccades (microsaccades) are rare in PSP. Our findings suggest that fixational saccades and SIs are generated by the same neural circuit. We also propose that, both in PSP patients and in healthy subjects, SWJs result from a coupling mechanism that generates a second corrective saccade shortly after a large fixation saccade. Due to brainstem and/or cerebellum impairment, fixational saccades in PSP are abnormally large, and thus more likely to trigger a second corrective saccade, giving rise to SWJs.

Disclosures: J. Otero-Millan, None; **A. Serra**, None; **R. Leigh**, None; **X.G. Troncoso**, None; **S.L. Macknik**, None; **S. Martinez-Conde**, None.

Nanosymposium

532. Saccades: Mechanisms and Role in Perception

Location: Room 33C

Time: Tuesday, November 16, 2010, 8:00 am - 10:45 am

Program Number: 532.10

Topic: D.06. Eye Movements

Support: NEI Grant EY-017592

Alfred P. Sloan Foundation

Title: Activity in globus pallidus and substantia nigra pars reticulata during voluntary and externally triggered saccades

Authors: *S. SHIN[1], M. A. SOMMER[1,2];
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Abstract: Dysfunctions in voluntary actions are a major symptom of basal ganglia disorders such as Parkinson's and Huntington's diseases. Neuronal activity related to the common action of making saccades is a well known property of substantia nigra pars reticulata (SNr), and we recently found it in both the external and internal segments of the globus pallidus (GPe & GPi) as well (Shin & Sommer, 2010). In this study, we asked whether neurons in GPe, GPi and SNr

are modulated differently during voluntary and externally triggered saccadic eye movements. We used four paradigms that elicited saccades in a range of contexts from highly stimulus-driven to highly voluntary. In order of increasing "voluntariness", we used visually-guided saccades, memory guided saccades, free scanning over an array of stimuli, and free scanning over a blank screen. Neurons from GPe, GPi, and SNr were recorded from three monkeys. Quantitative analysis of activity related to saccades of the same vectors (i.e. same direction and amplitude) made in the four tasks revealed that saccade-related activity in GPe and GPi differentiates between visually guided and memory guided saccades. Out of 46 GPe and 11 GPi saccade related neurons, 21% of GPe and 25% of GPi neurons had greater modulation for the visually guided saccade task, while 24% of GPe and 25% of GPi saccade modulated neurons preferred the memory-guided saccade task. GPe and GPi neurons, however, did not differentiate between the two scanning tasks, and 50% of both GPe and GPi fired less for those tasks than for themore stimulus-driven ones. Only small fractions (5% of GPe and 0% of GPi saccade-related neurons) fired more in the scanning paradigms. SNr neurons were similar and, if anything, even more stimulus-driven. Among saccade related neurons in SNr (n=20), 80% were modulated more for visually than memory guided saccades (only 7% with opposite preference), and 13% preferred these tasks over the scanning tasks, with none showing the opposite preference. In summary, our data confirm that neurons in GPe, GPi and SNr carry different strength of saccade signals in different contexts, but modulations are stronger for more externally triggered, not more voluntary, saccades.

Disclosures: S. Shin, None; **M.A. Sommer**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.1

Topic: D.17. Voluntary movements

Support: CIHR grant# MOP84293

Title: Decoding movement intentions from preparatory activity in human parietal and premotor cortex

Authors: *J. P. GALLIVAN, A. MCLEAN, K. F. VALYEAR, C. PETTYPIECE, J. C. CULHAM;
Univ. Western Ontario, London, ON, Canada

Abstract: A significant finding in monkey neurophysiology is the ability to predict upcoming sensorimotor behaviour from neural activity in parietal and premotor cortex, regions implicated in movement planning processes. Although functional magnetic resonance imaging (fMRI) has recently localized brain areas responsible for movement execution in humans, can the intention to make a particular movement be decoded in the planning phase? Here we employed multi-voxel pattern analysis (MVPA), a technique previously applied in decoding stimulus orientation, position, or shape in early visual areas, to investigate whether planning-related activity in human parietal and premotor cortex can be used to decode movement intentions. Using a slow event-related paradigm we had participants perform actions with their right hand towards a centrally located object positioned on a platform directly in front of them. The starting position of the hand and location of the object were constant across trials. Following visual presentation of an object consisting of a small cube atop a larger cube, subjects were instructed to perform one of three movements following a delay period: 1) Use a precision grip to grasp the top part of the object 2) Use a precision grip to grasp the bottom part of the object and 3) Reach to touch the object with the knuckles (without hand preshaping). The delay period allowed us to localize several planning-related regions-of-interest within parietal and premotor cortex and to further investigate whether the patterns of activations in these areas could be classified according to the upcoming movement. Using support vector machine (SVM) classification on individual trials, we found that activity patterns in these regions could reliably classify the specific hand action the subject was to perform several seconds before movement onset. Importantly, successful decoding prior to movement onset must result from differences in intentions rather than differences in kinematics or sensory feedback. To our knowledge, these findings are the first to show that movement intentions can be accurately decoded based on human brain activity using fMRI. These results take us one step closer to reconstructing desired movements in patient populations based on measurements of brain activity evoked by intended actions.

Disclosures: J.P. Gallivan, None; **A. McLean**, None; **K.F. Valyear**, None; **C. Pettypiece**, None; **J.C. Culham**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.2

Topic: D.17. Voluntary movements

Support: PRODEX (BELSPO, Belgium)

IAP (BELSPO, Belgium)

FNRS (Belgium)

ARC (Belgium)

ESA (European Union)

Title: Forces and movements control under changed gravity: Uncertainty in internal models modulates the motor strategy

Authors: F. CREVECOEUR[1], J. MCINTYRE[2], J.-L. THONNARD[1], *P. P. LEFEVRE[1];
[1]Univ. catholique Louvain, Louvain-la-Neuve, Belgium; [2]Univ. Paris Descartes, Paris, France

Abstract: The control of movement can be viewed as the generation of muscular forces that compensate for mechanical constraints and bring the movement towards the intended goal. In this respect, the internal models of gravitational forces are crucial given that these forces are omnipresent and act on any part of the body and on the object that we manipulate. This presentation gathers recent results collected under hyper- and microgravity conditions in order to elucidate the role played by gravity in sensorimotor coordination.
We investigated arm movement control while subjects were instructed to manipulate an object towards visual targets. The movements under hypergravity (1.8 g) were faster without any tendency to recover movement timing corresponding to normal gravity condition. Faster movements, in addition to the increase in the arm and object weight, reflect an important increase in the muscular forces in response to the increase in gravity. Simulation results suggest that this strategy is compatible with a re-optimization of the motor commands. To the contrary, movement performed under microgravity (0 g) were typically slower, tended to fall short and presented with skewed profiles that are a priori incompatible with the hypothesis of a re-optimization of the motor commands. The simulations suggest that this control strategy is compatible with a reduction in the intensity of the motor commands.
Why do the motor strategies in each gravitational condition differ? We found a correspondence between each adaptive process and the performances in anticipatory grip force scaling. Under hypergravity, performance was similar to that in normal Earth gravity showing that the subjects had a good online estimate of the state of the body based on internal models. Under microgravity, the grip force was less finely tuned to the load force variation reflecting greater uncertainty. This uncertainty could affect the internal online estimate of the state of the body and alter movement stability and trial-to-trial variability. Simulations based on optimal feedback control confirm that the movement slowing observed under microgravity condition may be a stabilizing mechanism under uncertain internal models of dynamics.
In conclusion, we hypothesize that the adaptive processes depend on the reliability of the internal models of dynamics. Hyper- and normal gravity appear to be similar conditions: confidence in the internal models allowed for high control performances. Instead, microgravity is a singular condition, in which the uncertainty in internal models possibly dictates a reduction in the intensity of the motor commands.

Disclosures: F. Crevecoeur, None; **P.P. Lefevre**, None; **J. Thonnard**, None; **J. McIntyre**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.3

Topic: D.17. Voluntary movements

Support: MIUR

Fondazione del Monte di Bologna e Ravenna

EU FP6-IST-027574 MATHESIS

Title: Vision for action in the medial parieto-occipital cortex: Visual responses to graspable objects in area V6A

Authors: P. FATTORI[1], V. RAOS[2], R. BREVEGLIERI[1], A. BOSCO[1], M. CIAVARRO[1], *C. GALLETTI[1];
[1]Dept Human and Gen. Physiol., Univ. Bologna, Bologna, Italy; [2]Dept of Basic Sci., Univ. of Crete, Med. Sch., Iraklion, Crete, Greece

Abstract: Recently, we have shown that the medial posterior parietal area V6A of the macaque is involved in encoding motor-related information for grasping objects with different grips (Fattori et al., 2010 J. Neurosci). Here, we examined whether V6A encodes also object-related information and, in case it does, what is the relationship between object and grip encoding in a reach-to-grasp task.
One hundred-ninety one neurons were recorded in V6A of 2 monkeys, both trained to perform 2 tasks which involved objects of different shapes requiring different grips to be grasped: ball, handle, ring, plate, stick-in-groove. Both tasks started with the monkey fixating a LED in dark; then, one of the objects was briefly illuminated (0.5 s). In one task, after a variable delay (0.5-2s) that followed object illumination, the animal reached and grasped the object in the dark while fixating the LED. In the second task, the monkey was required only to fixate the illuminated object (1- 1.5 s) without performing any grasping movement.
In the reach-to-grasp task, the majority of V6A cells showed visual responses to object presentation (131/191, 69%, t-test, $p<0.05$), and 32% of visual neurons displayed selectivity for an object or a set of objects (42/131, ANOVA 1 way, $p<0.05$). About half of the cells (105/191, 55%) responded to both object presentation and reach-to-grasp execution; 23% of them (24/105) displayed both object and grip selectivity. Cluster analysis revelead that: 1) the selectivity of the

responses during prehension follow a motor rule, namely, the use of index finger for the execution of the grip and 2) the selectivity of the responses during object presentation display a visuomotor rule: objects bearing a hole, thus requiring the fingers to be inserted in it for their grasping, were separated from the solid ones, requiring the fingers to wrap around the object. One hundred-eight cells were tested in the task of object-fixation without subsequent grasping. More than half of them were activated by object vision (69/108, 64%). Many of these cells were selective for objects (29/69, 42%). Visual selective responses of the fixation task displayed different clustering as compared with that of the visual responses of the reaching-to-grasp task. In the former case, clustering revealed a pure visual encoding based on the physical properties of the objects (flat/round/complex), with the grips being completely irrelevant.
These data reveals that area V6A is involved in coding both the grip required for grasping an object and the physical features of objects to be grasped. We suggest that object representation in V6A is purely visual during passive vision, and visuomotor during vision for action.

Disclosures: P. Fattori, None; **C. Galletti**, None; **R. Breveglieri**, None; **A. Bosco**, None; **M. Ciavarro**, None; **V. Raos**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.4

Topic: D.17. Voluntary movements

Support: Wellcome Trust

Title: Asymmetry in interactions of single neurons and local field potentials simultaneously recorded during visuomotor grasping task

Authors: *A. KRASKOV[1], C. MEHRING[2], T. BROCHIER[3], J. STOLL[2], R. N. LEMON[1]; [1]UCL Inst. Neurol., London, United Kingdom; [2]Biol., Albert-Ludwigs-University, Freiburg, Germany; [3]UMR 6193, Inst. de Neurosciences Cognitives de la Méditerranée, Marseille, France

Abstract: The ventral premotor cortex (area F5) and hand area of primary motor cortex (M1) are key components of the 'visuomotor grasping circuit'. These two cortical areas carry out different but clearly interrelated specific functions during preparation and execution of grasp. Although recent stimulation studies have shown that there are strong interactions between the two cortical areas, little is known about how information is transmitted between them. Here we used spike-field coherence analysis and estimated phase locking of single units (SUs) to the local field

potentials (LFPs) to examine functional interactions between F5 and M1.
Simultaneous recordings from hand representations of F5 and M1 were carried out in two awake behaving macaque monkeys trained to perform a delayed response visuomotor grasping task. They were trained to observe, reach out, grasp and hold differently shaped objects. A total of 202 single units (SU) were analysed (97 F5 and 105 M1) together with 151 local field potentials (LFPs) from 73 F5 and 78 M1 sites. Simultaneous recordings of SUs and LFPs in two different areas allowed us to analyse interactions within cortical areas (390 pairs in M1 and 304 in F5) and between them (166 pairs of SU in M1 with LFPs in F5 and 132 pairs of SU in F5 with LFPs in M1)
Our analysis concentrated on the observation and stable hold intervals of the task during which oscillatory LFP activity was well developed in the beta frequency band. Coherence analysis showed significant interaction of F5 SUs with LFPs in both F5 and M1. Spike-field coherence was found to be higher during stable hold compared with observation. In comparison to F5 SUs, SUs in M1 were much less coherent with LFPs within and in between areas. F5 and M1 SUs were significantly locked to LFPs oscillations within the same area. Between areas phase locking was observed for F5 units with LFPs in MI. M1 SUs had random phase relationship with LFPs in F5.
Our results show a significant task-related asymmetry between SUs and LFPs recorded simultaneously in two different but anatomically and functionally connected cortical areas. We hypothesise that during the observation period, F5 neurons represent a grasp-specific posture that is selected on the basis of the visual properties of the object to be grasped. This is then transmitted to M1 where it generates and maintains the appropriate grasp until such time as release of the object is required or a new posture needs to be adopted.

Disclosures: A. Kraskov, None; **R.N. Lemon**, None; **C. Mehring**, None; **J. Stoll**, None; **T. Brochier**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.5

Topic: D.17. Voluntary movements

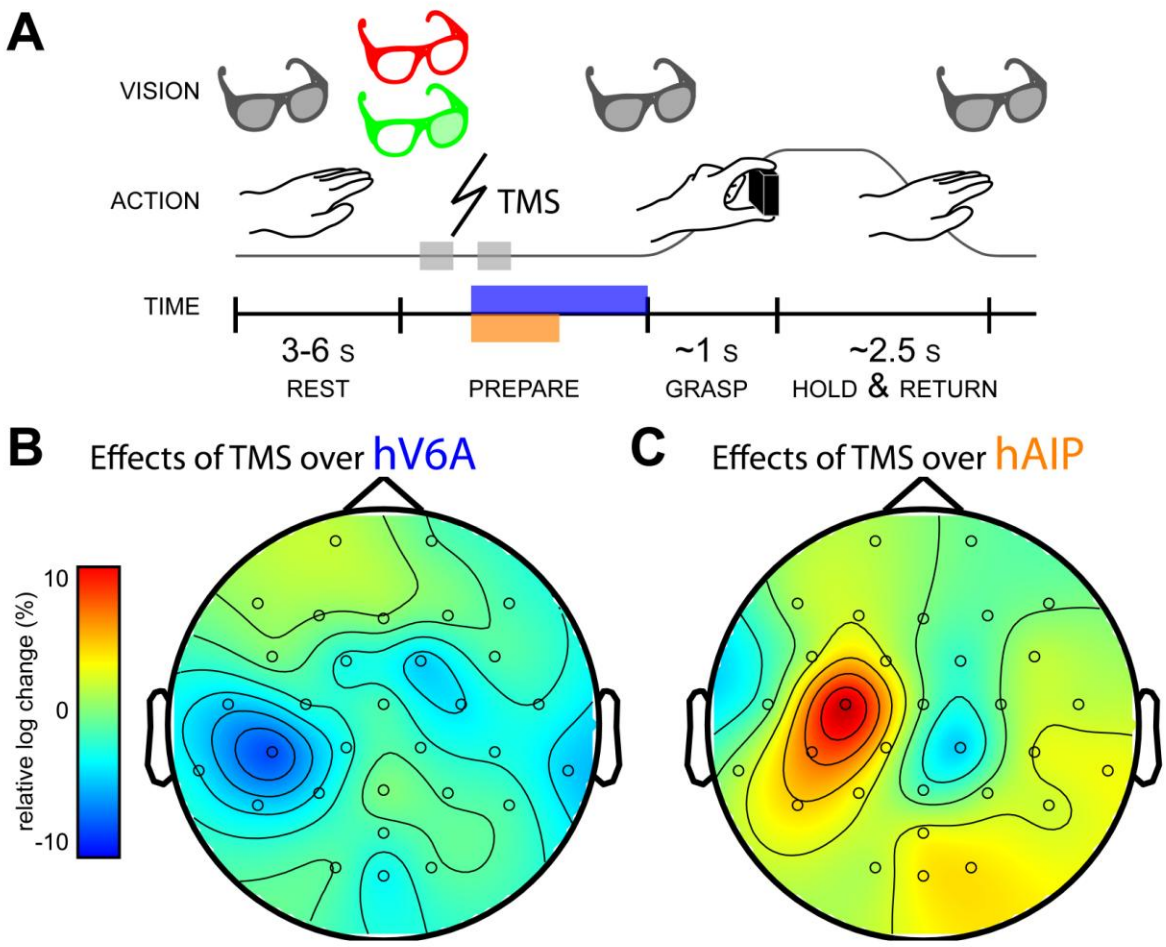
Support: The Netherlands Organisation for Scientific Research (NWO) Grant 400-04-379

Title: Causal contributions of hV6A and hAIP to goal-directed behavior - A TMS-EEG study

Authors: *L. VERHAGEN[1,2], C. DIJKERMAN[2], O. JENSEN[1], I. TONI[1];

[1]Donders Institute, Ctr. for Cognitive Neuroimaging, Radboud Univ., Nijmegen, Netherlands;
[2]Helmholtz Institute, Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands

Abstract: The dorsal visuomotor pathway supports goal-directed behavior and is anatomically subdivided in two streams, but their functional role remains unclear. An influential account distinguishes between transport and grip components of prehension movements, and it associates these components with medial and lateral streams of the dorsal visuomotor pathway, respectively[1]. Another proposal associates these two streams with advance planning and online motor control[2]. Here, we test these functional-anatomical dichotomies by measuring EEG responses to single pulse TMS perturbations delivered at critical nodes of these two streams, hV6A and hAIP[3]. We also manipulate reliance on perceptual depth cues and visuospatial accuracy requirements, asking subjects to grasp an object under monocular or binocular viewing conditions, while parametrically varying the slant of the grasped object (fig. 1A).
Planning of grasping movements induced sustained desynchronization in the alpha (9-12 Hz) and beta band (18-24 Hz). TMS over hV6A, compared to vertex stimulation, led to stronger alpha desynchronization over the hAIP region, irrespective of viewing conditions and accuracy requirements (fig. 1B). Yet, there were no changes in the prehension kinematics. This suggests that hAIP could compensate for transient alterations in hV6A, providing evidence against the notion of independent neural channels for reaching and grasping during the planning of prehension movements[1]. TMS over hAIP led to reduced beta desynchronization over C3 early in planning (fig. 1C), i.e. at a stage when the motor system is most likely to rely on prior motor experience and on perceptual cues of depth, rather than online motor control. This finding confirms that hAIP is necessary for integrating perceptual information into the prehension plan[3], and provides evidence against the notion that hAIP is necessary specifically during online control of motor behavior[2].
1. Culham et al. (2006) Neuropsychologia 44:2668-2684
2. Tunik et al. (2005) Nat Neurosci 8:505-511
3. Verhagen et al. (2008) J Neurosci 28:4726-4735



A. Experimental timing. Subjects were asked to grasp a rectangular prism (in black). The viewing conditions (binocular in red; monocular in green) and the prism orientation in depth were changed from trial to trial. Vision of the object was allowed only during the motor planning phase, turning opaque (gray glasses) at movement onset. We used single pulse TMS to disrupt processing in hV6A, hAIP and a control site (vertex), delivered randomly in the 100-200 or 300-400 ms interval after stimulus onset (gray boxes above the timeline).
B. hV6A stimulation. Topographical distribution of the differential relative power change in the alpha band (9-12 Hz), after hV6A stimulation (as compared to vertex stimulation), from 280 to 800 ms after stimulus presentation (see blue box in panel A; p=0.028).
C. hAIP stimulation. Topographical distribution of the differential beta (18-24 Hz) power change of the three way interaction between stimulation site (hAIP vs. vertex), vision and slant, from 280 to 480 ms (see orange box in panel A; p=0.01).

Disclosures: L. Verhagen, None; **C. Dijkerman**, None; **O. Jensen**, None; **I. Toni**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.6

Topic: D.17. Voluntary movements

Support: NSF IIS 0904504

Title: Skilled manipulations with a variable number of digits: evidence for motor equivalence

Authors: *M. SANTELLO[1], Z. HASAN[3], Q. FU[2];
[1]Arizona State Univ., TEMPE, AZ; [2]Arizona State Univ., Tempe, AZ; [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: The ability to perform the same task using different actuators is a manifestation of 'motor equivalence' - a phenomenon remarked upon in the early history of motor control - but studied only sporadically. The present study was designed to determine whether the phenomenon of motor equivalence occurs when transferring learned object manipulation. We asked subjects (n = 12) to reach to, grasp, lift, and release an inverted T-shaped object with an asymmetrical center of mass (CM) shifted to the left or right (L and R, respectively). Subjects were instructed to minimize object roll during lift. It has been shown that subjects learn this task by generating a compensatory torque at object lift onset (Fu et al., 2010; Zhang et al., 2010). Each subject performed the task under two experimental conditions: (1) two-digit grasping (thumb and index finger; 2d) and (2) three-digit grasping (thumb, index, and middle fingers; 3d). On the first experimental session, half of the subjects performed 10 2d trials followed by 3d trials for each object CM (2d→3d), whereas the other half started with 3d trials followed by 2d trials (3d→2d). Each subject was tested again two weeks later but on a trial sequence opposite to that experienced on his/her first experimental session. This experimental design allowed us to determine whether subjects are able to transfer the compensatory torque learned with a given number of digits to the same manipulation using a different number of digits. We hypothesized that subjects will be able to transfer the compensatory torque at object lift onset immediately after the change in grip type, therefore supporting the notion of motor equivalence. To further investigate the mechanisms underlying digit force and position modulation following a change in grip type, we also examined transfer of the compensatory torque components, one due to grip forces and digit position (grip torque), one due to load force sharing (lift moment).
We found that the compensatory torque on the first trial following a change in grip type was not significantly different from that exerted on the previous trial in both trial sequences (2d→3d and 3d→2d) ($p > 0.05$). Therefore, subjects were able to transfer the compensatory torque when switching grip type. However, the compensatory torque components changed significantly on the first trial following a change in grip type depending on the trial sequence and the CM (interaction Trial x Sequence x CM for grip torque and lift moment; $p < 0.01$ and $p < 0.05$, respectively). The flexible transfer to novel digit force-position coordination patterns suggests that the neural representation of learned object manipulations are object-, rather than effector-, centered.

Disclosures: M. Santello, None; **Z. Hasan**, None; **Q. Fu**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.7

Topic: D.17. Voluntary movements

Support: Wellcome Trust Grant WT083450

Title: PMv-M1 interactions during different contexts of grasping movements

Authors: *M. DAVARE[1], S. BESTMANN[1], J. C. ROTHWELL[1], J. DRIVER[2,3], R. N. LEMON[1];
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Abstract: The ventral premotor cortex (PMv) plays an important role in transforming an object's physical properties into a suitable motor command for grasp. PMv is more active when the object requires a precision grip (PG) compared to a whole-hand grasp (WHG), and when the grip force level is low compared to high. However, it is unclear whether this role of PMv in force scaling is independent of hand shaping because, so far, it has only been studied for precision grip.
To address this issue, we investigated in 15 volunteers how PMv interacts with M1 using a two coil conditioning-test TMS paradigm in a grip force visuomotor task where the hand shaping (PG vs WHG), the force (high vs low) and task difficulty (high vs low) were varied in a factorial design. PMv-M1 interactions were more facilitated when preparing for PG than WHG. This specific facilitation for PG was present for both force levels but was modulated by the task difficulty.
This suggests that hand shaping and task difficulty are key parameters processed by PMv, supporting the view that the influence of PMv onto M1 becomes larger with increasing requirements for visuomotor control.

Disclosures: M. Davare, Wellcome Trust, Research Grant; **S. Bestmann**, None; **J.C. Rothwell**, None; **J. Driver**, None; **R.N. Lemon**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.8

Topic: D.17. Voluntary movements

Support: Prodex Grants 90063, 90064, 90231, and 90232

Actions de Recherche Concerte´es (French community, Belgium)

NANOBIOTACT Project Grant EU-FPG-NMP-033287

Title: Optimal modulation of fingertip moisture during object manipulation

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Abstract: When handling an object or transporting it through space we apply an adequately large force normal to the grip surface (grip force) in relation to destabilizing forces tangential to the grip surface (load force). Accidental slips rarely occur because the grip force exceeds the minimal force required to prevent slip. It is well established that the minimal grip force required to prevent an object from slipping strongly depends on the frictional properties at the finger-object interface. Moreover, interindividual variation in the modulation of grip force suggests that the moisture level of the skin could influence grip force strategy. In the present study we asked subjects to perform a horizontal point-to-point task holding an object with a precision grip. The object was equipped with a moisture sensor. We found large inter- and intraindividual moisture level variations. There was a strong correlation between grip force exerted and moisture level at the fingertips. Indeed, the grip force was minimal when the fingertip moisture was optimal with respect to friction. Furthermore, fingertip moisture tended toward this optimal level at which grip force is minimal. In conclusion, we showed a modulation of the grip force with moisture level and hypothesized novel mechanisms of moisture regulation that tend to stabilize the moisture level toward the value that minimizes grip force.

Disclosures: J. Thonnard, None; **T. André**, None; **P. Lefèvre**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.9

Topic: D.17. Voluntary movements

Support: FWO G.0749.09

Title: Mirroring grasping: Force and muscle specific modulation of corticomotor excitability in M1 during movement observation

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Abstract: It was shown previously that movement observation modulates corticomotor excitability of the observer's primary motor cortex (M1). Here we investigated whether M1 excitability during movement observation "mimics" the neurophysiology of grasping. Previous studies investigating the preparation of different grip types have shown that the inferior frontal cortex facilitates M1 representations of the index finger (first dorsal interosseus, FDI) during a precision grip (PRG) and representations of the pink (aductor digiti minimi, ADM) during a whole-hand grip (WHG).
In study 1, we investigated whether a similar pattern of muscle specific facilitation is found when subjects passively observed either grip type. A single Transcranial Magnetic Stimulation (TMS) pulse was applied to left M1 while subjects observed videos showing how an actor grasped an object with either a PRG or a WHG grip. TMS was applied (1) when the hand had just entered the scene, (2) when the hand was opened, (3) during grip formation, (4) during the late grasp phase, and (5) when the object was lifted in the air. We found that during the grip formation/grasping phase, corticomotor excitability of the ADM was significantly higher for the WHG than for the PRG ($p<0.05$) whereas FDI excitability tended to be higher for the precision grip. Previous research has shown that the observer's M1 reflects also the force requirements of an observed movement. In study 2, we measured M1 excitability during different phases of grasping and lifting either a light or a heavy object. Using the same general setup as in study 1, TMS was applied over the thumb representation (m. opponens pollicis, OP) (1) in the beginning of the grasp phase, (2) at the end of the grasp phase (i.e. just before the object was lifted), (3) at the highest point of the lift trajectory, and (4) when the object was put down. Importantly, both objects looked identical such that only the kinematic profile of the grasp-and-lift movement differed between conditions. We found that OP excitability was higher when subjects observed how a heavy object was lifted compared to a light object. However, this difference reached significance only during the lift phase. Our data suggest that the weight-dependent facilitation of the observer's M1 reflects the integration of several kinematic cues such as a longer grasp phase, lower lifting velocity and lower elevation when the heavy object was moved. In summary, our findings indicate that the observation of grasping movements seems to activate human M1 in a

similar way as grasp execution.

Disclosures: N. Wenderoth, None; **K. Alaerts**, None; **S.P. Swinnen**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.10

Topic: D.17. Voluntary movements

Title: Spatial and temporal coordination of digit placement distribution during reach-to-grasp

Authors: *J. R. LUKOS[1], M. SANTELLO[2];
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Abstract: We previously reported that subjects adopt a distinct spatial distribution of digit contact points on an object at lift onset as a function of its center of mass (CM) location when it could be anticipated (Lukos et al., 2007). Here, we examined hand kinematics from reach onset through object contact to determine the temporal and spatial coordination of the trajectories of the thumb and index. The goal of this analysis was to determine whether the effect of CM on digit placement at contact requires viewing both hand and object for accurate positioning of the digits. If so, one would expect fingertip trajectories to diverge closer to object contact. Alternatively, the coordination of digit kinematics might not require online visual feedback of both hand and object. Within this scenario, a difference in fingertip trajectories would emerge early in the reach. Based on studies of hand posture modulation to object geometry, we hypothesized that the trajectories of thumb and index fingertips would exhibit a modulation to object CM early in the reach.
Subjects (n = 12) reached to, grasped, and lifted an object with a left (L) or right (R) CM for 6 consecutive trials. The only task requirement was to minimize object roll. during lift. We computed the difference between the time-normalized fingertip velocities between L and R trials and determined the time at which the difference was greater or smaller than 2 standard deviations of the mean of the first 2% of the reach duration. Consistent with our hypothesis, both the thumb and index fingertip trajectories diverged within the initial 10% of the reach duration. Interestingly, we found considerable trial-to-trial variability in the time course of fingertip velocities during the reach, even though subjects significantly modulated digit placement to CM location by the 2nd trial in a consistent fashion (Lukos et al., 2007). To verify that this modulation was not solely due to changes in the wrist trajectory, we performed the same analyses in an intrinsic hand frame of reference by subtracting wrist trajectories from the

fingertip kinematic data. This analysis confirmed the results of kinematic analyses in an extrinsic frame of reference.
These preliminary analyses indicate that the CM-dependent modulation of digit placement at contact does not require online visual feedback about hand and object, and a consistent distribution of digit contact points at the end of the reach is attained by compensating for trial-to-trial variability of wrist and/or fingertip trajectories during the reach. Further analyses will extend the above analyses to all digits as well as digit-digit coordination.

Disclosures: J.R. Lukos, None; **M. Santello**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.11

Topic: D.17. Voluntary movements

Support: NIH Grant 035032

Title: Effect of fatigue on finger force variance in a two-finger pressing task

Authors: *T. SINGH[1], V. ZATSIORSKY[2], M. LATASH[2];
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Abstract: A previous study has shown that when all four fingers (I,M,R and L) involved in a pressing task are affected by fatigue, the system's ability to compensate for the error induced due to the increased variance in the force output of the individual fingers diminished compared to when only one finger (I) was fatigued. In a previous study we also found that fatigue led to an increase in inter-trial variability in one-finger (I) pressing tasks while its effects of the four-finger tasks were smaller. In this study, we tested the effects of fatigue on force variance and a co-variation of finger forces in a two-finger (IM) pressing task without (*stable* setup) and with an additional requirement to maintain a certain value of the total moment of force (referred to as the *unstable* setup). The purpose was to study whether the adaptive strategy of increasing force variance of individual finger simultaneously with an increase in force co-variation can be applied in such a marginally redundant task
The fatiguing exercise by the I finger induced a significant drop in the maximum force production in both one-finger and two-finger tasks. On average, the peak MVC of the I finger dropped by 36.8% ($p<0.001$), M finger by 20.5% ($p<0.001$) and of the IM combination by 27.3%

($p<0.001$). Unintentional force production by non-task fingers (enslaving) increased during fatigue by about 75% ($p<0.01$).
Fatigue caused the RMS error of the total force for the IM condition to increase, on average, by about 6.7% ($p<0.05$). The variance of the force produced by the I and M fingers increased during fatigue by 60% for the I finger and 30% for the M finger. However, the finger forces co-varied such that the force variance that did not affect the total force (V_{GOOD}) increased more than the variance that affected the total force (V_{BAD}). V_{GOOD} increased by about 200% during fatigue ($p<0.05$), while V_{BAD} increased only by about 23% (not significant). V_{GOOD} was higher for the stable setup compared to the unstable setup ($p<0.01$) but there was no fatigue × setup interaction. This study shows that an increase in compensated variability (V_{GOOD}) of finger forces in a two-finger task can prevent fatigue from having adverse effects on the combined output of the two-finger task. This happens even in a marginally redundant task where the space of possible solutions is reduced by the additional constraint.

Disclosures: T. Singh, None; **V. Zatsiorsky**, None; **M. Latash**, None.

Nanosymposium

533. Finger and Grasp Control

Location: Room 1B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 533.12

Topic: D.17. Voluntary movements

Support: NIH Grant AG-018751

NIH Grant NS-035032

NIH Grant AR-048563

Title: Age related differences in finger interaction during accurate object rotation

Authors: *V. SKM[1], W. ZHANG[3], V. M. ZATSIORSKY[2], M. L. LATASH[2];
[1]Kinesiology, The Pennsylvania State Univ., UNIVERSITY PK, PA; [2]Kinesiology, The Pennsylvania State Univ., University pk, PA; [3]Arizona State Univ., Tempe, AZ

Abstract: Aging leads to a general decline in hand dexterity. In this study, we explored the effects of aging on digit coordination during accurate object rotation. We studied the mechanical variables and analyzed hierarchies of multidigit synergies at two levels of a hypothetical control hierarchy, namely the virtual finger-thumb level (VF-TH) and the individual finger level (IF).

Healthy young and elderly participants (nine in each group) performed series of accurate 30° pronation and 30° supination movements using a handle instrumented with six-component force/torque sensors. The young participants performed the task at two speeds, at a natural speed and as quickly and accurately as possible. Elderly participants performed the task only at the fast speed. The weight of the object was counterbalanced. Indices of multidigit synergies were defined as covaried changes in elemental variables (such as digit forces and moments of force) that stabilize a particular performance variable (such as total force and total moment of force) at each of the two levels, VF-TH and IF. Fast actions of the elderly were slower than the natural speed actions of the young participants. Elderly participants produced higher grip forces and were less accurate as compared to the younger group. During all phases of movement, indices of synergies were not significantly lower in elderly as compared to young at both the VF-TH level and the IF level. This result is inconsistent with previously published results in pressing tasks. We explain these results within the framework of the referent configuration hypothesis and suggest that earlier results in studies with isometric tasks might be due to the lack of salient feedback on the referent configuration (referent values of performance variables), while in the current task the subjects could see their movements and this feedback could help them in the organization of better co-variation of digit forces.

Disclosures: V. Skm, None; **W. Zhang**, None; **V.M. Zatsiorsky**, None; **M.L. Latash**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.1

Topic: F.01. Human Cognition and Behavior

Support: McDonnell foundation

NIH R01 NS060776

Title: Multifocal fMRI hypoactivation on a working memory task after diffuse mild traumatic brain injury involves regions associated with task-relevant attention

Authors: *F. G. YANG[1], S. C. LAHUE[1], S. R. COOPER[1], G. T. MANLEY[2], P. MUKHERJEE[1], T. L. LUKS[1];
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Abstract: The present study aims to investigate the impact of mild Traumatic Brain Injury (TBI) on neural circuits of working memory (WM). Previous Functional Magnetic Resonance Imaging (fMRI) research on severe TBI in adults reported decreased frontal activations. However, little research has focused on the neural representation of WM in adult mild TBI patients. We administered an n-back WM task for letter identity with memory load ranging from 0- to 2-back conditions on 6 patients 1 month post-injury, and 6 healthy controls matched for age, gender and years of education. During the experiment, a fixed pseudorandom sequence of letters was presented centrally on a computer screen, one letter at a time. Each letter was presented for 500 msec, with an interval of 2000 msec. Participants were instructed to decide if the currently presented letter was a target letter (0-back), or matched the letter that was presented 1 back in sequence, or 2-back in sequence. Participants responded to matches and no-matches by pressing buttons with the middle finger and the index finger of the right hand respectively. Four 60 second tasks blocks were interleaved with 30 second rest blocks for each condition. All image processing was performed using SPM8. Our results showed that in the 0-back condition, in comparison with healthy controls, mild TBI patients demonstrated decreased activations in bilateral cingulate, bilateral pre-supplementary motor area (pre-SMA) and left insula. Patients did not show significant difference in activation patterns from controls in response to the 1-back condition. In response to the 2-back condition, patients exhibited right-lateralized decreased activations in the superior frontal and orbitofrontal gyri and bilateral activations in inferior frontal junction (IFJ) and temporal-parietal junction (TPJ). Comparison of the 2-back condition to the 0-back condition revealed patients' hypoactivations in bilateral IFJ, TPJ, left hippocampus, and right insula. These results suggest that mild TBI patients' working memory functions might not be significantly affected, as the 2-back>0-back contrast showed decreased activations mostly in regions found to be involved with task-relevant high-level attention in previous research (e.g., IFJ and TPJ). Furthermore, cingulate and pre-SMA showed impact of TBI in the 0-back condition, which requires basic-level attention and little WM load. The decreased activation in the left hippocampus in the 2-back>0-back contrast is consistent with decreased activation in high WM load conditions found in previous TBI research**.**

Disclosures: F.G. Yang, None; **S.C. LaHue**, None; **S.R. Cooper**, None; **G.T. Manley**, None; **P. Mukherjee**, None; **T.L. Luks**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.2

Topic: F.01. Human Cognition and Behavior

Support: Marie Curie Research Fellowship

Wellcome Trust

Title: Direct evidence for top-down prefrontal control on memory representations in the presence of external interference

Authors: *E. FEREDOES[1], K. HEINEN[1], C. RUFF[3], J. DRIVER[2];
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Abstract: Current accounts of working memory (WM) posit a key role for dorsolateral prefrontal cortex (dlPFC) in preventing external distractors from interfering with maintenance of task-relevant stimuli in posterior association cortex. Two accounts suggest how this may be achieved. "Protection" accounts suggest that dlPFC acts on memory representations to enhance them over distractors whereas "suppression" accounts suggest that dlPFC acts on distractor representations to minimise their disruptive effects. To test such accounts causally, we combined transcranial magnetic stimulation (TMS) over dlPFC concurrent with fMRI to produce BOLD signal modulations in functionally connected regions during performance of a WM task. Subjects performed a visual delayed recognition task, with TMS applied to right dlPFC in the delay period of each trial. A 2x2x2 factorial design varied the category of the memory target (face, house); delay period visual distractor (present, absent); and TMS intensity (high, low). A burst of 3 TMS pulses was applied in the middle of the delay period, at the point when a visual distractor could appear. Any such distractor was always in the opposite category to the target (i.e., face distractor for house target trials and vice-versa). This allowed us to test whether dlPFC TMS would affect remote brain regions responsive to the target category, the distractor category, or both. Initial analysis of the fMRI data identified, at the single subject level, category-selective regions by a face>house contrast, or house>face contrast for distractor absent trials, revealing typical fusiform (FG) and parahippocampal (PH) regions respectively. We then assessed the impact of right dlPFC TMS (high>low intensity) on the BOLD signal in each of these regions. Results from 14 participants showed significant effects as follows. In face-sensitive FG voxels for face-memory trials, high intensity TMS produced a significant increase in BOLD signal for house-distractor present versus absent trials. Conversely, in house-sensitive PH voxels for house-memory trials there was a significant TMS-dependent increase in BOLD signal for face-distractor present versus absent trials. Considering both the FG and PH results, high dlPFC TMS increased activity in regions representing current memory targets, but not in regions representing the distractors. These results provide novel and direct evidence for top-down influences of dlPFC over posterior association areas, and the specific pattern of TMS effects on target representations during the delay period provide a new line of evidence for a protection account of dlPFC control over WM.

Disclosures: E. Feredoes, None; **K. Heinen**, None; **C. Ruff**, None; **J. Driver**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.3

Topic: F.01. Human Cognition and Behavior

Support: NSF 0924636

Title: Modeling maintained activity in dorsal and ventral stream neurons using just bottom-up processes

Authors: *S. S. PATEL, A. B. SERENO;
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Abstract: Many visual neurons in the dorsal and the ventral stream have the property that after a brief visual stimulus presentation in their receptive field, the spiking activity in these neurons persist above their baseline levels for several seconds. The origin of this elevated baseline spiking acitivity (also called maintained activity) is unknown. We have previously proposed a simple neural network model for bottom-up spatial attention which was based on shape selective neurons in monkey lateral intraparietal cortex. In this model, after stimulation by a brief visual stimulus, a shape selective neuron exhibited maintained activity similar to that observed in many shape selective cells in LIP and AIT. In order to investigate the mechanisms underlying the maintained activity, we focussed on the network circuitry corresponding to a single spatial location in the model. This circuitry consisted of a pair of shape selective neurons (A, B) that were mutually inhibited via inhibitory inter-neurons (A excited C and was inhibited by D; B excited D and was inhibited by C). The membrane activity of each neuron was described by the shunting equation. A thresholding non-linearity converted the membrane activity to firing rate. A and B had non-zero baseline firing rate. A and B had adaptive gain mechanisms within their excitatory and inhibitory synapses. Upon stimulation of A by a 50 ms pulse (input to B was zero), the firing rate of A increases rapidly, reaches a peak and then decays back to a steady-state level higher than the baseline level. This maintained activity was not due to mechanisms within A but was due to the pattern of network activation. Closer examination reveals that baseline activity, mutual inhibition and threshold non-linearity were necessary for A's maintained activity. The level of maintained activity was also modulated by the passive membrane decay constants of all the neurons. Despite their important role in bottom-up attention, adapative gain mechanisms were not necessary for maintained activity. We conclude that maintained activity or short-term memory of visual events may result from a bottom-up process without any external or top-down modulatory signal. This conclusion is consistent with the existence of maintained activity in many LIP and AIT neurons with little correlation with the monkey's task. The model thus provides a unified explanation of reflexive spatial attention, its dependence on shape and short-term memory of visual events.

Disclosures: S.S. Patel, None; **A.B. Sereno**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.4

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH068721

Title: EEG correlates of item and temporal order information in working memory

Authors: *L.-T. HSIEH[1], A. D. EKSTROM[2,1], C. RANGANATH[2,1];
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Abstract: The ability to maintain temporal order information in working memory (WM) is crucial in our daily life. For instance, when dialing a recently learned phone number, one must maintain not only the relevant items (i.e., the digits), but also their temporal order (i.e., the sequence of digits). Results from scalp electroencephalography (EEG) and intracranial EEG (iEEG) studies have indicated that oscillatory activity in the theta (4-8 Hz) and alpha (9-13 Hz) bands is correlated with WM maintenance. However, little is known about the differences in oscillatory activity between the maintenance of item and temporal order information in WM. One challenge in addressing this question is that differences in task difficulty usually complicate the interpretation of brain activity differences between tests of WM for item information and tests of WM for order information. Accordingly, in the present study, we attempted to compare the neural correlates of maintenance of item and order information while controlling for overall task difficulty. We recorded EEG while participants completed two types of WM trials: ITEM trials and ORDER trials. On each trial, participants see an instruction word (either "ITEM" or "ORDER"), followed by four sequentially presented fractals, and then a test display. On ORDER trials, the test display consisted of two fractals from the previous sequence in which participants were asked to identify which fractal came earlier in the sequence. On ITEM trials, the test display consisted of one previously presented fractal and another visually similar foil fractal that was not in the sequence with participants identifying the old fractal on ITEM trials. Behavioral results revealed that accuracy and reaction times were similar for ITEM and ORDER trials, suggesting that task difficulty was matched between the two conditions. Preliminary scalp EEG analyses indicate that oscillatory activity, particularly in the theta band, was modulated by maintenance of item and order information. We have also adapted the task for testing with patients who have implanted electrodes for seizure monitoring. We obtained similar results to

our scalp EEG with one patient, confirming that theta oscillations play an important role in maintaining temporal information in WM. Together, these data underscore the importance of coordinated neural activity in the theta-band for correctly maintaining the order of information in a recently learned list.

Disclosures: L. Hsieh, None; **A.D. Ekstrom**, None; **C. Ranganath**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.5

Topic: F.01. Human Cognition and Behavior

Title: Interaction between alpha and gamma oscillations correlates with individual working memory capacity

Authors: *F. ROUX[1], H. MOHR[2], M. WIBRAL[3], W. SINGER[1,4], P. J. UHLHAAS[1,2,5];
[1]Neurophysiol., Max Planck Inst. For Brain Res., Frankfurt Am Main, Germany;
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Abstract: Recent evidence from electrophysiological recordings suggests that neural oscillations in the theta, alpha and gamma frequency-ranges reflect the neural dynamics of WM representations. However, the precise functional roles of these frequency bands remains to be resolved.
In the current experiment, we employed a visuo-spatial delayed match to sample paradigm. Participants were instructed to encode and maintain the spatial position of either three or six red discs. On one third of the trials, three red discs were presented together with three blue discs and participants were instructed to ignore the blue items. During the retrieval, a single disc was presented and participants had to indicate whether the probe matched one of the spatial positions from the target display. During the task, we recorded magnetoencephalographic (MEG) data from 18 participants. In addition, structural magnetic-resonance (MR) data were obtained. MEG data were analyzed in the time-frequency domain (3-250 Hz) using wavelets. Spectral power for oscillations in the alpha-band (9-14 Hz) and gamma-band (40-150 Hz) was computed using multi-taper methods and the cortical sources of oscillatory activity in these frequency bands was localized with a dynamic imaging of coherent sources (DICS) algorithm.
Behavioral performances decreased with increasing WM load and significant differences were

found between participants with high and low WM capacity. Gamma activity was strongest over parietal and central sensors in the presence of distractors during the early delay period, while alpha oscillations increased with WM load over left frontal sensors. For participants with high and low WM capacity, we found a statistical interaction between capacity, oscillation-frequency and WM load. Alpha-activity showed a load-dependent modulation only in participants with low WM capacity, while gamma-activity increased with WM load only in participants with high WM capacity. The cortical sources of activity in the gamma-band were localized in parietal, pre-frontal and frontal areas, while oscillations in the alpha-band were found predominantly in pre-frontal and frontal areas.
Our results represent novel evidence for an interaction between WM load and oscillations in the alpha and gamma frequency-band which correlates with WM capacity. Specifically, our data suggests that participants with higher WM capacity may be more efficient at preventing distracting information from accessing WM thereby increasing WM capacity through the modulation of gamma-band oscillations.

Disclosures: F. Roux, None; **H. Mohr**, None; **M. Wibral**, None; **W. Singer**, None; **P.J. Uhlhaas**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.6

Topic: F.01. Human Cognition and Behavior

Support: NIST 70NANB

1R01AG024059

Title: Unobtrusive assessment of working memory using continuous monitoring of computer games

Authors: *M. PAVEL[1], H. JIMISON[2], D. AUSTIN[1], D. ERDOGMUS[3];
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Abstract: Working memory is one of the key cognitive functions underlying executive function, for tasks where it is necessary to manipulate representations of objects that are not present in the sensory inputs. Since most research on working memory is performed in a laboratory setting,

there are many issues concerning the characterization of working memory in situations where an individual must perform more than one concurrent task. This is especially important in assessing the effects of aging, frequently associated with a slow decline of working memory. As a part of a large longitudinal study focused on the early detection of cognitive decline in the aging population, we have been investigating ways to monitor working memory using unobtrusive techniques based on self-motivating activities that require the participants to perform tasks that rely on their working memory. In particular, we investigated how people play a computerized version of the game "Concentration". At the beginning of each game the players are confronted with a matrix of images of playing cards displayed face-down and asked to "turn" two cards on each trial. The player's goal on each trial is to identify (i.e., to turn) two matching cards using the player's memory of previously exposed card face value and location. Correct and incorrect moves can be use as indicators of whether a given item (a card) is in working memory. The difficulty of the task can be manipulated over a block of trials (games) as well as over a subset of individual trials. In order to assess each participant's memory, we developed a computational model based on the notion of survival analysis. The memory trace duration of an item in working memory is described in terms of survival analysis and characterized by a hazard function derived from a Weibull distribution. In the first experiment, the participants in the study played this computer game for a year and the number of games ranged from hundreds to thousands. After removing the effects of guessing (i.e., a model of a random player), the parameters of the maximum likelihood fit of the distribution are used to characterize the working memory of each player. The results suggest relatively that we can obtain stable estimates of the working memory capacity and that these are highly correlated with the participants' neurophysiological measurements. We will also discuss the results of a similar experimental task performed in the laboratory, with concurrent measurement of EEG. The results of this experiment are interpreted in terms of cognitive reserve.

Disclosures: M. Pavel, None; **H. Jimison**, None; **D. Austin**, None; **D. Erdogmus**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.7

Topic: F.01. Human Cognition and Behavior

Support: CELEST, an NSF Science of Learning Center (SBE-0354378)

The SyNAPSE Program of DARPA (HR0011-09-C-0001)

Title: How prefrontal spatial working memory and the supplementary eye fields control sequences of eye movements: Microstimulation with and without latency changes

Authors: *M. R. SILVER, D. BULLOCK, S. GROSSBERG;
Dept. of Cognitive and Neural Systems and Ctr. for Adaptive Systems, Boston Univ., BOSTON, MA

Abstract: Microstimulation in the supplementary eye fields (SEF) changes the order in which remembered sequences of saccades are produced, while leaving saccade metrics such as latency, accuracy, and peak velocity intact (Histed & Miller, 2006). That low-level features of the saccades were unchanged by microstimulation suggests that the SEF does high-level processing, such as selection of saccade targets. However, microstimulation sometimes does alter saccade latency (Yang, Heinen, & Missal, 2008). Are these data inconsistent with the hypothesis that the SEF selects targets? Do they suggest that the SEF is involved with saccade production or other low-level processes? Both effects of microstimulation can be quantitatively simulated by a competitive queuing model of prefrontal spatial working memory in which the SEF selects saccade plans from working memory and passes them to downstream oculomotor regions such as the frontal eye fields. Differences in the structure of these tasks, particularly the proximity of microstimulation to saccade initiation, are responsible for the variable effects of microstimulation on saccade latency. More generally, the model proposes answers to the following general questions: How do working memory circuits store multiple spatial locations for the control of planned sequences of eye movements? How does using rank-sensitive coding contribute to sequence storage and recall when the same movement repeats at multiple list positions, or ranks, during the sequence? The model utilizes rank-sensitive prefrontal working memory representations (Averbeck et al, 2003), which depend upon rank-related activity in parietal cortex (Sawamura et al., 2002; Grossberg & Pearson, 2008), to produce spatial sequences in which the same action is repeated several times. The model shows how the SEF (Schlag & Schlag-Rey, 1987) could mediate the selection of saccade plans from working memory by simulating both behavioral results and SEF cell dynamics (Isoda & Tanji, 2002: Lu et al., 2002).

Disclosures: M.R. Silver, None; **D. Bullock**, None; **S. Grossberg**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.8

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH085444

NIH Grant MH064498

Title: Tracking the evolution of a thought: stimulus encoding automatically engages multiple domains of information representation that are flexibly retained in short-term memory

Authors: *J. A. LEWIS-PEACOCK, A. T. DRYSDALE, B. R. POSTLE;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: What is the representational basis of the temporary storage of information in the human brain? We designed a fMRI study to test whether the activation of long-term memory (LTM) prompted by stimulus encoding can be extended to multiple domains of information representation. First, a pattern classifier was trained to dissociate delay-period activity corresponding to phonological, visual, and conceptual representations in short-term memory (STM). Then, the trained classifier was used to track the evolution of multiple, concurrent representations elicited by the encoding and retention in STM of a single pictorial stimulus. Information-based decoding of delay-period activity confirmed that pictures of familiar objects were encoded visually but augmented by concurrent recoding into phonological (e.g., Tversky, 1969) and conceptual (e.g, Wickens, 1973) forms. Although the visual representation was strongest at the beginning of the delay period, across the delay, it along with the associated conceptual representation dropped off such that only a phonological representation of the stimulus remained (an outcome consistent with accounts suggesting a privileged role for verbal encoding in STM, e.g., Baddeley and Hitch, 1974). The trained classifier was also used to decode fMRI data from a second short-term recognition task which required the prioritization in STM of a randomly selected subset of representations that all referred to a single stimulus. One stimulus characteristic was selected as relevant (e.g., visual) for the first half of the trial, but any characteristic was potentially relevant for the second half (phonological, visual, or conceptual). The pattern classifier's decoding of task-related activity showed that only the currently relevant memory representation was reflected in the activation pattern of the fMRI signal. However, temporarily irrelevant representations were reinstated back into the focus of attention when they were signaled as relevant for the second half of the trial. Thus, sustained activity may reflect the momentary contents of consciousness, rather than the contents of STM. Together, these results provide strong support for accounts of STM as the temporary reactivation of LTM representations (e.g., Anderson, 1983; Cowan, 1995; Postle, 2006). In conclusion, the present study identified direct neural evidence for flexible coding in STM, consisting of multiple, concurrent representational formats, each of which can be selectively sustained in the focus of attention in accordance with the demands of the task.

Disclosures: J.A. Lewis-Peacock, None; **B.R. Postle**, None; **A.T. Drysdale**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.9

Topic: F.01. Human Cognition and Behavior

Support: NIDCD R21DC009871-01

Arizona Biomedical Research Commission #8-043

Title: Repetition enhances single neuron responses to word recognition in human anterior cingulate cortex

Authors: *P. N. STEINMETZ[1], S. GOLDINGER[3], M. PAPESH[3], D. M. TREIMAN[2]; [1]Neurol. and Neurosurg., [2]Neurol., Barrow Neurolog. Inst., PHOENIX, AZ; [3]Psychology, Arizona State Univ., Tempe, AZ

Abstract: While the anterior cingulate has a well established role in error and conflict monitoring, its role in memory monitoring by judging confidence during recollection, is less well understood. We investigated the role of single neurons in the human anterior cingulate cortex during continuous recognition memory (CRM) for words. In each session, printed words were presented twice, with a variable number of other words (1-32) between presentations. Each word was shown for 1000 ms, after which subjects indicated, via a button press, whether they had seen it previously during the experimental session. The subjects were epilepsy patients with implanted depth and microwire electrodes.

Recordings from 112 well-isolated neurons were obtained in 19 sessions performed by 10 patients. Four of the sessions were second sessions using the same set of words tested previously in the patient. For first sessions using a given set of words, the difference between the average firing rates (200-1000 ms after stimulus onset) evoked by the first and second presentations of a word were statistically different for 7 of 69 neurons (10%, $p<0.05$ 1-way ANOVA). During second sessions presenting the same set of words, 6 of 43 (14%) showed a significant difference between responses to the first and second presentation. Recordings of multi-unit activity (MUA; failing strict criteria for well-isolated single neuron activity) during the first session using a given set of words showed that 9 of 154 (6%) had significant differences between first and second presentations of words. By contrast, 11 of 72 (15%) of clusters of MUA had significant differences during the second session using a given word set. Such a contrast in the fraction of units with reliable differences between first and second word presentations is highly significant (p=10^-9, Fisher's Exact Test).

Taken together, these results show that neurons in the human anterior cingulate cortex encode the memory of printed words, and that the strength of this encoding is increased by repeated presentation of the same set of words.

Disclosures: P.N. Steinmetz, None; **S. Goldinger**, None; **M. Papesh**, None; **D.M. Treiman**, None.

Nanosymposium

534. Human Memory

Location: Room 4

Time: Tuesday, November 16, 2010, 8:00 am - 10:30 am

Program Number: 534.10

Topic: F.01. Human Cognition and Behavior

Support: NIH 2R01MH066078

NIA R21AG030795

Uehara Foundation (K.J.)

Title: Common prefrontal mechanisms govern both decision difficulty in intertemporal choice behavior and working memory load

Authors: M. S. CHUSHAK[1], K. JIMURA[1], B. MARTINS[1], *T. S. BRAVER[2]; [1]Psychology, Washington Univ., Saint louis, MO; [2]Psychol, Washington Univ., SAINT LOUIS, MO

Abstract: Prior research has suggested that individual differences in intertemporal choice behavior are associated with prefrontal cortex (PFC) activity during active maintenance of task-relevant information (working memory). However, it is still unclear whether these two processes involve common neural mechanisms. In the current study, event-related fMRI was conducted while subjects performed working memory (WM) and delay discounting tasks, on inter-mixed trials. Delay discounting trials varied parametrically in decision difficulty, based on distance from the individually-derived subjective value indifference point (estimated in a prior behavioral session). WM trials varied in load (high= 5 items vs. low = 2 items). Behaviorally, decision difficulty and WM load effects (reaction time slowing) were correlated across subjects, suggesting common mechanisms. More direct evidence was obtained in a conjunction analysis that identified a region in right anterior prefrontal cortex in which activation was increased both by delay discounting decision difficulty and WM load. These two neural effects were again positively correlated across subjects, indicating that the individuals most affected by WM load were also most affected by decision difficulty. Further, cross-task brain-behavioral analysis also revealed that behavioral difficulty effect predicted the neural WM load effect in lateral PFC and posterior regions, while the behavioral WM load effect predicted a neural difficulty effect in a

similar set of areas. Finally, stronger decision difficulty effects were also correlated with a steeper delay discounting function, suggesting that decision difficulty contributes to impulsivity and poor self-control. Together, the results demonstrate a tight link between neural mechanisms of WM load and intertemporal decision-making. The primary locus of effect within anterior PFC is consistent with the view that the same mechanisms that contribute to the integration of information within WM may also be recruited to enable comparisons of choices differing in reward amount and delay, based on integration of these variables into actively maintained internal representations of subjective value.

Disclosures: M.S. Chushak, None; **T.S. Braver**, None; **K. Jimura**, None; **B. Martins**, None.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.1

Topic: F.02. Animal Cognition and Behavior

Support: UW's work is funded by an Open University studentship

Title: Inhibition of return in the rat using the covert orienting of attention task

Authors: *U. WAGNER, C. L. ROSTRON;
Life Sci., The Open Univ., Milton Keynes, United Kingdom

Abstract: Covert orienting of visual attention describes switching of attention without head or eye movement. In humans this process is investigated using the covert orienting of attention task (COVAT) where participants have to respond to a cued target occurring left or right of a central fixation point. These targets can be validly cued (target and cue occur on the same side) or invalidly cued (target and cue occur on opposite sides). Typically, when the time between cue and target is short (up to 200-300 ms), responses to validly cued targets are faster than to invalidly cued targets. However, with a longer cue to target interval (CTOA), reaction times to invalidly cued targets are faster than reaction times to validly cued targets. This pattern of reaction times at longer cue to target intervals has been termed inhibition of return (IOR). The COVAT is an important paradigm in attention research, and the effects of different diseases (e.g. Alzheimer's and Parkinson's) on inhibition of return have been investigated using it. While a version of the COVAT does exist for rats, inhibition of return has, to our knowledge, never before been demonstrated in rats.
We therefore trained 24 male Lister Hooded rats on a version of the COVAT that could be

conducted in 5-hole wall operant chambers in an attempt to elicit inhibition of return. In this task 50% of the cues were valid and 50% invalid, thus cues did not generate expectations about the location of the target. CTOAs were 200ms, 400ms, 600ms, 800ms and 1000ms. Data were analysed using the conventions for human participants; by calculating the magnitude of the validity effect (validity effect = reaction time invalid - reaction time valid) across CTOAs. Results suggest that the COVAT can successfully elicit inhibition of return in Lister Hooded rats, as demonstrated by a positive validity effect at short CTOAs and a negative validity effect at longer CTOAs. Given the importance of covert orienting and inhibition of return in neuroscience, an animal model of inhibition of return is potentially very useful for studying neural substrates of attention as well as for applied research, for example of the cognitive effects of neurodegenerative disorders.

Disclosures: U. Wagner, None; **C.L. Rostron**, None.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.2

Topic: F.02. Animal Cognition and Behavior

Support: U01-CA141549-01 NCI

Title: Attention deficits in neurofibromatosis-1 mutant mice results from dopamine system abnormalities

Authors: *J. A. BROWN[1], R. J. EMNETT[2], C. R. WHITE[2], S. HARMON[3], C. YUEDE[4], S. CONYERS[4], K. L. O'MALLEY[3], D. F. WOZNIAK[4], D. H. GUTMANN*[2];
[1]Neurol. Washington Univ. Sch. of Med., St Louis, MO; [2]Neurol., [3]Anat. & Neurobio., [4]Psychiatry, Washington Univ. Sch. of Med., Saint louis, MO

Abstract: Over half of children with the neurofibromatosis-1 (NF1) inherited cancer syndrome have problems with learning and memory as well as attention system dysfunction (attention deficit disorder; ADD). Previous studies have employed Nf1 genetically-engineered mice to identify the biochemical and electrophysiologic deficits underlying the cognitive phenotypes. In contrast, comparatively little is known about the cellular and molecular etiologies for the attention deficits. Using a unique Nf1 optic glioma mouse model, we now report deficits in non-selective and selective attention system function without an accompanying hyperactivity phenotype. Nf1 optic glioma (OPG) mice exhibit reduced interest in novel objects and

environmental stimuli. Indices of attention system dysfunction in these mice are greatly improved by treatment with methylphenidate (MPH; Ritalin®) similar to that observed in children with NF1, suggesting a defect in brain catecholamine homeostasis. We next established that this attention system abnormality is the consequence of reduced dopamine levels in the striatum, which are restored following either MPH or L-dopa administration. Finally, Nf1 OPG mice have reduced striatal expression of tyrosine hydroxylase, the rate limited enzyme in dopamine synthesis without any dopaminergic cell loss. Taken together, these data provide a possible mechanistic explanation for the attention system dysfunction in Nf1 mutant mice relevant to the treatment of ADD in children with NF1.

Disclosures: J.A. Brown, None; **R.J. Emnett**, None; **C.R. White**, None; **S. Harmon**, None; **C. Yuede**, None; **S. Conyers**, None; **K.L. O'Malley**, None; **D.F. Wozniak**, None; **D.H. Gutmann***, None.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.3

Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust Grant WT079314MF

Title: Role of dose in attentional performance of rats prenatally exposed to ethanol

Authors: *L. BIZARRO[1], I. BRYS[2], S. PUPE[2], P. J. E. ASHERSON[3], I. P. STOLERMAN[4]; [1]Psicologia do Desenvolvimento e da Personalidade, [2]Programa de Pós-graduação em Psicologia, Univ. Federal Do Rio Grande Do Sul, Porto Alegre, Brazil; [3]Social, Genet. & Developmental Psychiatry Ctr., [4]Dept. of Addictions, Inst. of Psychiatry King´s Col. London, London, United Kingdom

Abstract: There are numerous reports in the literature about cognitive and neuroanatomical abnormalities associated with prenatal exposure to ethanol (PEE). Epidemiological studies testing for an association between PEE and the occurrence of symptoms that are characteristic of attention deficit hyperactivity disorder (ADHD) have yielded inconsistent results. The objective of the present study is to investigate inattentive and impulsive responses in rats prenatally exposed to ethanol, using the 5-choice serial time task (5-CSRTT). Subjects were 46 male two months old Wistar rats, born from dams exposed to one of the four treatments during pregnancy: A35-liquid diet with 35% ethanol-derived calories (35% EDC, 6.7% v/v); A10-liquid diet with

10% ethanol-derived calories (10% EDC, 1.8% v/v) with caloric intake limited by pair-feeding to match that in the A35 group; controls pair-fed with liquid diet without ethanol; controls with free access to laboratory chow and water. The animals were trained with food rewards to detect and respond to brief (1s) visual stimuli presented every 5s in one of five holes until a stable baseline was achieved. After that, four test sessions were performed with shorter stimulus durations (0.5, 0.25s) or manipulations in inter-trial-interval duration (7s, 2s), in a randomized order. The results provide evidence of impairments in the performance of rats from A35 group. At the baseline, the percentage of omission errors from these animals was greater than all other groups ($F(3,42)=4.2$, $p<0.05$). Compared to baseline, under test conditions A35 showed more omission errors than control groups (GLM/LSD, $p<0.05$) at every manipulation of stimulus duration and inter-trial-interval. Rats from A10 group did not present detectable impairment at baseline or in test sessions; these animals did not differ in accuracy, latency to respond, latency to collect reward, omission errors, premature, incorrect or perseverative responses ($p>0.05$). These results suggest attentional impairment in rats prenatally exposed to the higher dose of ethanol: although these rats were able to learn the task just as easily, they had a higher rate of omissions in the final parameters of training and test sessions. Interestingly, their visual accuracy was intact. Moreover, neither dose of ethanol increased premature responses, suggesting that the control of impulsive behavior was not affected. The identified attentional impairment is comparable to findings from equivalent cognitive tasks used in people with ADHD, supporting a potential role for PEE in the cognitive deficits associated with ADHD.

Disclosures: L. Bizarro, None; **I. Brys**, None; **S. Pupe**, None; **P.J.E. Asherson**, None; **I.P. Stolerman**, None.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.4

Topic: F.02. Animal Cognition and Behavior

Support: ARC Grant DP1092442

ARC Grant DP1093968

Title: Multichannel recordings in the fly brain reveal frequency dynamics in response to visual stimuli

Authors: *B. VAN SWINDEREN[1], T. POLLAK[2], C. REID[2], A. PAULK[2];

[1]St Lucia, Australia; [2]Queensland Brain Inst., St Lucia, Australia

Abstract: We have developed a method to record up to 16 channels simultaneously throughout the Drosophila brain, from tethered flies presented with visual stimuli. This method allows for the first time a comprehensive analysis of whole fly brain dynamics in response to visual stimuli - much like human electroencephalogram (EEG) studies. To record local field potentials (LFPs) from multiple sites in the fly brain, we used multichannel silicon probes (Neuronexus.com), where each recording site is separated by 50 microns. Thus, the recording sites transect the entire fly brain, from left eye to right eye. The value of this "skewer" preparation lies in the accessibility of the entire fly visual system, where different layers of visual processing (retina, medulla, lobula, central brain) are sampled, from the periphery to the center, for both hemispheres simultaneously. In a separate set of experiments, we transected the fly brain dorso-ventrally, and this typically provided 3-4 LFP recording sites through the brain, from the mushroom body calyces to the sub-esophageal ganglion.
We found that recording site and electrode orientation significantly influenced the amplitude of responses to moving visual stimuli displayed on an LED arena. Surprisingly, responses to visual stimuli (measured as power of the 10-100 Hz frequency domain) were often greater in the central brain, in the vicinity of the mushroom bodies (MB) and central complex (CC), than in the optic lobes. We performed coherence analyses to determine whether different parts of the brain were phase-locking in specified frequency domains in response to moving visual stimuli, and found some that some frequency bands that showed increased power in response to visuals (e.g., 20-30 Hz) also showed increased coherence, and that these effects could be distributed across widely separated (>100 microns) recording sites in the fly brain. Recordings from transgenic flies where synaptic activity could be transiently silenced revealed that coherence effects were lost when key central brain structures (the MB and CC) were targeted.
Flickering visual stimuli have been used to generate steady state visually evoked potentials (SSVEPs) in human EEG studies of visual attention. To develop a similar visual attention paradigm in Drosophila, we adapted our multichannel recording paradigm to track such frequency tags in the fly brain. Our first experiments with competing frequency tags reveal that dynamics of power and coherence between competing flickering visuals can be used to measure attention-like states in the fly. We demonstrate how our whole-brain recording paradigm can be used to map these states in the Drosophila brain.

Disclosures: B. Van Swinderen, None; **T. Pollak**, None; **C. Reid**, None; **A. Paulk**, None.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.5

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

NSERC

EJLB

Vanier Graduate Scholarship

Title: Dorsolateral prefrontal cortical neurons encode speed and accuracy of target selection through response suppression

Authors: *T. LENNERT, J. C. MARTINEZ-TRUJILLO;
Physiol., McGill Univ., Montreal, QC, Canada

Abstract: When human select the greater of two digits, choices are faster and more accurate the larger the numerical distance between them, a phenomenon known as the distance effect. The same phenomenon has been observed in humans when comparing the rank of alphabetically ordered letters, and in monkeys when comparing quantities, suggesting that it is a general phenomenon that affects the speed and accuracy of target selection. We investigated their neural mechanisms by recording responses of dorsolateral prefrontal cortex (dlPFC) neurons of two rhesus monkeys while they performed a rank-order selection task. The animals were presented with two white moving random dot patterns (RDPs) to the left and right of a central fixation cross. After a variable interval both RDPs simultaneously changed, each to a different color. Prior to training, we chose a set of colors and arbitrarily assigned to each a fixed ordinal position (rank) within a scale (grey < pink < green < blue < red < cyan). Animals were rewarded for keeping gaze on the cross and releasing a lever after the higher-ranked RDP (the target) changed motion direction while ignoring changes in the lower-ranked RDP (the distracter). After training both animals showed stable performance with higher proportions of correct responses (Se: $p < 0.0001$, Ra: $p = 0.0012$, one-way ANOVA) and shorter reaction times (Se: $p < 0.0001$, Ra: $p = 0.012$, one-way ANOVA) as the ordinal distance between target and distracter increased, revealing a distance effect. This suggests that the animals used the ordinal representation of colors, and compared the rank of the two stimuli to select the target.
We recorded the responses of 222 dlPFC neurons while the animals performed the task. 122 (55%) units reliably signaled the position of the target. Following the color change onset, responses to targets increased by a similar amount, however, responses to distracters were progressively suppressed as their ordinal distance from the target increased. We quantified whether these firing rate modulation influenced the neurons' ability to discriminate targets and distracters by computing receiver-operating-characteristic curves between responses to targets and distracters as a function of distance (d1, d2, and d3), and derived estimates of the latency and accuracy of the neurometric performance. Mean latency was significantly lower and accuracy larger for d3, followed by d2, and d1 ($p = 0.002$, one-way ANOVA). Thus, neurons selected the target faster and more accurately the greater the ordinal distance to the distracter, mimicking the animals' behavior. These results demonstrate that the activity of dlPFC neurons reflect the speed

and accuracy of target selection.

Disclosures: T. Lennert, None; **J.C. Martinez-Trujillo**, None.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.6

Topic: F.02. Animal Cognition and Behavior

Title: Brain-state dependent attentional modulation of firing rate, response variability and choice probability of inferior temporal neurons in monkeys performing categorization of ambiguous visual stimuli

Authors: N. EMADI, *H. ESTEKY;
IPM Sch. of Cognitive Sci., Tehran, Iran, Islamic Republic of

Abstract: To explore the interaction and dynamics of visual attention, sensory cortical processing, brain state and behavioral choice we recorded single unit responses from inferior temporal (IT) cortex of two macaque monkeys during passive fixation (passive) and two-alternative forced-choice body/non-body categorization (active) tasks. The stimuli were 180 grayscale photographs of bodies and objects each presented with 4 different levels of ambiguity (10, 30, 45 and 60% noise). In each recording session these 720 (4x180) noisy stimuli and 90 full noise images (0% visual signal) were randomly presented to the monkey in interleaving blocks of passive and active tasks. Comparison of cells' activity in passive and active tasks revealed that body cells (d' for body vs. object stimuli >0) showed significantly more response enhancement for body than object images. Non-body cells (d'<0) showed the reverse. No such response enhancement was observed in trials when the monkeys made a wrong choice. Magnitude of the response enhancement was larger for more noisy stimuli and was usually observed 150-400ms after stimulus onset. More importantly, in trials with high baseline activity, but not those with low activity, responses of body selective cells to presentation of body images were enhanced while responses of non-body cells were suppressed. Also neural response variability decreased in the active compared with the passive task. Larger effects were observed at higher noise levels. Choice probability measure showed that cells' firing rates were correlated with monkeys' choice, particularly in trials with high baseline activity. Our data show that attentional enhancement or suppression of IT cells' firing rate, improvement of response reliability and correlation of neural activity with monkeys' behavioral choice depend on cells' stimulus category selectivity, baseline spontaneous activity and task difficulty.

Disclosures: N. Emadi, None; **H. Esteky**, None.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.7

Topic: F.02. Animal Cognition and Behavior

Support: MRC NIRG G0700980

RCUK Academic Fellowship

British Pharmacology Society Integrative Pharmacology Fund

Title: Selective inhibition of the noradrenaline transporter improves attention and impulse control in a non-paced version of the 5-choice serial reaction time task

Authors: *E. S. ROBINSON;
Univ. Bristol, Bristol, United Kingdom

Abstract: Previous studies have shown that atomoxetine, a noradrenaline re-uptake inhibitor (NaRI) and treatment for attention deficit hyperactivity disorder, improves impulse control in three different operant animal models. The present study has further investigated the effects of NaRI in the 5-choice serial reaction time task (5CSRTT) using atomoxetine and reboxetine. In the first part of the study, rats were trained in a standard, fixed inter-trial interval (ITI), 5CSRTT and then tested at baseline and under conditions to challenge attention and/or impulse control. For the second part of the study, rats were re-baselined and then tested using a non-paced, variable ITI version of the task. Male Lister-hooded rats (n = 24) were tested in standard Med Associates 5-hole boxes and KLimbic software (Conclusive solutions Ltd). Following training and 10 baseline sessions, animals were tested using a series of attentional and impulse control challenges (baseline, short stimulus, noise distracter, variable inter-trial interval and long inter-trial interval) under vehicle or 0.3mg/kg atomoxetine (i.p, t=-30min) treatment. Data were analysed using a paired t-test. At the end of this study, all rats were re-baselined using a variable ITI schedule (4, 5 and 6 sec). Atomoxetine (0.0-0.3mg/kg, i.p.) and reboxetine (0.0-0.3mg/kg, i.p.) were tested using a within-subject fully randomised study design with at least 2 weeks washout and re-baseline between drug treatments. Data were analysed using a repeated measure ANOVA with TREATMENT as the within subjects factor. Subpopulation analyses were also

carried out using a median split of the population based on accuracy under vehicle conditions. Atomoxetine (0.3mg/kg) significantly improved impulse control under all challenge conditions ($p<0.05$) but had no effect on accuracy. In contrast in the variable ITI study, atomoxetine (0.0-0.3mg/kg) induced a dose-dependent improvement in accuracy ($p<0.05$) and reduction in premature responses ($p<0.05$). Reboxetine (0.0-1.0) did not significantly improve accuracy in the whole population but median split analysis revealed a significant improvement with both reboxetine (0.1 and 0.3mg/kg) and atomoxetine (0.1 and 0.3mg/kg) in the poor performing animals ($p<0.05$). These data suggest that blockade of noradrenaline re-uptake sites is an important target in terms of improvements in impulse control. Under conditions that increase the visual attention required to perform the task, both treatments also improved accuracy in poor performing animal and suggest an interaction with endogenous catecholamine levels.

Disclosures: E.S. Robinson: Employment; University of Bristol. Research Grant; Medical Research Council, Biology and Biotechnology Science Research Council, Wellcome Trust. Other Research Support; MSD Newhouse, Scotland, Pfizer UK.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.8

Topic: F.02. Animal Cognition and Behavior

Support: Canada Institutes of Health Research (CIHR) to EDR

Title: Cholinergic modulation of both visual and olfactory attention with the five-choice serial reaction time test

Authors: *V. LJUBOJEVIC[1,2], P. LUU[3], E. DE ROSA[2];
[1]Toronto, ON, Canada; [2]Psychology, [3]Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The nucleus basalis magnocellularis (NBM) sends acetylcholine (ACh) to neocortical regions that are involved in attentional cognitive processes. Using the five choice serial reaction time task (5CSRTT), the rodent analog of sustained attention in the human cognitive literature, it has been shown that a loss of cholinergic cells in the NBM causes impaired visual attentional performance in rats (Lehmann et al., 2003; McGaughy et al., 2002).
The present research examined the neurochemical modulation of attentional processes using both a visual and an olfactory version of the 5CSRTT. To that purpose, we trained 14 male adult Long-Evans rats to attend and react to the briefly presented visual or odor stimuli until they

achieved a stable performance under the baseline task conditions, i.e., low attentional demand with stimulus duration (SD) of 1s. Following the successful acquisition of both versions of the 5CSRTT, the rats were subjected to selective cholinergic lesions of the NBM with the cholinergic immunotoxin 192 IgG-saporin to remove the cholinergic innervation from the neocortical mantle. This allowed an examination of the role of ACh in modulation of visual and olfactory attention.
After the two week post-surgical recovery period, we compared the attentional performance of the saporin-lesioned (SAP) group (N=8) to that of the sham-lesioned (SHAM) group (N=6) on the two versions of the 5CSRTT task. We observed the impaired attentional performance of the SAP rats on the visual 5CSRTT under the baseline conditions (SD=1s); shortening the SD = 0.5s increased the extent of their deficits. With the olfactory 5CSRTT, the SAP impairment was only observed under the attentional challenge of SD=0.5s. However, in both modalities the difference between two groups trended toward statistical significance due to the low number of the experimental subjects in each group. We are currently performing further parametric manipulations to further challenge the rats in both modalities. We will then collect data from an additional 14 rats to increase the statistical power of our experiment.
After the completion of the behavioral data collection, we will conduct acetylcholinesterase histochemistry and choline acetyltransferase immunohistochemistry in order to determine the extent of the loss of cholinergic afferents in fronto-parietal target cortical areas and the loss of cholinergic cell bodies in the NBM, respectively. In addition, parvalbumin immunohistochemistry will be carried out to quantify GABA-releasing neurons colocalized in NBM to confirm the selectivity of our lesion.

Disclosures: V. Ljubojevic, None; **P. Luu**, None; **E. De Rosa**, None.

Nanosymposium

535. Attention: Animal Studies

Location: Room 2

Time: Tuesday, November 16, 2010, 8:00 am - 10:15 am

Program Number: 535.9

Topic: F.02. Animal Cognition and Behavior

Support: Feodor Lynen Research Fellowship from the Humboldt Foundation

Irma T Hirschl & Monique Will-Caulier Trusts Award

Title: Cortical area PITd, a ventral pathway area for the control of spatial visual attention?

Authors: *H. STEMMANN, W. FREIWALD;

Lab. of Neural Systems, The Rockefeller Univ., New York, NY

Abstract: Selective attention is the process of selecting information deemed important for current behavioural goals at the expense of other behaviourally irrelevant information. The control of spatial visual attention is generally thought to be exerted by a fronto-parietal circuit, chiefly encompassing structures of the occulomotor system. In support of this notion, Moore and Fallah showed that electrical stimulation of prefrontal area FEF leads to a decrease in psychophysical thresholds for stimulus detection in the corresponding area of the visual field (1) and enhances visual responses of V4 neurons in a similar way as spatial attention does (2). Bisley and Goldberg (3) proposed a saliency map to be located in parietal area LIP.
Here we present evidence from fMRI, electrophysiology, and electrical microstimulation experiments in the macaque monkey suggesting that a third major cortical contributor to the control of spatial attention may be located in the temporal lobe. Monkeys performed an attention demanding motion processing task that required spatial attention to be directed to one of two random dot surfaces undergoing a rapid series of motion direction changes. A prolonged translation event within this RSVP stream had to be detected, discriminated, and reported by a saccadic response to one of several saccade targets. FMRI experiments revealed strong and consistent attentional modulation within a subregion of PITd in this task. Electrophysiological recordings targeted to this subregion of PITd showed much larger attentional modulations of neural activity than in motion-selective area MT. These modulations persisted across changes in task requirements (detection versus discrimination) and task-relevant feature dimensions (color versus motion). PITd neurons exhibited little if any tuning to motion direction, color or shape; receptive fields were spatially confined and easily mapped with a simple random dot stimulus. Together, these visual and attentional properties suggest PITd contains a specialized subregion that may not subserve feature-specific processing, but may instead serve a more general role in attention. As a direct test of this idea, we found that microstimulation in the subregion of PITd modulated by attention in fMRI experiments led to specific behavioural changes most parsimoniously explained by a switch of the attentional focus. Thus, we propose that the temporal lobe may contain a hitherto unsuspected area for controlling attention.
Moore, T. and Fallah, M. (2001). Proc. Natl. Acad. Sci. USA. 98: 1273-6.)
(Moore, T. and Armstrong, K.M. (2003). Nature 421: 370-373.)
(Bisley,J.W., and Goldberg,M.E. (2003). Science 299, 81-86.)

Disclosures: H. Stemmann, None; **W. Freiwald**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.1

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant RO1 ES015747

Title: A pharmacological screen in Drosophila may identify novel drugs to treat mood and attention deficit disorders

Authors: H. O. LAWAL[1], V. SHAHI[1], A. N. TERRELL[3], R. S. HADI[1], J. JANG[1], L. ROBERTS[1], B. HUANG[1], M.-T. CHOU[1], A. CHEN[1], *D. E. KRANTZ[2];
[1]Univ. California Los Angeles, Los angeles, CA; [2]Univ. California Los Angeles, LOS ANGELES, CA; [3]Div. of Envrn. Hlth. Sci., Univ. of Minnesota, Minneapolis, MN

Abstract: Drugs that target aminergic signaling are the mainstay for the treatment of depression, anxiety and attention deficit disorder, but methods to identify new targets are limited. We are using Drosophila to identify novel drugs that potentiate monoamine signaling. In flies, as well as mammals, VMATs are required to package into synaptic vesicles all monoamine neurotransmitters, including dopamine and serotonin. dVMAT mutants show several behavioral deficits including reduced larval locomotion. The locomotion deficit of the dVMAT mutant provides a sensitized background to screen for novel drugs that potentiate amine release or post-synaptic signaling. We screened a panel of 1040 drugs and identified 42 compounds that increase larval locomotion. In a secondary screen comparing the effects of dVMAT null and hypomorphic alleles, we showed that 11 of the 42 are likely to act via increasing aminergic signaling, and at least one may employ a novel mechanism to potentiate amine release. A second set of drugs is likely to act post-synaptically to directly activate aminergic signaling pathway. We will now validate our results in rodents. Our screen represents an important new way to identify potentially new drugs and targets for the treatments for depression, anxiety and attention deficit disorder.

Disclosures: H.O. Lawal, None; **D.E. Krantz**, None; **V. Shahi**, None; **R.S. Hadi**, None; **A.N. Terrell**, None; **J. Jang**, None; **M. Chou**, None; **L. Roberts**, None; **B. Huang**, None; **A. Chen**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.2

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Roberto and Renata Ruhman

Israel Science Foundation

Israel Ministry of Health

Title: A genetic approach for intracerebroventricular delivery: Choroid plexus-specific lentiviral-based system

Authors: *L. REGEV, E. EZRIELEV, E. GERSHON, S. GIL, A. CHEN;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Administration of synthetic or purified peptides directly into the brain ventricles is a method commonly used by neuroscientists for exploring physiological and behavioral functions of gene products. i.v. administration is controlled by the blood-brain barrier, which limits its effectiveness, and current approaches for acute or chronic intracerebroventricular delivery have significant technical drawbacks resulting from both the chemical properties of the delivered substance and the experimental procedures. Here we describe a genetic approach for the delivery of secreted peptides or proteins into the cerebrospinal fluid (CSF). Using a choroid plexus-specific promoter, we established a lentiviral-based system, which offers inducible and reversible delivery of a gene product into the CSF. The functionality of this system was demonstrated by using the over-expression of the two established neuropeptides, corticotropin-releasing factor and gonadotropin-releasing hormone, modulating anxiety-like behavior and estrus cycle, respectively. We show that this choroid plexus specific lentiviral-based system is a reliable, effective, and adaptable research tool for intracerebroventricular delivery.

Disclosures: L. Regev, None; **E. Ezrielev**, None; **E. Gershon**, None; **S. Gil**, None; **A. Chen**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.3

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Development of a novel non-radiometric assay for nucleic acid binding to TDP-43 suitable for high-throughput screening using AlphaScreen technology

Authors: *A. C. PAWLYK, J. A. CASSEL, B. E. BLASS, A. B. REITZ;
ALS Biopharma, Doylestown, PA

Abstract: TAR DNA binding protein 43 (TDP-43) is a nucleic acid binding protein that is associated with the pathology of amyotrophic lateral sclerosis (ALS), the related disorder frontotemporal lobar dementia, certain forms of cystic fibrosis, and HIV infection. Assays to examine nucleic acid binding to TDP-43 typically use qualitative techniques that are not amenable to the high-throughput screening techniques necessary to discover small molecule probes or therapeutics. We have developed a robust, quantitative, non-radiometric high-throughput assay measuring oligonucleotide binding to TDP-43 using AlphaScreen technology. AlphaScreen technology works by detecting the proximity of TDP-43 tethered to an acceptor bead and a DNA oligonucleotide tethered to a donor bead. This permits energy transfer via excited singlet oxygen when the two macromolecules are associated. We have established this assay in a 384-well plate format allowing the detection direct binding of biotinylated oligonucleotides or competitive binding of a test oligonucleotide or small molecule. Biotinylated single-stranded TAR DNA (bt-TAR-32) and six TG repeats (bt-TG6) bound with high affinity to TDP-43, with K_D values of 0.75 nM and 0.63 nM, respectively. Both oligonucleotides exhibited slow dissociation rates, with half-lives of 750 min for bt-TAR-32 and 150 min for bt-TG6. The relative affinities of unlabeled DNA and RNA oligonucleotides, as determined by displacement of either bt-TAR-32 or bt-TG6, were consistent with previous reports of nucleic acid interactions with TDP-43, where increasing TG or UG repeats yields greater affinity. We found that DNA oligonucleotides bound with a greater affinity than RNA oligonucleotides. Screening a library of 7,360 compounds for inhibition of TDP-43 binding to bt-TAR-32 identified a series of compounds with nascent SAR and IC_{50} values ranging from 100 nM to 10 μM. We have established a homogenous, quantitative, high-throughput assay for the binding of nucleic acids to TDP-43 and demonstrated that this assay can be used to assess both direct and competitive binding interactions. We have demonstrated it is capable of identifying small molecule inhibitors of the nucleic acid-TDP-43 interaction from compound libraries. These compounds may prove to be useful biochemical tools to facilitate the elucidation of the function of TDP-43 and may lead to novel therapeutics for indications where the TDP-43-nucleic acid interaction is causal to the associated pathology. Screening of additional compound libraries and the development of downstream assays of TDP-43 function will enhance our understanding of the pathogenic role of TDP-43 in ALS and other diseases.

Disclosures: **A.C. Pawlyk**, ALS Biopharma, LLC, Employment; ALS Biopharma, LLC, Ownership Interest; **J.A. Cassel**, ALS Biopharma, LLC, Employment; **B.E. Blass**, ALS Biopharma, LLC, Employment; **A.B. Reitz**, ALS Biopharma, LLC, Employment; ALS Biopharma, LLC, Ownership Interest.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.4

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Howard Hughes Medical Institute

Title: Simultaneous multicolor brightly fluorescent labeling of axonal arbors, synaptic terminals and neuronal nuclei: Rabies virus as an anterograde tracer

Authors: *I. R. WICKERSHAM[1,2], H. S. SEUNG[1,2];
[1]Brain & Cognitive Sci., MIT, CAMBRIDGE, MA; [2]Howard Hughes Med. Inst., Cambridge, MA

Abstract: Determining the projection targets of a given brain region requires anterograde tracing: injecting some substance that will be taken up by neurons at the injection site and label their axonal arbors. The best tracers currently available are viral vectors based on lentivirus and adeno-associated virus. These vectors are nevertheless limited by their low expression levels and the long waiting times required for sufficient expression. In addition, their limited payload capacities have prevented effective simultaneous labeling of subcellular compartments such as presynaptic terminals, for increasing confidence in probable synapses, and nuclei, to provide a normalization factor for comparing the results of different injections. Here we show that pseudotyping a deletion-mutant rabies virus with the vesicular stomatitis virus envelope protein drastically attenuates its otherwise outstanding ability to infect retrogradely, transforming it into a vector that infects cells almost entirely local to the injection site, but leaves intact its signature ability to drive rapid, extremely high level transgene expression. We further demonstrate that, because of rabies virus's extensible structure, its genome can easily accommodate multiple transgenes, in this case encoding fluorophores separately labeling the cytoplasm, nucleus, and presynaptic terminals. These modifications make such vectors excellent candidates for simple anterograde tracing in their own right. In addition, however, because other versions can similarly be engineered to fluorescently label the postsynaptic densities and cytoplasms of neurons targeted either retrogradely or genetically, they could be used in combination to separately label putatively connected pre- and postsynaptic populations with complementary fluorophores for jointly visualizing axons, dendrites, and likely synaptic contacts. Rabies viral vectors are therefore extremely versatile tools for targeted high-resolution assays of neural circuitry.

Disclosures: I.R. Wickersham, None; **H.S. Seung**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.5

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: CIRM Grant RN1-00577

NIH Grant DP2OD004744

March of Dimes Grant 5-FY08-110

Title: Probing ion channels with genetically encoded unnatural amino acids

Authors: *L. WANG, B. SHEN, J.-Y. KANG;
The Salk Inst., LA JOLLA, CA

Abstract: Genetically encoding unnatural amino acids provides a unique way to selectively introduce novel chemical and physical properties into proteins for the investigation of proteins and protein-involved biological processes in live cells. We developed new strategies for generating orthogonal tRNA-synthetase pairs: A type 3 polymerase III promoter was identified for the efficient and functional expression of orthogonal prokaryotic tRNAs in mammalian cells, and aminoacyl-tRNA synthetase mutants evolved in yeast or bacteria were transferred for direct use in mammalian cells. These strategies made possible the genetic incorporation of diverse unnatural amino acids into proteins in primary neurons and neural stem cells. Using unnatural amino acids with extended side chains, we studied the inactivation mechanism of Kv1.4, and found that the bulkiness of the residues in the inactivation peptide is essential for fast channel inactivation. Using unnatural amino acids with photocaged side chains, we showed the ability to control ion channel function and thus neuronal activity with light. Lastly, fluorescent unnatural amino acids were used to probe how ion channels respond to membrane depolarization by combining fluorescence microscopy and patch clamp.

Disclosures: L. Wang, None; **B. Shen**, None; **J. Kang**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.6

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Development of a sustained release biodegradable microparticle formulation of the dopamine reuptake inhibitor GBR12909 for longer-term intervention of drug abuse

Authors: *J. S. RODEFER[1], S. R. D'MELLO[2], T. E. PRISINZANO[3], A. K. SALEM[2]; [1]Psychology & Neurosci., Florida State Univ., TALLAHASSEE, FL; [2]Pharmaceutics, Univ. of Iowa, Col. of Pharm., Iowa City, IA; [3]Medicinal Chem., Univ. of Kansas, Lawrence, KS

Abstract: Cocaine and methamphetamine are widely abused drugs with serious effects on public health, including the spread of human immunodeficiency virus (HIV), hepatitis, and tuberculosis. On a biochemical level, cocaine inhibits the reuptake of dopamine (DA), serotonin (5-HT), and norepinephrine (NE). Evidence suggests, however, that cocaine binding to the dopamine transporter (DAT) and subsequent inhibition of DA reuptake may be responsible for its reinforcing properties and thus represents a suitable target for the design of an agonist substitution-type medication for cocaine and methamphetamine abuse.
Dopamine Reuptake Inhibitors (DRIs) have shown significant potential in the treatment of stimulant abuse. GBR12909 and its derivatives represent suitable model compounds in DRI treatment of cocaine abuse. We formulated GBR 12909 to be released at sustained and controlled rates similar to repeated daily administration of GBR 12909 with the exception that only a single administration is necessary. Given non-compliance issues with cocaine and methamphetamine treatment, this once daily treatment regimen is of great advantage.
Poly alpha hydroxy acid polymers were prepared with controlled molecular weights and lactic acid (LA) /glycolic acid (GA) ratios that produced defined degradation rates and had extended effects on drug seeking and related behaviors that were defined by the physico-chemical properties of the polymer. Particles prepared from these polymers are non-toxic and can be packaged, scaled up, and stored easily.
Water soluble GBR12909 HCl was entrapped into poly lactic-co-glycolic acid (PLGA) particles using the water in oil in water (w/o/w) solvent evaporation technique. The method involves the use of three phases: 1) an inner water phase containing the GBR12909.HCl to be incorporated, 2) an intermediate organic phase consisting of a polymer/methylene chloride solution, and 3) an outer water phase containing an emulsifying agent.
Preliminary validation of our work suggests that GBR12909 microparticles were effective in locomotor behavioral assessments. Administration of GBR12909 microparticles was observed to significantly increase locomotor behavior in rats in a concentration-dependent fashion that resembled increasing doses of acute peripheral administration of GBR12909. These behavioral data suggest that these pharmaceutical methods are effective in producing sustained release of compounds and may serve as an effective model for novel medication development for the treatment of cocaine and methamphetamine abuse.

Disclosures: J.S. Rodefer, None; **S.R. D'Mello**, None; **T.E. Prisinzano**, None; **A.K. Salem**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.7

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH

Title: Chromosome conformation capture at the grin2b locus reveals a conserved chromatin loop with a possible function in transcriptional regulation

Authors: *R. BHARADWAJ[1,2], Y. JIANG[1,2], S. AKBARIAN[3];
[1]UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL, WORCESTER, MA;
[2]BRUDNICK NEUROPSYCHIATRIC RESEARCH INSTITUTE, Worcester, MA;
[3]BRUDNICK NEUROPSYCHIATRIC RESEARCH INSTITUTE, PSYCHIATRY, WORCESTER, MA

Abstract: Chromosome conformation capture (3C) provides a semi-quantitative assessment of the level of physical interaction between specific regions of the genome, both in cis (within) and in trans (between different chromosomes). 3C entails formaldehyde cross-linking of chromatin followed by restriction enzyme digestion and intra-molecular ligation resulting in a library of ligated products. These are then analyzed, by PCR across the ligation junctions, in order to assess the level of interaction between any two regions specified by restriction fragments, in the genome. Here, we illustrate an example of this approach. We identified a specific binding site for Setdb1 (SET domain, bifurcated 1)/Kmt1e/Eset, a histone 3 lysine 9 specific methyltransferase, within the third intron of Grin2b, encoding the NMDA receptor 2B subunit. We performed 3C analysis to capture potential interactions around 50kb of the Grin2b transcription start site (TSS) in mouse brain nuclei . A single loop was identified, tethering the Setdb1 target site in intron no. 3 to the Grin2b TSS. Of note, chromatin surrounding the TSS was highly enriched with KAP1 (KRAB-associated protein 1), a known transcriptional co-repressor that physically interacts with Setdb1. Interestingly, 3C on forebrain nuclei of CamkII-Setdb1 transgenic mice showed a significant broadening of the cis-interacting part of the loop. This correlated with downregualated expression of Grin2b mRNA and protein in transgenic forebrain. Of note, we found evidence that a similar loop formation may exist in human cerebral cortex, because 3C on postmortem tissue indicated that the GRIN2b TSS is in physical contact with an intronic fragment located 26 kb downstream that shows 78% sequence conservation with Setdb1 target site in the murine Grin2b intron. We conclude that 3C is a useful tool to uncover higher order chromatin structures that are potentially conserved across species and that could play a role in

transcriptional regulation. Of note, GRIN2B sequence polymorphisms are associated with genetic risk for bipolar disorder and schizophrenia, and GRIN2B-specific antagonists may exert therapeutic effects in treatment resistant depression. Therefore, the findings presented here could provide novel insights into transcriptional regulation of an important NMDA receptor subunit in human and mouse brain, with important implications for the neurobiology of major psychiatric disease.

Disclosures: R. Bharadwaj, None; **Y. Jiang**, None; **S. Akbarian**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.8

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Creation of neurological rat models using zinc finger nucleases

Authors: *X. CUI, D. JI, D. FISHER, A. MCCOY, Y. WU, E. J. WEINSTEIN;
Sigma Advanced Genet. Engin. Labs, Sigma-Aldrich Corp., Saint Louis, MO

Abstract: The laboratory rat is the preferred model system in many neuronal studies for its physiology and larger size. However, until recently, the lack of genetic manipulation tools for the rat genome forced researchers to use the mouse instead. The creation of the first knockout rats via microinjection of zinc finger nucleases (ZFNs) into single-cell embryos was the beginning of a revolution in the rat research world and neuroresearch. Being the only tool to engineer the rat genome in a targeted fashion, ZFN technology allows specific and efficient introduction of desired mutations to the gene of interest.
ZFNs are fusion proteins of a zinc finger protein and the DNA endonuclease domain of a type II restriction enzyme, *Fok*I. ZFNs are engineered to bind and cleave at specific chromosomal locus to generate double strand breaks, repair of which results either insertions/deletions (potentially gene knockouts) by the non homologous end joining pathway or targeted integration/gene replacement by homologous recombination.
Using ZFN technology, we have generated knockout rat models for schizophrenia and related disorders (e.g. DISC1), Alzheimer's (such as ApoE, BDNF, APP), Parkinson's (Park2, Park7, SNCA, LRRK2, PINK1). More knockouts of genes involved in pain and addiction as well as Autism are in the make. In general, 10-30% live births from microinjected embryos were founders with various lengths of deletions, and 100% modified alleles tested transmitted to germline. Injection statistics, current status of each model, and available phenotyping data will

be discussed.
More importantly, we have successfully achieved ZFN-mediated targeted integration/knockin in rat embryos and generated animals bearing site-specific insertions. We will also report current progresses on introducing point mutations, conditional knockouts and knockins in the rat genome.

Disclosures: **X. Cui**, Sigma-Aldrich Corporation, Employment; **D. Ji**, Sigma-Aldrich Corporation, Employment; **D. Fisher**, Sigma-Aldrich Corporation, Employment; **A. McCoy**, Sigma-Aldrich Corporation, Employment; **Y. Wu**, Sigma-Aldrich Corporation, Employment; **E.J. Weinstein**, Sigma-Aldrich Corporation, Employment.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.9

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R21 MH076289

DOD Grant W81XWH-09-2-0114

Title: Generation and characterization of transgenic mice expressing both a neuronal silencer and transynaptic tracer

Authors: D. AIKATH[1], *C. G. KENTROS[2];
[1]Univ. of Oregon, Eugene, OR; [2]Inst. Neurosci., Univ. Oregon, EUGENE, OR

Abstract: Recent work with genetically modified mice has led to new tools for analyzing the connectivity and function of neural circuits, from genetically encoded transynaptic tracers for studying connectivity to heterologous ligand- or light-gated channels for electrophysiological manipulations. Since circuit analysis requires knowledge of both anatomical and functional connectivity, we present mice that enable the expression of both kinds of transgenes with cellular specificity. We have previously shown that transgenically expressed drosophila allatostatin receptors (AlstR) can be used for targeted neuronal silencing in a reversible, short-term manner. We created several lines of tetO mice using an IRES site to combine AlstR with a fluorophore-labeled neuronal tracer wheat germ agglutinin (DSRed-WGA), which is transported transynaptically to downstream neurons. The silencer and the tracer are both controlled by a tTA-tetO inducible system, allowing for cell-type specific expression through the promoter

driving the tTA, and also temporal regulation of transgene expression through the use of doxycycline. Thus, when crossed to cell-specific tTA lines, both the silencer and the tracer are expressed in the primary cell expressing the tTA. Thus the neurons can be reversibly silenced by application of the AL ligand, and due to the primarily anterograde transynaptic transport of the DSRed-WGA, their postsynaptic partners can be visualized by fluorescence microscopy either postmortem or in vitro. Moreover, doxycycline can be used to control the onset of WGA-expression, enabling one to distinguish major, direct synaptic partners from minor or indirect ones. These mice thereby enable the comparison of the functional connectivity of particular neurons to their anatomical connectivity by allowing one to examine the effects of turning off particular neurons on their identified downstream synaptic partners. This is possible both in real time in vitro and over extended periods in vivo, allowing for the direct examination of the dynamics of native neural circuits in both the long and short term.

Disclosures: D. Aikath, None; **C.G. Kentros**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.10

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Striatal gene expression in C57BL/6J and DBA/2J inbred mouse strains: Comparison of array and next generation sequencing platforms

Authors: *J. E. HUNTER[1], D. BOTTOMLY[2], P. DARAKJIAN[3], N. WALTER[4], R. SEARLES[5], S. MCWEENEY[2], R. HITZEMANN[4], K. BUCK[4];
[1]Behavioral Neurosci., [2]Oregon Clin. and Translational Res. Institute, Portland Alcohol Res. Ctr., [3]Behavioral Neuroscience, Portland Alcohol Reseach Ctr., [4]Behavioral Neuroscience, Portland Alcohol Res. Ctr., [5]Massively Parallel Sequencing Shared Resource, Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: C57BL/6J (B6) and DBA/2J (D2) are two of the most commonly used inbred mouse strains in neuroscience research. However, the only currently available mouse genome is based entirely on the B6 strain sequence (NCBI m37, April 2007). Subsequently, oligonucleotide microarray probes are based solely on this B6 reference sequence, making their application for gene expression profiling comparisons across mouse strains dubious due to their allelic sequence differences, including single nucleotide polymorphisms (SNPs) (Nature Methods 4:679,2007). Next generation transcriptome sequencing, termed RNA-Seq, has emerged as an alternate high-

throughput approach to overcome this limitation. Using RNA-Seq, one generates millions of short sequencing reads which are aligned to a reference sequence and 'digital RNA counting' is applied based on reads that map to exons. The aim of this study is to assess biological replicates of brain striatum RNA from B6 and D2 mice using an RNA-Seq platform, Illumina Genome Analyzer IIx, and two oligonucleotide microarray platforms, Affymetrix GeneChip 430 2.0 and Illumina WG 6.1. We overlap the genes queried by each platform and determine correlations of gene expression measures across platforms within strains as well as differential expression between the two strains. Using RNA-Seq, roughly 18 million 72 base pair sequencing reads were generated per sample. Aligning these reads to the mouse reference sequence allowed 17,846 genes annotated in Ensembl (www.ensembl.org) to be queried in both strains and 852 and 930 genes to be queried only in the B6 and D2 strains, respectively. Interestingly, more than half of the sequencing reads that aligned represented only a subset of all genes queried (roughly 10% in both strains). In preliminary analyses comparing the RNA-Seq data to the microarray platforms, we saw significant correlation among the platforms for differential expression between the two strains. These results in addition to coding SNPs detected using RNA-Seq will be discussed. [Supported by AA011114, DA005228, AA010760, AA07468, AA11034, AA13484, MH51372 ,and BX000222]

Disclosures: J.E. Hunter, None; **D. Bottomly**, None; **P. Darakjian**, None; **N. Walter**, None; **S. McWeeney**, None; **R. Hitzemann**, None; **K. Buck**, None; **R. Searles**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.11

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: HHMI

Title: Imaging neural activity with genetically encoded calcium indicators

Authors: *L. TIAN, S. A. HIRES, T. MAO, D. HUBER, J. AKERBOOM, K. SVOBODA, L. LOOGER;
Howard Hughes Med. Inst. Janelia Farm Res. Campus, Ashburn, VA

Abstract: Understanding neural coding, learning and memory requires precise, simultaneous observation of multiple neurons in awake, behaving animals. These requirements can be met by optical recording of brain activity using genetically encoded calcium indicators (GECIs). Since

they are encoded by DNA, GECIs can be delivered to the intact brain non-invasively, and targeted to defined populations of neurons and specific sub-cellular compartments for long-term, repeated, in vivo measurements. Over the last decade GECIs have been iteratively improved, and are now useful for quantitative imaging of neural activity in vivo. For example, GCaMP3 is being used to image large populations of neurons in behaving mice over months. Using GCaMP3 as an example, we describe the design and optimization of a GECI, including tuning indicators for specific applications. We will discuss the strengths and limitations of GCaMP3 in neural imaging, and propose strategies that might mitigate these limitations in next generation GECIs.

Disclosures: L. Tian, None; **S.A. Hires**, None; **T. Mao**, None; **D. Huber**, None; **J. Akerboom**, None; **K. Svoboda**, None; **L. Looger**, None.

Nanosymposium

536. Development of Novel Cellular and Molecular Tools

Location: Room 7B

Time: Tuesday, November 16, 2010, 8:00 am - 11:00 am

Program Number: 536.12

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R01 HD050735

NIH EB008432

NIH EB008281

NIH EB007813

NHMRC 486682, Australia

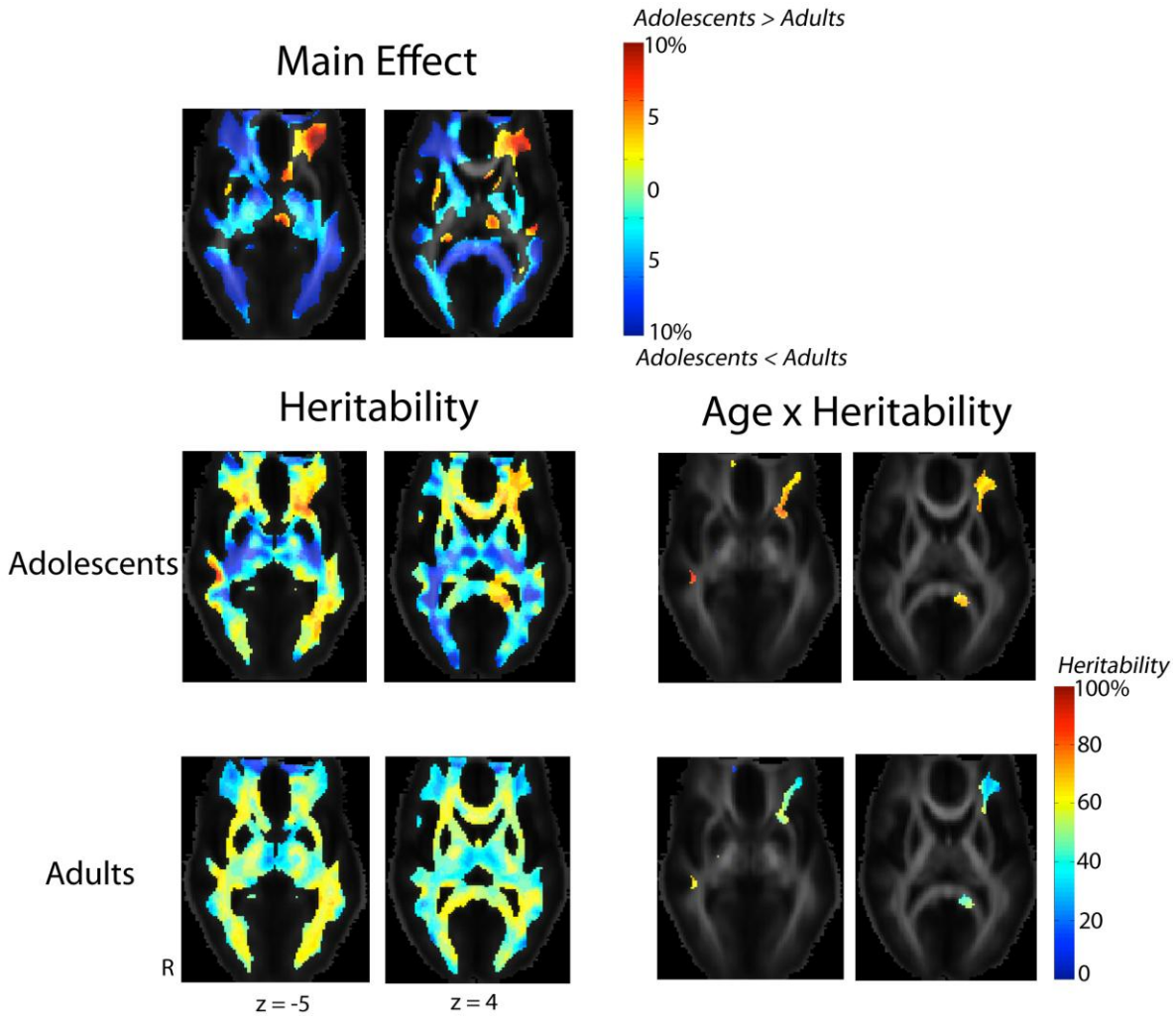
Title: Environmental factors surpass genetic influences in determining fiber microstructure as the brain develops: Diffusion tensor imaging in 705 twins

Authors: *M.-C. CHIANG[1], K. L. MCMAHON[2], G. I. DE ZUBICARAY[2], N. G. MARTIN[3], M. J. WRIGHT[3], I. HICKIE[4], A. W. TOGA[1], P. M. THOMPSON[1];
[1]UCLA, Los Angeles, CA; [2]Univ. of Queensland, Functional Magnetic Resonance Imaging Laboratory, Ctr. for Magnetic Resonance, Brisbane, Australia; [3]Queensland Inst. of Med. Res., Brisbane, Australia; [4]Brain and Mind Res. Institute, Univ. of Sydney, Sydney, Australia

Abstract: In young adults, white matter fiber integrity is under strong genetic control and

correlates with intellectual performance [1]. However, it is still unclear how genetic influences vary as the brain's fiber circuitry develops. Here we scanned 705 twins and their siblings, including 531 healthy adults (aged 20 or older) and 174 adolescents (aged 12 and 16), using 30-gradient diffusion tensor imaging (DTI) at 4 Tesla, an imaging method exquisitely sensitive to white matter integrity, quantified by the fractional anisotropy (FA) of water diffusion. At each point of the brain, we assessed age-related differences in heritability of white matter integrity between adolescence (age < 20 years) and adulthood (age ≥ 20 years) by modeling age as a moderator that linearly interacts with additive genetic and unique environmental variance components [2]. Direct age effects on FA were modeled. We used the false discovery rate method to adjust for multiple comparisons across voxels. Between age 12 and adulthood, brain fiber organization and coherence, measured by FA, increased by up to 10% in most of the white matter (Figure 1; MNI coordinates of the slices, in mm, are shown at the bottom). We also detected significant age x heritability interaction. White matter integrity in the left inferior and middle frontal gyri, the splenium of the corpus callosum on the left, and the right inferior fronto-occipital fasciculus, was significantly more heritable in the adolescents than adults. In adolescents, around 70-80% of the variation in FA was attributable to genetic factors, but in adults, only 30-40% of the variation in FA was attributable to genetic factors. In conclusion, we mapped dynamic changes in white matter heritability as the brain matures. Heritability of white matter integrity decreases as subjects get older. Environmental influences, e.g., learning, education, life experiences, diet, and exercise, start to dominate and increasingly determine brain fiber networks as one matures into adulthood.

[1] Chiang MC et al. (2009). J Neurosci. 29:2212-2224.
[2] Purcell S (2002). Twin Res. 5:554-571.



Disclosures: M. Chiang, None; **A.W. Toga**, None; **P.M. Thompson**, None; **K.L. McMahon**, None; **G.I. de Zubicaray**, None; **N.G. Martin**, None; **M.J. Wright**, None; **I. Hickie**, None.

Nanosymposium

624. Forebrain Neurogenesis and Patterning

Location: Room 25A

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 624.1

Topic: A.01. Brain Patterning

Support: Allen Institute for Brain Science

Title: Profiling the expression of 2,000 genes over embryonic and postnatal brain development identified clusters of co-regulated genes

Authors: *C. L. THOMPSON[1], L. NG[1], C. LEE[1], K. GLATTFELDER[1], A. HENRY[1], C. LAU[1], L. PUELLES[2], J. RUBENSTEIN[3], J. HOHMANN[1], A. JONES[1];
[1]Allen Inst. for Brain Sci., SEATTLE, WA; [2]Univ. of Murcia, Murcia, Spain; [3]UCSF, San Francisco, CA

Abstract: During brain development, numerous cascades of genes are activated in precise order to control each of the steps from proliferation to terminal differentiation, specific to each brain region. To gain insight into the transcriptional cascades underpinning brain development, in situ hybridization data was generated over four embryonic and three postnatal ages for 2000 genes, including ~700 transcription factors. These data were registered to a coordinate framework with classically annotated reference atlases, and expression information was quantified and summarized per brain voxel (80-200 micron voxel size). The complexity of this dataset, made possible by the large number of genes (2000 genes), high temporal resolution (7 ages), and spatial resolution (80-200 micron voxel size) enabled resolution of co-regulated gene sets across the brain.
At each developmental stage and for each of four major regions (telencephalic vesicle, diencephalon, midbrain, and hindbrain), the degree of co-regulation between genes was determined as a correlation value based upon the spatial similarity of gene expression patterns at the voxel level. The set of 2000 genes were hierarchically clustered based to identify co-regulated gene sets. In the E13.5 diencephalon, both small and large clusters of genes were identified. A handful of highly-correlated genes consisting primarily of transcription factors (e.g., Pax6), identified the alar plate of prosomere 3 (corresponding to the future location of the GABA-ergic reticular nucleus of the thalamus). These genes maintained close correlation over multiple timepoints suggesting that this gene set provides a cellular identity of the reticular nucleus that persists into adulthood. On the other hand, a larger cluster of ~150 genes was expressed in the ventricular zone of the E13.5 diencephalon, but by E18.5 these genes divided into multiple smaller un-related clusters, suggesting that the gene expression in the E13.5 ventricular zone represented a very heterogenous cell population that differentiated into diverse cellular populations with unique spatial characteristics by E18.5.
Examination of the co-regulation of genes over time with high spatial resolution may provide a launching point for future analyses of transcriptional regulation and network analyses. In this dataset, several well-known transcription factors cluster tightly over time with their downstream targets, suggesting that novel relationships between transcription factors and putative target genes may be identifiable. The ISH dataset is available as part of the Allen Developing Mouse Brain Atlas (www.brain-map.org).

Disclosures: C.L. Thompson, None; **L. Ng**, None; **C. Lee**, None; **K. Glattfelder**, None; **A. Henry**, None; **C. Lau**, None; **L. Puelles**, None; **J. Rubenstein**, None; **J. Hohmann**, None; **A. Jones**, None.

Nanosymposium

624. Forebrain Neurogenesis and Patterning

Location: Room 25A

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 624.2

Topic: A.07. Development of Motor, Sensory and Limbic Systems

Support: Stanley Medical Research Institute

Title: Age-dependent and region-specific gene expression profiles in the prefrontal cortex and the caudate nucleus of human brain

Authors: *K.-H. CHOI[1], B. HIGGS[3], L. ZHANG[4], S. DIGLISIC[2], C. WEICKERT[5], M. WEBSTER[2];
[1]Psychiatry, Stanley Med. Res. Inst., ROCKVILLE, MD; [2]Stanley Med. Res. Inst., Rockville, MD; [3]Elashoff Consulting, Redwood City, CA; [4]Psychiatry, USUHS, Bethesda, MD; [5]Schizophrenia Res. Inst. (SRI), Univ. of New South Wales, Sydney, Australia

Abstract: We have reported that a substantial number of schizophrenia susceptibility genes undergo expression changes in the prefrontal cortex (PFC) during postnatal development (Choi et al. 2009). Here, we investigated region-specific and developmental gene expression profiles between the PFC and the caudate nucleus (CN) of human brain. Genome-wide expression profiles were compared between these regions in humans ranging in age from 1 month to 49 years using the Affymetrix microarrays (>54,000 transcripts). Using stringent criteria of significance (adjusted coefficient r2 > 0.6 and FDR-adjusted q-value < 0.05), we identified 1,236 and 1,745 genes that show age-dependent expression changes in the PFC and the CN, respectively. We also identified 517 common genes that show same directional changes in expression in both regions. Biological pathway analysis of these age-dependent genes revealed that categories such as nervous system development (PFC: adj.p=4.7E-6, and CN: adj.p=0.005) and the mitochondrion (PFC: adj.p=3.9E-10, and CN: adj.p=0.009) were enriched in both regions. Strikingly, a majority of the mitochondrial genes in the PFC (91%), but not in the CN, showed gradual increase in expression during postnatal development (see below figure). Taken together, our results suggest that a substantial number of genes undergo region-specific and age-dependent expression changes during brain development. Thus, disturbances in normal expression patterns of disease susceptibility genes during sensitive period of brain development may result in early onset of psychiatric symptoms in humans.$$graphic_{BA15762D-8C71-4B21-937A-963B6E44973F}$$

Disclosures: K. Choi, None; **B. Higgs**, None; **S. Diglisic**, None; **C. Weickert**, None; **M. Webster**, None; **L. Zhang**, None.

Nanosymposium

624. Forebrain Neurogenesis and Patterning

Location: Room 25A

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 624.3

Topic: A.01. Brain Patterning

Support: NIH Grant 5F32HD053198

NIH Grant 5R01HD036404

NIH Grant 5R01MH081187

Title: ENU mutagenesis identifies three novel genes required for forebrain development

Authors: *R. W. STOTTMANN, T. SIGGERS, D. BEIER;
Genet., Brigham & Women's Hospital/ Harvard Med. Sch., Boston, MA

Abstract: We have performed an ENU mutagenesis screen in the mouse specifically focusing on mutations affecting neurodevelopment. We have identified eight mutations in our screen and here we discuss our current studies on three of these novel genes affecting cortical development. The most remarkable phenotype uncovered to date is the rudolph mutation, which has severe developmental defects in both the CNS and appendicular skeleton. The organization of the neocortex is profoundly disrupted and contains clustered cell bodies that appear to be neurogenic foci. The causal gene is the cholesterol biosynthesis enzyme Hsd17b7, which is notable given the recent implication of a role for oxysterols in mediating intracellular components of Hedgehog signaling. We see decreased induction of known Sonic hedgehog (Shh) target genes both in vivo and in vitro, revealing a requirement for embryonic cholesterol metabolism in both CNS development and normal Shh signaling. Notably, we find that introduction of a mutation which results in Shh ligand-independent upregulation of hedgehog signaling (the alien mutation in Ttc21b) ameliorates the mutant phenotype. This result supports an evolving model in which intracellular cholesterol synthesis mediates activity of Smoothened; a hypothesis we are exploring further in the rudolph mutant.
We also have discovered a line we call brain dimple (brdp), which carrys a mutation in beta tubulin, 2b (Tubb2b). This mutant has an extreme reduction in cerebral cortex tissue, most severely affecting the caudo-lateral portion of the cortex. Mutations in TUBB2B have recently been identified in human patients with asymmetrical poymicrogyria, but no mouse allele has previously been characterized. The brdp phenotype emerges after the onset of mouse

neurogenesis (E11.5) and we are currently studying the mechanism of Tubb2b function in the cortex. Brdp/+ adult mice have a hyperactivity phenotype and we show they have cortical hypocellularity and defects in cortical lamination.
Additionally, we describe a mutation in grainyhead-like 2 (Grhl2), which results in small embryos with hypoplastic cortices and incompletely penetrant craniofacial defects and encephaloceles. Current studies are aimed at identifying the mechanism leading to the reduction in cortical size. We have examined the expression of the Grhl1, Grhl2, and Grhl3 genes in the developing mouse cortex and are using Protein-Binding Microarrays (PBMs) in conjunction with traditional RNA expression microarrays to identify the targets of Grlh2 that are required for cortical development.

Disclosures: R.W. Stottmann, None; **T. Siggers**, None; **D. Beier**, None.

Nanosymposium

624. Forebrain Neurogenesis and Patterning

Location: Room 25A

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 624.4

Topic: A.01. Brain Patterning

Support: KAKENHI 22590055

KAKENHI 20200011

Title: IgSF molecule MDGA1 is involved in radial migration and positioning of a subset of cortical upper-layer neurons

Authors: *T. YAMAMOTO[1], T. ISHIKAWA[1], H. MATSUMOTO[1], N. GOTOH[1], C. MURAYAMA[1], M. IWASHITA[2], F. MATSUZAKI[2], T. SUZUKI[1];
[1]Fac. of Pharmaceut. Sci., Hokkaido Univ., Sapporo, Japan; [2]Ctr. for Developmental Biol., RIKEN, Kobe, Japan

Abstract: MDGA1 encodes a GPI-anchored IgSF molecule containing MAM (meprin, A5 protein, PTPμ) domain, which we isolated as a gene expressed by a specific subset of spinal and DRG neurons. In mouse cerebral cortex, expression of MDGA1 is also specifically observed in neurons located in the upper layer. To investigate the functional role of MDGA1 in neural development, we generated the LacZ-KI-KO mice, which were subsequently backcrossed to C57BL6 mice more than ten generations to make the resultant strain congenic. Although the homozygous mice showed no readily detectable alteration in gross anatomy of nervous system,

MDGA1 mutant neurons behaved differently during developmental process of cerebral cortex formation. At E16.5, MDGA1-expressing wild type cells were observed throughout the cortical plate. However, in homozygous littermate embryos, majority of LacZ-expressing MDGA1 mutant cells resided in deeper layer of the cortical plate, underneath the Er81-expressing earlier born future layer V neurons. At E17.5, a part of mutant cells migrated to the upper layer but some population still resided in the deeper layer in homozygous embryos, while virtually all of MDGA1-expressing cells migrated to the uppermost area of the cortical plate in wild type littermates. These observations collectively indicate that MDGA1 is involved in proper radial migration of MDGA1-expressing neurons. By E18.5, majority of MDGA1 mutant neurons migrated to the upper layer; however, their position in the upper layer is apparently lower than those of wild type neurons. This positioning is unchanged until P0, and corrected by P7. Interestingly, Cux2-expressing upper layer neurons did not show alterations between wild type and homozygous littermates during the process, indicating that MDGA1 is expressed and required by a subset of radially migrating neurons, and further suggesting that radial migration and positioning might be differently regulated among cell types in upper layer neurons.

Disclosures: T. Yamamoto, None; **T. Ishikawa**, None; **H. Matsumoto**, None; **N. Gotoh**, None; **C. Murayama**, None; **T. Suzuki**, None; **M. Iwashita**, None; **F. Matsuzaki**, None.

Nanosymposium

624. Forebrain Neurogenesis and Patterning

Location: Room 25A

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 624.5

Topic: A.09. Evolution of Developmental Mechanisms

Support: CIRM Predoctoral Fellowship

Title: Neurogenic radial glia in the outer subventricular zone of human neocortex

Authors: *J. H. LUI[1], D. V. HANSEN[2], A. R. KRIEGSTEIN[2];
[1]Eli and Edythe Broad Ctr. of Regeneration Med. and Stem Cell Res., UCSF, SAN FRANCISCO, CA; [2]Eli and Edythe Broad Ctr. of Regeneration Med. and Stem Cell Res., UCSF, San francisco, CA

Abstract: Neurons in the developing rodent cortex are generated from radial glial cells that function as neural stem cells. These epithelial cells line the cerebral ventricles and generate intermediate progenitor cells that migrate into the subventricular zone (SVZ) and proliferate to increase neuronal number. The developing human SVZ has a massively expanded outer region

(OSVZ) thought to contribute to cortical size and complexity. However, OSVZ progenitor cell types and their contribution to neurogenesis are not well understood. Here we show that large numbers of radial glia-like cells and intermediate progenitor cells populate the human OSVZ. We find that OSVZ radial glia-like cells have a long basal process but, surprisingly, are non-epithelial as they lack contact with the ventricular surface. Using real-time imaging and clonal analysis, we demonstrate that these cells can undergo proliferative divisions and self-renewing asymmetric divisions to generate neuronal progenitor cells that can proliferate further. We also show that inhibition of Notch signalling in OSVZ progenitor cells induces their neuronal differentiation. The establishment of non-ventricular radial glia-like cells may have been a critical evolutionary advance underlying increased cortical size and complexity in the human brain. We are currently investigating the evolutionary mechanisms that gave rise to this cell population.

Disclosures: J.H. Lui, None; **D.V. Hansen**, None; **A.R. Kriegstein**, None.

Nanosymposium

624. Forebrain Neurogenesis and Patterning

Location: Room 25A

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 624.6

Topic: A.01. Brain Patterning

Support: NIH/NINDS Grant R01 NS 055064

Title: Morphometric analysis of the lateral ventricles in the human fetal brain: An in utero MRI study

Authors: *J. A. SCOTT, V. RAJAGOPALAN, P. HABAS, K. KIM, J. CORBETT-DETIG, O. GLENN, A. J. BARKOVICH, C. STUDHOLME;
Radiology and Biomed. Imaging, Univ. of California, San Francisco, San Francisco, CA

Abstract: The lateral ventricles (LV) of the fetal brain begin as dilated compartments and gradually become narrow and wing-shaped. Since the LV are non-rigid fluid filled spaces deep in the brain, growth of surrounding structures may strongly influence the shape under normal development. To date, the progression of the LV to its mature shape was studied qualitatively. Here we quantified and modeled changes in volume and shape of the LV. We studied 39 normal human fetal brains from 20 to 28 gestational weeks by motion-corrected *in utero* structural MRI reconstructed into 3D image volumes. We characterized local volume changes using tensor-based morphometry (TBM) and local changes in shape by statistical analysis of surface

curvature. The images were automatically segmented into ventricles and brain tissue classes. A symmetric, group-wise, non-rigid registration was used to bring all tissue maps into an average coordinate system. Local volume growth was characterized by the Jacobian determinant of the non-rigid mappings to each individual. Triangular meshes of the LV surfaces were used to calculate mean curvature at each vertex. General linear modeling was used to investigate age-related changes in both global and local LV volume and surface curvature. Two TBM analyses of local volume change were conducted. Firstly, looking at growth relative to the overall brain size and secondly, relative to the global volume of the LV. Global LV volume increases at a rate of 0.5 cm^3/wk (4.1 +/- 1.3 cm^3) and surface area increases linearly at a rate of 0.24 cm^2/wk (2.64 +/- 0.59 cm^2). However, the ratio of LV to brain volume decreases by approximately 2% from 20 to 28 weeks. Relative to whole brain growth, TBM shows that the LV grew slower along the lateral and dorsal aspects in the frontal, parietal and occipital lobes. Relative to total LV growth, significantly greater expansion of the LV is found in the ventro-medial part of the anterior horn. While the average curvature over the whole surface does not change significantly, local convexity increases in the frontal horns, ventro-lateral aspect bordering the thalamus, and dorso-lateral aspect deep to the emerging intraparietal sulcus. These data indicate symmetrical patterns of shape change and largely uniform volume gains in the LV during the latter half of gestation, a period in which LV growth is not proportional to brain tissue growth. The locations of curvature change in the LV suggest expansion of surrounding tissue structures and cortical folding play a role in shaping the LV. The model of LV development generated here may be used for comparison in the study of ventriculomegaly and other developmental abnormalities that may alter LV shape and size.

Disclosures: J.A. Scott, None; **V. Rajagopalan**, None; **P. Habas**, None; **K. Kim**, None; **J. Corbett-Detig**, None; **O. Glenn**, None; **A.J. Barkovich**, None; **C. Studholme**, None.

Nanosymposium

624. Forebrain Neurogenesis and Patterning

Location: Room 25A

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 624.7

Topic: A.02. Neurogenesis and Gliogenesis

Support: Ministerio de Educación y Ciencia FEDER AGL2006-10160

Title: Serum albumin and alpha-fetoprotein regulate brain development

Authors: *A. G. GARCÍA-GARCÍA, E. POLO-HERNÁNDEZ, A. VELASCO, A. TABERNERO, J. M. MEDINA;

Univ. of Salamanca / INCYL, Salamanca, Spain

Abstract: We have previously found that albumin induces in astrocytes the synthesis of the neurotrophic factor oleic acid. Albumin is internalized in astrocytes through endocytosis mediated by the receptor megalin and in a process dependent on caveolins. Inside the astrocyte, albumin sequestrates oleic acid in the endoplasmatic reticulum, thus activating the transcription factor SREBP-1. This transcription factor, induces stearoyl-CoA desaturase-1 (SCD-1), which is the rate-limiting enzyme in the synthesis of oleic acid. Oleic acid is released by astrocytes to the extracellular space, thus being available for neurons.
In fact, oleic acid induces the growth of the neurites and the expression of the axonal growth-associated protein 43 (GAP-43) together with the microtubule-associated protein 2 (MAP-2) , markers of axonal and dendritic growth respectively.
This effect is mediated by the nuclear receptor PPAR-alpha, Protein Kinase C and and the bHLH transcription factor NeuroD2, suggesting that oleic acid is a neurotrophic factor during late neurogenesis.
Albumin is a plasma protein that has its highest concentrations in brain during the embrionary and neonatal life. Albumin reaches maximum levels in brain by day 1 after birth, coinciding with the maximum expression of SCD-1 and the increase of MAP-2 and GAP-43.
Alfa-fetoprotein (AFP), is a plasma protein similar to albumin in structure and properties, with high concentrations in plasma, during the embrionary period. In fact, we have demonstrated by Western Blot analysis, that the pattern of serum alpha-fetoprotein showed two peaks in rat brain at E15.5 and E19.5. In addition, our results show that oleic acid concentrations, determined by HPLC, increases in brain when the ratio of AFP/ albumin concentrations plunges. This result correlates with the appearance of the first developing structures in the rat encephalon. In vitro, purified AFP reduced dramatically neuronal viability, by promoting apoptosis. Moreover, under these circumstances, AFP reduced the effect of oleic acid on neuronal differentiation by decreasing the levels of MAP-2 and GAP-43. Taken together these results suggest that AFP and albumin may play a role in regulating brain development.

Disclosures: A.G. García-García, None; **E. Polo-Hernández**, None; **A. Velasco**, None; **A. Tabernero**, None; **J.M. Medina**, None.

Nanosymposium

625. Synaptic Transmission Dynamics

Location: Room 6F

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 625.1

Topic: B.07. Synaptic Transmission

Support: NIH Grant DC008125

Title: Developmental mechanisms for suppressing the effects of delayed release at the endbulb of Held

Authors: H. YANG[1], *M. A. XU-FRIEDMAN[2];
[1]Dept. Biol. Sci., [2]SUNY Buffalo, BUFFALO, NY

Abstract: Delayed release of neurotransmitter, also called asynchronous release, is commonly observed at synapses, yet it is not known if it plays any function in information processing. We examined this issue at endbulb of Held synapses, which are formed by auditory nerve fibers onto bushy cells in the cochlear nucleus. Endbulbs from CBA/CaJ mice aged P6-49 show prominent delayed release when driven at physiologically-relevant rates. In bushy cells from mice before the onset of hearing (P6-12), spikes appear to be driven by delayed release as long as 100 ms after a train of presynaptic activity. However, no such spikes are observed in bushy cells from mice after the onset of hearing (>P14). Dynamic-clamp experiments indicated that delayed release can drive spikes in older bushy cells provided synchronous release is absent, suggesting that activity normally suppresses these spikes. We investigated the contribution of different potassium channels to this effect, and found that apamin and α-dendrotoxin revealed late spikes. This suggests that in older bushy cells, postsynaptic activity triggered by synchronous release activates $K_V1.x$ and SK channels, which suppresses the subsequent effects of delayed release. We also considered the presynaptic interaction between synchronous and delayed release. Enhancement of delayed release using strontium was correlated with a drop in the synchronous EPSC in voltage clamp and a drop in firing probability in current clamp, while EGTA-AM had the opposite effects. These effects were consistent with delayed and synchronous release competing for a single vesicle pool.

Disclosures: H. Yang, None; **M.A. Xu-Friedman**, None.

Nanosymposium

625. Synaptic Transmission Dynamics

Location: Room 6F

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 625.2

Topic: D.14. Cerebellum

Support: NIH grant R01EY011027

HHMI

Title: Shared intrinsic electrophysiological signature of diverse cerebellar mossy fiber neurons

Authors: *K. E. KOLKMAN[1,2,3], L. E. MCELVAIN[1,2,3], A. SAKATOS[1], B. ZINGG[1,3], S. DU LAC[1,2,3];
[1]Salk Inst., LA JOLLA, CA; [2]Neurosciences Grad. Program, UCSD, La Jolla, CA; [3]Howard Hughes Med. Inst., La Jolla, CA

Abstract: The cerebellum receives sensory, motor and cortical signals via distinct populations of mossy fiber cerebellar-projecting neurons (CPNs). CPNs provide input to granule, Golgi and unipolar brush cells, but despite their importance, little is known about how their intrinsic properties transform inputs into firing rate. The in vivo firing of CPNs varies widely, and a key question is whether CPNs simply relay information to the cerebellum or perform more complex computations. In this study, we examine the intrinsic firing of CPNs and their underlying currents by performing dextran injections into the mouse cerebellum and targeting whole-cell patch-clamp recordings to retrogradely labeled CPNs. We recorded CPNs in eight precerebellar nuclei: the external cuneate, lateral reticular, medial vestibular, pontine, supragenual, Roller/intercalatus, prepositus hypoglossi and reticularis tegmenti pontis (NRTP). CPNs in all nuclei could fire high frequency bursts and sustain firing over 100 Hz throughout a 1 second-long somatic depolarizing current step. Depolarizing current pulses resulted in a linear or bilinear firing rate relationship over a wide range of currents, and when probed with sinusoidally modulating current, CPNs responded with constant gain across all tested frequencies. Therefore, although CPNs convey diverse signals to the cerebellum, their electrophysiological properties are remarkably similar; they perform a linear or bilinear scaling of somatic current into neuronal firing rate.
To determine what specific ion channels mediate the linear current-to-firing rate relationship over a wide dynamic range, we assessed the effects of potassium channel blockers and recorded changes in firing. We find that slow-conductance calcium-activated potassium (SK) and voltage-activated potassium (Kv3) currents are crucially important for shaping the relationship between current input and CPN firing rate. Blocking SK channels resulted in an increase in gain and a decrease in the current range over which CPNs could modulate firing rate. Blocking Kv3 currents caused an overall decrease in the current range over which CPNs could fire. Blockade of both currents renders neurons incapable of sustained firing, and even partial blockade of both results in a dramatic decrease in the range over which CPNs can fire. We conclude that CPNs share common channel expression that enables them to scale widely diverse inputs and faithfully convey a wide range of inputs to the cerebellum.

Disclosures: K.E. Kolkman, None; **L.E. McElvain**, None; **A. Sakatos**, None; **B. Zingg**, None; **S. du Lac**, None.

Nanosymposium

625. Synaptic Transmission Dynamics

Location: Room 6F

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 625.3

Topic: B.07. Synaptic Transmission

Support: Swedish research council project no. 126000

Title: Development of synaptic connectivity onto interneurons in the CA1 region of the rat hippocampus

Authors: *E. L. HANSE[1], I. RIEBE[2];
[2]Neurosci. and Physiol., [1]Goteborg Univ., 40530 Goteborg, Sweden

Abstract: The impact of a given presynaptic neuron on the firing probability of the postsynaptic neuron critically depends on the number of functional release sites that connect the two neurons. In the hippocampal CA1 region this synaptic connectivity between glutamatergic CA3 and CA1 pyramidal neurons has been found to increase from single connections in the neonatal rat, to multiple connections in the young adult rat. On the other hand, GABAergic interneurons form multiple connections onto CA1 pyramidal neurons already in the neonatal rat. In the present study, we have examined glutamate and GABA connectivity onto GABAergic CA1 stratum radiatum interneurons in the hippocampal slice, by comparing the amplitudes of action potential dependent and independent spontaneous synaptic currents. In contrast to the multiple glutamate connectivity previously found onto pyramidal neurons in young adult rats, we found single glutamate connectivity onto interneurons. For GABA connectivity, on the other hand, we found multiple synaptic connections, as onto the pyramidal neurons. These results suggest that the developments of glutamate synaptic connectivity onto pyramidal neurons and onto interneurons are governed by different mechanisms, despite similar origin of the presynaptic input. This difference in the development of glutamate synaptic connectivity adds to the notion that whereas glutamatergic transmission onto pyramidal neurons undergoes substantial developmental changes, glutamatergic transmission onto interneurons does not.

Disclosures: E.L. Hanse, None; **I. Riebe**, None.

Nanosymposium

625. Synaptic Transmission Dynamics

Location: Room 6F

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 625.4

Topic: D.14. Cerebellum

Support: NIH Grant EY018561

NIH Grant DC006668

Title: Vestibular climbing fiber signals modulate complex and simple spikes in Purkinje cells in mouse uvula-nodulus

Authors: *V. A. YAKHNITSA, N. H. BARMACK;
Physiol.& Pharmacol., OHSU, Portland, OR

Abstract: A universal feature of cerebellar physiology is that Purkinje cell complex and simple spikes (CSs, SSs) discharge antiphasically. Accordingly, ipsilateral roll-tilt increases the discharge of CSs and decreases that of SSs in Purkinje cells in the uvula-nodulus. CSs reflect the modulated climbing fiber input from the contralateral inferior olive. Modulation of SSs is less well understood. Vestibular primary afferent mossy fibers terminate on granule cells, whose ascending axons bifurcate into parallel fibers that make excitatory synapses on Purkinje cell dendrites. However, parallel fibers are only one of several synaptic influences on SSs. Purkinje cell spontaneous activity and inputs from inhibitory interneurons, stellate and basket cells, also influences Purkinje cell discharge. The antiphasic discharge of CSs and SSs could be generated by independent activation of climbing and mossy fibers or one of these two pathways could regulate the other. We tested these possibilities by separately manipulating afferent signals conveyed by mossy and climbing fibers in the anesthetized mice. We blocked modulation of the vestibular primary afferent mossy fiber pathway by making a unilateral labyrinthectomy (UL), leaving intact the vestibular climbing fiber input that originates from the contralateral labyrinth. After UL, modulated SSs persisted in Purkinje cells with modulated CSs (50% of ipsilateral Purkinje cells, 20% of contralateral Purkinje cells). We blocked modulation of the vestibular climbing fiber input to the uvula-nodulus unilaterally by making microlesions of β-nucleus. We identified each recorded Purkinje cell by juxtacellular labeling with neurobiotin. Even partial microlesions disrupted vestibular modulation of both CSs and SSs. In 54 Purkinje cells contralateral to olivary microlesions, 15 lacked CSs, 22 had CSs unmodulated by vestibular stimulation and 17 had modulated CSs. In 8/15 Purkinje cells with no CSs, SSs also were not modulated, 4/15 were weakly modulated in phase with ipsilateral roll-tilt and 3/15 were weakly modulated out of phase. In 20/22 Purkinje cells with unmodulated CSs, SSs also were not modulated. In 14/17 cells with modulated CSs, SSs were modulated. A secondary effect of the microlesions was increased spontaneous discharge of SSs (CSs absent- 48.6±15.9 imp/s vs. CSs present- 20.8±12.7 imp/s, $P<0.0001$). In sum, loss of mossy fiber inputs did not abolish vestibular modulation of SSs. Loss of climbing fiber inputs reduced SS modulation. We conclude that SS modulation is a consequence of climbing fiber activity. Based upon interneuronal recordings we speculate that this effect is mediated primarily by stellate cells.

Disclosures: V.A. Yakhnitsa, None; **N.H. Barmack**, None.

Nanosymposium

625. Synaptic Transmission Dynamics

Location: Room 6F

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 625.5

Topic: B.07. Synaptic Transmission

Title: Inhibitory inputs regulate the temporal fidelity and contribute to the expression of input-timing-dependent plasticity in hippocampal CA1 neurons

Authors: *J. BASU[1], S. A. SIEGELBAUM[1,2,3];
[1]Dept. of Neurosci., [2]Dept. of Pharmacol., [3]Howard Hughes Med. Inst., Columbia Univ., New York, NY

Abstract: CA1 pyramidal neurons, which provide the major output of the hippocampus, receive direct sensory information from layer III neurons of the entorhinal cortex (EC) via the perforant pathway (PP) and indirect sensory information from layer II EC neurons through the trisynaptic pathway via the Schaffer Collateral (SC) inputs. Whereas the SC inputs synapse on the proximal dendrites of CA1 neurons and provide a strong excitatory drive, the PP inputs synapse on the distal dendrites of the CA1 neurons and provide only weak excitation of the CA1 neurons. Pairing the SC inputs with the PP inputs at a 20 ms timing delay (PP before SC), which matches the delay inherent to the trisynaptic SC path, leads to a robust long-term potentiation of the SC EPSPs (termed input-timing-dependent plasticity or ITDP; Dudman et al 2007). Here we report that the potentiation of the proximal postsynaptic depolarization during ITDP involves two separate mechanisms: i) an enhancement of the SC-evoked EPSP (eLTP), and ii) a long-term depression of SC evoked feed-forward inhibitory synaptic transmission (iLTD) onto the CA1 pyramidal neurons. Using whole cell recordings from CA1 neurons in mouse hippocampal slices, we found that the magnitude of ITDP was reduced by more than 50% and the strict timing dependence relaxed when inhibition was blocked using $GABA_A$ and $GABA_B$ receptor antagonists. In control slices application of GABA blockers caused a large (~100%) increase in the peak depolarization elicited by SC stimulation due to elimination of feed-forward inhibition. In contrast following induction of ITDP, the GABA blockers caused little or no increase in the peak depolarization, consistent with a reduction in feed-forward inhibition. In a separate set of experiments, we directly monitored inhibitory postsynaptic currents (IPSCs) (at +10 mV) before and after induction of ITDP. We observed a significant long-term depression of the IPSC that was highly specific to the 15-20 ms pairing interval. Moreover, both eLTP and iLTD required NMDA receptor activation and postsynaptic CA1 neuron depolarization as ITDP was suppressed with APV or by voltage clamping the cell at hyperpolarized membrane potentials. Finally, using specific antagonists, we found that iLTD also required activation of CB1 cannabinoid receptors

and mGluR1a receptors. Our study thus demonstrates how inhibitory inputs in the hippocampal circuit both determine the precise temporal fidelity of the ITDP learning rule and participate in the expression of ITDP through iLTD.

Disclosures: J. Basu, None; **S.A. Siegelbaum**, None.

Nanosymposium

625. Synaptic Transmission Dynamics

Location: Room 6F

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 625.6

Topic: B.07. Synaptic Transmission

Support: NIH Grant EY017836

NIH Grant EY019828

Alfred P. Sloan Foundation

Title: Signal transfer at a retinal ribbon synapse

Authors: T. JARSKY[1], J. DEMB[3], *J. H. SINGER[2];
[1]Ophthalmology, [2]Ophthalmology and Physiol., Northwestern Univ., Chicago, IL;
[3]Ophthalmology and Molecular, Cellular, and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: During night vision in mammals, rod photoreceptor output is conveyed to ganglion cells (GCs) by the rod bipolar (RB) pathway: rod -> RB -> AII amacrine cell -> ON cone bipolar cell -> GC. A gain control mechanism at the RB-AII synapse permits the RB to transfer rod signals without saturating as ambient light intensity varies. Here, we use voltage clamp recording from synaptically coupled RBs and AIIs in a slice preparation of mouse retina to examine the presynaptic mechanisms underlying the synapse's dynamic range. RBs were stimulated for ~3 s with a time-varying voltage [a filtered (0-50 Hz) white noise stimulus with SD = 3 mV] superimposed upon tonic depolarizations between the Vhold of -60 mV and -42 mV; this protocol was designed to mimic RB responses to rod inputs at varying levels of background illumination. EPSCs were recorded in AIIs. With increasing depolarization, the EPSCs elicited by the fluctuating stimulus decreased in amplitude, but the postsynaptic response remained well-correlated with the stimulus. The transfer function of the synapse was well-approximated by a linear-nonlinear (L-N) model. The linear filter at all potentials was well-described as a delta

function with a peak at ~2 ms. The slope of the static nonlinearity, however, was reduced significantly with membrane depolarization. This is consistent with a reduction in synaptic gain reflecting the diminution of EPSC amplitude with depolarization. We found that the reduction in gain accompanied the depletion of the readily-releasable pool (RRP) of vesicles. Thus, we conclude that the RB-AII synapse acts a high fidelity relay of rod signals throughout its operating range and that the size of the RRP is a determinant of synaptic gain.

Disclosures: T. Jarsky, None; **J.H. Singer**, None; **J. Demb**, None.

Nanosymposium

625. Synaptic Transmission Dynamics

Location: Room 6F

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 625.7

Topic: D.14. Cerebellum

Support: NWO-ALW

NWO-ZON-MW

NeuroBsik

EEC-SENSOPAC

Prinses Beatrix Fonds

CMSB

NGI-NWO

Title: Enhanced granule cell output and irregular purkinje cell firing in gain-of-function cacna1a^{S218L} mutant mice

Authors: *F. E. HOEBEEK[1], Z. GAO[2], B. TODOROV[3], C. F. BARRETT[3,4], S. VAN DORP[5], M. D. FERRARI[4], A. M. J. M. VAN DEN MAAGDENBERG[3,4], C. I. DE ZEEUW[2,5]; [2]Neurosci., [1]Erasmus MC, Rotterdam, Netherlands; [3]Human Genet., [4]Neurol., Leiden Univ. Med. Ctr., Leiden, Netherlands; [5]Netherlands Inst. for Neurosci., Royal Acad. of Arts and Sci., Amsterdam, Netherlands

Abstract: Mutations in the *CACNA1A* gene are associated with neurological disorders, such as ataxia, hemiplegic migraine, and epilepsy. These mutations can be categorized by their effects on Ca^{2+}-channel function as loss- or gain-of-function mutations. Whereas recent evidence demonstrates that loss-of-function mutations decrease the regularity of cerebellar Purkinje cell activity and thereby induce cerebellar ataxia, it is unknown how gain-of-function mutations induce ataxia. Using gain-of-function *Cacna1a*^{S218L} knock-in mice we here show that both synaptic connectivity and transmission between granule cells and Purkinje cells are increased. Additionally, *Cacna1a*^{S218L} Purkinje cells show hyperexcitable action potential and dendritic Ca^{2+}-spike firing, which deregulates their spontaneous firing pattern and can be counteracted by Ca^{2+}-dependent K^{+}-channel activators. Our findings illustrate the underlying mechanisms of ataxia with gain-of-function mutations, which are surprisingly similar to those in loss-of-function *Cacna1a* mutants. This commonality reveals the existence of a narrow window for optimal Ca^{2+}-homeostasis: sufficiently increased or decreased calcium-influx induces ataxia.

Disclosures: F.E. Hoebeek, None; **Z. Gao**, None; **B. Todorov**, None; **C.F. Barrett**, None; **A.M.J.M. van den Maagdenberg**, None; **M.D. Ferrari**, None; **C.I. De Zeeuw**, None; **S. van Dorp**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.1

Topic: B.08. Synaptic Plasticity

Support: stowers institute support

Title: Drosophila Orb2, a gene required for stable long-term memory, has Prion-like properties

Authors: *A. MAJUMDAR[1], K. SI[2];
[1]Si lab, Stowers Inst. For Med. Res., Kansas City, MO; [2]stowers institute for medical research, Kansas city, MO

Abstract: The Drosophila Orb2 gene belongs to a family of RNA binding proteins, known as Cytoplasmic Polyadenylation Element Binding Protein or CPEB, which regulates translation of subset of cellular mRNAs. Intriguingly previously we have observed that the Aplysia homologue of Drosophila Orb2, ApCPEB, has the properties of a prion-like protein and the prion-like state is required to stabilize synaptic facilitation, a cellular correlate of long-term memory. Based on these observations we proposed that the prion-like properties of ApCPEB and its homologue's in

other species provide a self sustaining mechanism for synaptic protein synthesis that is essential for the persistence of memory. This model predicts that Drosophila Orb2 would be necessary for maintenance but not formation of memory and will have the properties of a prion. We and others have found that indeed the Orb2 gene is required for the persistence of memory for long-term but not for the formation of memory. Now using biochemical and cell biological assays we find that the Drosophila Orb2 indeed has some of the properties of a dominant self-perpetuating prion-like protein. We find that Orb2 exist in two distinct conformational states, one of which is monomeric and the other is multimeric and amyloid in nature. The multimeric state of Orb2 can act as a dominant seed to convert the monomeric to the multimeric state. However unlike conventional prions that are inactive or toxic in the prion state, Orb2 is active and non toxic in the prion-like state. To our knowledge this is the first example of a prion-like molecule in Drosophila that might serve a normal physiological function.

Disclosures: A. Majumdar, None; **K. Si**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.2

Topic: B.08. Synaptic Plasticity

Support: CIHR Grant MOP 15121

FRSQ studentship

Title: Eukaryotic elongation factor 2 kinase links target of rapamycin complex 1 to translational elongation and decreased elongation causes a paradoxical increase in the ratio of cap-dependent to internal-ribosomal-entry-site-dependent translation in Aplysia sensory neurons

Authors: *D. B. WEATHERILL[1], W. S. SOSSIN[2];
[2]Neurol. & Neurosurg., [1]McGill Univ., Montreal, QC, Canada

Abstract: Long-term facilitation (LTF) in *Aplysia* is a leading cellular model for elucidating the biochemical mechanisms of synaptic plasticity underlying learning. LTF requires translational control downstream of the target of rapamycin (TOR) complex 1 (TORC1). Our lab has previously shown that treatment with the facilitating neurotransmitter, 5-hydroxytryptamine (5-HT), causes a TORC1-mediated decrease in phosphorylation of eukaryotic elongation factor 2 (eEF2), within synaptosomes isolated from the sensory neurons (SNs) involved in LTF. This

effect on eEF2 phosphorylation, which is known to stimulate translational elongation, was also observed in the SN neurites, but only if the neurites had previously been axotomized or treated with high concentrations of potassium chloride- two conditions which increase levels of intracellular calcium. Because eEF2 kinase (eEF2K) is a calcium-calmodulin-dependent kinase that in other systems has been shown to be regulated by a target of TORC1 signalling, ribosomal protein S6 kinase (S6K), and because we have previously shown that S6K-regulation is important for at least one phase of LTF (LTF measured at 24 hours; 24-hr LTF), in the present study we set out to characterize the *Aplysia* orthologue of eEF2K. Here we show that this eEF2K orthologue contains a similar consensus S6K phosphorylation site, that this site is indeed regulated by S6K, and that exogenous expression of a mutant form of eEF2K, containing a serine-to-alanine mutation at this site, blocks the ability of 5-HT to decrease eEF2 phosphorylation. Therefore, in *Aplysia* SNs, as in other systems, S6K and eEF2K provide a link between TORC1 signalling and eEF2 dephosphorylation/stimulation of translational elongation. Surprisingly, and in contrast to what would be expected to occur in SN neurites given our findings concerning eEF2 phosphorylation, we found that within the SN cell soma 5-HT treatment actually results in a decrease in eEF2K phosphorylation and a concomitant increase in eEF2 phosphorylation. The decrease in eEF2K phosphorylation is not occluded by treatment with the TORC1 inhibitor, rapamycin, suggesting that, within the SN soma, 5-HT acts through a second, parallel pathway. Lastly, we found that overexpression of eEF2K increases the ratio of cap- to internal-ribosomal-entry-site- (IRES-) dependent translation. This provides novel evidence that inhibiting translational elongation, which can result in an increase in the levels of free translation initiation factors, can result in a biased effect on the initiation of cap- versus IRES-dependent translation.

Disclosures: D.B. Weatherill, None; **W.S. Sossin**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.3

Topic: B.08. Synaptic Plasticity

Support: HHMI

Broitman Program for Cognitive Disorders

Title: Non-proteolytic ubiquitination by neuralized activates cpeb3: A novel function of the ubiquitin system in synaptic plasticity and memory storage

Authors: *E. PAVLOPOULOS[1], P. TRIFILIEFF[1], V. CHEVALEYRE[1], S. ZAIRIS[1], G. MALLERET[2], E. R. KANDEL[1,3,4];
[1]Columbia Univ., NEW YORK, NY; [2]Univ. Claude Bernard, 5UMR5167 CNRS, Lyon, France; [3]HHMI, New York, NY; [4]Kavli Inst. for Brain Sci., New York, NY

Abstract: Ubiquitination is important for modifying the molecular composition of synapses. In addition to its well established role in proteasomal degradation, ubiquitination can regulate protein function and activity. This role of ubiquitination, however, has not been explored in the context of synaptic plasticity. Here, we provide evidence based on studies in wild type and genetically modified mice and in neuronal cultures that ubiquitination directly stimulates protein synthesis and the growth of new synaptic contacts. We find that Neuralized (Neurl), an E3 ubiquitin ligase, ubiquitinates and activates the cytoplasmic polyadenylation element-binding protein 3 (CPEB3), a regulator of protein synthesis. The ubiquitination of CPEB3 by Neurl is direct and requires the physical interaction of the two proteins mediated by the prion-like domain of CPEB3. Once ubiquitinated by Neuralized, activated CPEB3 leads both to 1) an increase in two CPEB3 targets that are essential components in synaptic plasticity in hippocampal neurons: the GluR1 and GluR2 subunits of AMPA receptors, and 2) a facilitation of the growth of new spines. Conditional overexpression of Neuralized similarly increases both the levels of GluR1 and GluR2 and the number of spines and functional synapses in the adult hippocampus. In addition, overexpression of Neurl leads to enhanced hippocampal-dependent memory and enhanced LTP in the Schaffer-collateral pathway. Consistently, inhibition of the ubiquitin ligase activity of Neuralized in the adult hippocampus results in reduction of GluR1 and GluR2 protein levels and deficits in both long-term memory and L-LTP. These results suggest a model whereby Neurl-dependent ubiquitination facilitates hippocampal plasticity and hippocampal-dependent memory by modulating the activity of CPEB3 and CPEB3-dependent protein synthesis and synapse formation.

Disclosures: E. Pavlopoulos, None; **P. Trifilieff**, None; **V. Chevaleyre**, None; **S. Zairis**, None; **G. Malleret**, None; **E.R. Kandel**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.4

Topic: B.08. Synaptic Plasticity

Support: NS046769

DA026110

Title: Determinants of conditional RNA targeting in neurons

Authors: *I. A. MUSLIMOV, S. PINTOVA, H. TIEDGE;
State Univ. of New York, Hlth. Sci. Ctr. At Brooklyn, BROOKLYN, NY

Abstract: In neurons, the targeted delivery of select RNAs to synapto-dendritic domains is an important mechanism underlying local translational control of gene expression, and thus the long-term structural and functional plasticity of synapses. Spatial codes have been identified that specify differential dendritic RNA targeting. How is spatial RNA coding modulated over time in an activity-dependent manner? Here we report the discovery of RNA codes that exert temporal control over spatial codes. In dendritic BC1 RNA, constitutive targeting is specified by spatial codes in the 5' domain. ID elements are recurrent genetic elements that are phylogenetically derived from the targeting-competent 5' BC1 domain. We found that a subtype of ID elements (ID4) confers dendritic targeting competence to a host mRNA. In contrast to constitutive BC1 targeting, however, ID4 targeting was found to be activity-dependent. ID4, while retaining 5' BC1 targeting-determinant motifs, features additional non-canonical attributes that are responsible for the switch from constitutive to conditional targeting. Our work shows that physical characteristics of RNA nucleotide interactions can serve as determinants of conditional subcellular RNA targeting in neurons. Supported by NS046769 and DA026110.

Disclosures: I.A. Muslimov, None; **S. Pintova**, None; **H. Tiedge**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.5

Topic: B.08. Synaptic Plasticity

Support: NINDS NS037731

NINDS NS050634

Title: Local protein translation during presynaptic bouton maturation

Authors: *K. HSIAO[1,2], D. L. BENSON[3];

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Abstract: Neurons can generate new proteins at sites distant from the cell body in order to achieve rapid or synapse-specific responses. Neurons may be particularly reliant on this form of regulation since a variety of human neurological diseases are caused by mutations that can disrupt the local regulation of translation. We have shown recently that developing synapses rely on a continuous supply of new proteins: following brief periods of protein synthesis inhibition, presynaptic function decreases and synapse elimination increases. Similar outcomes are observed whether protein synthesis is inhibited globally (including cell bodies and processes) or locally (restricted to processes), but it has been unclear whether the source for new proteins in young neurons is axonal, dendritic or both. Using optical isolation in live hippocampal neuron cultures, and translation reporters that are targeted to different sites of new synthesis via well characterized 3'UTRs, we have compared local synthesis in axons and dendrites at different stages of development and in response to activity. We find that new protein synthesis can be detected in axons as well as dendrites and that synthesis rates are differentially regulated in each compartment by neural activity. These findings indicate that in addition to postsynaptic sites, presynaptic compartments also have the capacity to synthesize new proteins at sites distant from growth cones. Together with our previous work these findings suggest that local presynaptic synthesis can regulate synapse stability.

Disclosures: K. Hsiao, None; **D.L. Benson**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.6

Topic: B.08. Synaptic Plasticity

Support: NS046769

DA026110

Title: Interaction of dendritic BC1 RNA with translation initiation factors

Authors: *T. EOM[1,2], V. BERARDI[1,2,4], J. ZHONG[1,2], G. RISULEO[4], H. TIEDGE[1,2,3]; [1]Physiol. and Pharmacol., State Univ. of New York, Hlth. Sci. Ctr. At Brooklyn, Brooklyn, NY; [2]The Robert F. Furchgott Ctr. for Neural and Behavioral Sci., [3]Neuology, State Univ. of New

York, Hlth. Sci. Ctr. at Brooklyn, Brooklyn, NY; [4]Genet. and molecular biology, La Sapienza university of Rome, Rome, Italy

Abstract: Local protein synthesis forms a basis for long-term synaptic plasticity in neuron. Dendritic BC1 RNA represses translation by interacting with initiation factors. Localized to synapto-dendritic domains, it participates in translational control through interactions with eukaryotic initiation factor 4A (eIF4A). eIF4B, a modulator of eIF4A, may also contribute to BC1-mediated translational regulation. We examined binding of eIF4B to BC1 RNA. Electrophoretic mobility shift assays (EMSAs) with BC1 RNA and BC1 derivatives showed that the 3' domain of BC1 RNA is important for binding to eIF4B. Helicase assays and in vitro translation assays revealed functional contributions of the BC1 3' domain in BC1-mediated translational control. In addition, the punctuated A-rich central domain is required for translational repression competence through its interactions with eIF4A. The combined results provide molecular insight into functional regulatory RNA interactions with the translation initiation machinery.

Disclosures: T. Eom, None; **V. Berardi**, None; **J. Zhong**, None; **G. Risuleo**, None; **H. Tiedge**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.7

Topic: B.08. Synaptic Plasticity

Support: NIH Grant DA026067 (J.S.M.)

NIH Grant P30-HD03352

Title: Pin1 regulates synaptic plasticity by influencing cell signaling and spine density and morphology

Authors: *P. R. WESTMARK[1], C. J. WESTMARK[1], K. J. O'RIORDAN[2], C. BURGER[2], J. S. MALTER[1];
[1]Pathol, [2]Neurol., Univ. Wisconsin, MADISON, WI

Abstract: The underlying molecular and cellular basis for synaptic plasticity and long-term memory consolidation may be the regulation of protein synthesis. Pin1, a peptidyl prolyl

isomerase, is present in postsynaptic dendritic terminals and regulates glutamatergic induced protein synthesis and spine density and morphology. Genetic knockout or pharmacological inhibition of Pin1 upregulated translation, possibly through 4E-BP1/2 and eIF4E, and influenced spine density and morphology. Consistent with increased protein synthesis, hippocampal slices from $Pin^{-/-}$ mice had normal E-LTP but significantly enhanced L-LTP compared to WT controls. The plasticity related proteins PKCζ and PKMζ were elevated in $Pin1^{-/-}$ cortex and hippocampus compared to WT. Once induced, PKMζ directly regulated dendritic translation as well as Pin1 activity. In addition, primary cortical neurons from $Pin^{-/-}$ mice were found to have increased spine density and a higher percentage of mature spines than $Pin1^{+/+}$ or $Pin1^{+/-}$ mice. Therefore, Pin1 not only regulates dendritic protein synthesis induced by glutamate signaling and the synthesis of proteins that contribute to long-term forms of synaptic plasticity, but also influences spine density and morphology.

Disclosures: P.R. Westmark, None; **C.J. Westmark**, None; **K.J. O'Riordan**, None; **C. Burger**, None; **J.S. Malter**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.8

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS50465

Title: Exercise affects BDNF-related plasticity using epigenetic mechanisms

Authors: *F. GOMEZ-PINILLA[1], Y. ZHUANG[2], J. FENG[3], Z. YING[2], G. FAN[3]; [1]Integrative Biol. and Physiol., UCLA, LOS ANGELES, CA; [2]Integrative Biol. and Physiol., UCLA, Los angeles, CA; [3]Human Genet., David Geffen Sch. of Medicine, Univ. of California Los Angeles, Los angeles, CA

Abstract: The role of exercise in promoting brain plasticity and cognitive enhancement has been associated with the action of brain-derived neurotrophic factor (BDNF). Based on recent evidence that the action of BDNF on activity-dependent plasticity is under the scope of epigenetic regulation, we have examined whether the action of exercise on the brain could involve mechanisms of epigenetic regulation. Animals were exposed to one-week period of voluntary exercise shown to enhance learning and memory performance involving hippocampal BDNF-mediated synaptic plasticity. We have focused these studies on the BDNF promoter III,

as this region is highly responsive to neuronal activity and is susceptible to epigenetic regulation. We have found that exercise promoted DNA demethylation in BDNF promoter III, and elevated levels of MeCP2 and BDNF mRNA and protein in the hippocampus. In addition, we used chromatin immunoprecipitation assay to evaluate modification at histone 3 following exercise, and found that exercise increased acetylation of histone 3 in BDNF promoter III region. Western blot analysis showed that exercise elevated the ratio of acetylated/total for histone 3 but had no effects on histone 4 levels, indicating some level of specificity for the action of exercise. In addition, exercise reduced levels of the deacetylase HDAC5, which has been shown to be associated with the regulation of BDNF gene. Exercise elevated the expression of CaMKII and p- CREB, which are implicated on the pathways by which neural activity stimulates BDNF transcription using mechanisms of epigenetic regulation. Our results show that exercise influences remodeling of chromatin containing the BDNF gene. Results are consistent with the idea that exercise can promote stable changes in gene expression which consequences for brain function and plasticity.

Disclosures: F. Gomez-Pinilla, None; **Y. Zhuang**, None; **J. Feng**, None; **Z. Ying**, None; **G. Fan**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.9

Topic: B.08. Synaptic Plasticity

Support: Nancy Lurie Marks Postdoctoral Fellowship

NINDS Grant

HHMI

Title: Activity-dependent regulation of Arc in wild-type and Tsc1-lacking hippocampal neurons

Authors: *H. S. BATEUP, C. L. DENEFRIO, B. L. SABATINI;
Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Proper neural circuit function is dependent upon the ability of neurons to adapt to changes in network activity during development. The term homeostatic plasticity refers to the process by which chronic changes in activity result in neuron-wide scaling up or down of

synaptic strength to maintain firing levels within a desired range. Homeostatic plasticity is essential for maintaining balanced excitation and inhibition, a process proposed to be disrupted in neurodevelopmental disorders.
The immediate early gene Arc has been identified as a putative mediator of homeostatic plasticity. However, the molecular mechanisms that link changes in activity to regulation of Arc have not been well characterized. Here we examine the bi-directional regulation of Arc in response to blockade of activity with TTX or increased activity following picrotoxin treatment in dissociated hippocampal cultures. We find that while homeostatic changes in synaptic receptors are observed 24-48 hours after activity manipulation, Arc protein levels are rapidly and robustly increased in response to picrotoxin or decreased in response to TTX. We further determined that Erk1/2 is responsible for the activity-dependent regulation of Arc as blockade of Erk1/2 phosphorylation with U0126 was capable of disrupting this process. Arc is known to be regulated at both the transcriptional and translational level. We find that the activity-dependent regulation of Arc occurs at the level of gene transcription. Quantitative RT-PCR results show robust regulation of Arc mRNA by activity which is also blocked by U0126. Interestingly, we observed that Arc exhibits a high basal turn over rate as transcriptional blockade with actinomycin D resulted in a very rapid loss of both Arc mRNA and protein levels.
Since activity-dependent gene transcription is thought to be vital for early brain development, we investigated whether this molecular mechanism was disrupted in a model of the autism spectrum disorder Tuberous Sclerosis Complex (TSC). Using dissociated cultures from conditional Tsc1 knock-out (Tsc1 KO) mice we find that Arc mRNA and protein levels, as well as Erk1/2 phosphorylation, are basally increased. Furthermore, the activity-dependent regulation of this pathway is altered compared to control neurons. In gene array experiments from cultures treated with TTX or picrotoxin, we find that a number of genes normally induced by activity in control neurons are basally up-regulated in Tsc1 KO neurons. Such perturbations could alter the ability of neurons to respond to changes in network activity levels during development and may contribute to cognitive impairments in TSC.

Disclosures: H.S. Bateup, None; **C.L. Denefrio**, None; **B.L. Sabatini**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.10

Topic: B.08. Synaptic Plasticity

Support: This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences

Title: Promoter proximal RNA polymerase II stalling may determine temporal expression kinetics of neuronal immediate early genes

Authors: *R. N. SAHA, E. R. BAILEY, K. ADELMAN, S. M. DUDEK;
NIEHS, NIH, RTP, NC

Abstract: Transcription of immediate early genes (IEGs) in neurons is exquisitely sensitive to neuronal activity, but the mechanisms underlying the very earliest of these transcription events are largely unknown. Neuronal activity, induced *in vitro* by withdrawing Tetrodotoxin (TTX) after its application for 48 hours, asynchronously produces IEG products that may be detected from anywhere between 2 minutes and 1 hour. Using microarray and intron-based real-time PCR screens, we have identified a group of 24 very fast IEG (VF-IEGs) whose pre-mRNA is up-regulated 2-fold or more within 5 min of TTX withdrawal with eventual 2-fold increase in mRNA within 15 min. These screens have also revealed another group of 43 fast IEGs (F-IEGs) that we define as having a 2-fold increase in mRNA by 45 min following TTX withdrawal, but not at 15 min. In this study, we tested the hypothesis that very fast expression kinetics is attained by pre-engaging (stalling) transcription competent RNA Pol II in promoter proximity, whereas a lack of stalling results in comparatively slower kinetics.
To test this hypothesis, Chromatin ImmunoPrecipitation (ChIP) assays were performed in neurons with antibodies against 3 different Pol II epitopes. Pol II enrichment was detected near the transcription start site (TSS) in 5 out of 6 tested VF-IEGs. The contrasting lack of Pol II within gene bodies suggests that VF-IEGs possess promoter proximal stalled polymerase (PPPS). No such enrichment was detected in five tested F-IEGs. To substantiate these findings globally, ChIP-seq was performed with a Pol II antibody that detects Pol II irrespective of the phosphorylation status of its C-terminal domain. Our ChIP-seq data strongly couples very fast induction with paused Pol-II as the significant majority (>85%) of VF-IEGs have PPPS. F-IEGs in contrast, have few cases of stalled Pol II. Thus it appears that Pol II stalling is not sufficient but necessary for very fast responses.
To address any biological role of Pol II stalling, RNAi was used to knock-down 2 subunits of the Negative Elongation Factor (NELF) complex, a known mediator of stalling. NELFa or NELFe depletion reduced promoter proximal Pol II occupancy and attenuated rapid induction of every VF-IEG, thus highlighting the dependence of fast expression on Pol II stalling. Similarly, slower expression of F-IEGs was not affected by NELFa depletion, suggesting that these genes are not regulated by this mechanism. Taken together, these data strongly correlate the fast kinetics of IEG expression in neurons with stalled Pol II and suggest a role of this mechanism in transcription-dependent learning and memory.

Disclosures: R.N. Saha, None; **E.R. Bailey**, None; **S.M. Dudek**, None; **K. Adelman**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.11

Topic: B.08. Synaptic Plasticity

Support: HFSP CDA

Title: Spatiotemporal regulation of activity-dependent genes in post-mitotic neurons

Authors: *T. TAKIZAWA, M. TAKAGI, K. ITOH, K. NAKASHIMA;
Grad. Sch. of Biol. Sci., Nara Inst. of Sci. and Technol., Ikoma/Nara, Japan

Abstract: An increasing body of evidence shows that sub-nuclear spatial gene positioning is of great relevance in a wide range of cellular functions such as differentiation and gene expression. For instance, the nuclear periphery has been shown to constitute a repressive environment for gene transcription in mammalian cells. Neurons express a number of specific genes upon depolarization in response to external stimuli. Although signaling pathways and transcription factors involved in activity-dependent gene expression in neurons have been intensively studied, spatial positioning and chromatin regulation of activity-dependent neuronal genes remain elusive. We have mapped the sub-nuclear spatial positioning and chromatin states of activity-dependent genes in mouse hippocampal neurons and delineated their spatio-temporal regulation. Using microarray analysis we have identified sets of genes which are upregulated within 30 minutes (designated as the early genes) while another set of genes is upregulated around180 minutes (the late genes) after depolarization . DNA fluorescence in situ hybridization revealed that the late genes are preferentially located at the nuclear periphery while the early genes are not. Surprisingly the late genes were transcribed at the periphery after depolarization, indicating the nuclear periphery in neurons is a transcriptionally permissive environment. The late genes are enriched in a repressive histone marker, di-methyl lysine 9 of histone H3, and get phosphorylated on serine 10 of H3 upon depolarization. The early genes are enriched in promoter-initiated RNA polymerase II (RNAP II) phosphorylated on serine 5 of its C-terminal domain. Negative elongation factor (NELF) also occupies the proximal region of the early genes and plays a critical role in RNAP II stalling. These results demonstrate that the temporal regulation of activity-dependent genes in post-mitotic neurons correlates with the sub-nuclear spatial positioning and chromatin states.

Disclosures: T. Takizawa, None; **M. Takagi**, None; **K. Itoh**, None; **K. Nakashima**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.12

Topic: B.08. Synaptic Plasticity

Support: NIH Grant GM84805

Title: Quantification of stimulation-dependent rapid translation of synaptic proteins in a mouse model of autism

Authors: C. D'HULST[1], V. DROUET[2], H. WU[3], *J. B. DICTENBERG[4];
[1]Biol. Sci., [2]Biol., [3]Hunter College/CUNY, New York, NY; [4]Biol Sci., Hunter Coll, NEW YORK, NY

Abstract: Fragile X syndrome (FXS) is the main cause of inherited mental retardation and the leading known genetic form of autism affecting 1/2500 individuals. The disorder is caused by a CGG triplet expansion (dynamic mutation), located within the 5' untranslated region of the *Fragile X mental retardation gene 1 (FMR1)*, to more than 200 repeats. The concomitant hypermethylation of the CpG island in the promoter region of the gene causes transcriptional silencing of *FMR1* and consequently absence of the protein FMRP. FMRP plays a role in regulation of translation, transport and stability of various neural mRNAs. By affecting synaptic plasticity, FMRP is important in learning and memory and absence of the protein leads to mental retardation.
Given that FMRP plays an important role in synaptic local protein synthesis, our interest turned to synaptic proteins that are known to function in activity-dependent mechanisms at the synapse. Although few mRNA targets of FMRP are well characterized, one of the more important *bona fide* mRNAs encodes the post synaptic density protein of 95 kDa (PSD95), which is associated with the augmentation of excitatory synapse maturation and serves to cluster ion channels and scaffold regulatory protein complexes at the synapse region. PSD95 is a binding partner at the synapse for neuroligins, a small family of post-synaptic transmembrane proteins, which interact with pre-synaptic cell-adhesion proteins called neurexins. By simultaneously binding to PSD95, neuroligins link the post-synaptic density and control of ionotropic neurotransmitter receptors to the exocytotic machinery of the presynaptic terminal. The neuroligin gene was previously associated with autism in X-linked chromosomal deletions and mutations in specific isoforms. Several groups already showed that PSD95 levels are altered in fragile X syndrome and we hypothesize that this could lead to changes in neuroligins levels and ultimately neuron homeostasis. Clearly altered PSD95 can dramatically alter synapses in fragile X syndrome, but it is not currently shown if particular synapses are altered. Using fluorescent imaging techniques, western blot and real-time PCR techniques we were able to demonstrate deficient rapid (20 min) stimulus-induced changes in the expression of synaptic proteins between fragile X KO mice and their WT littermates. Preliminary data show a decrease in NL1 positive vGlut-puncta, a marker for excitatory synapses, in DIV7 but not DIV14 KO dendrites. These results suggest that changes in synaptic cell-adhesion molecules at specific developmental stages contribute to neuronal

homeostasis in FXS.

Disclosures: C. D'Hulst: None. **V. Drouet:** None. **H. Wu:** None. **J.B. Dictenberg:** None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.13

Topic: B.08. Synaptic Plasticity

Title: Activity- and translation-dependent degradation of Arc mRNA in neuronal dendrites

Authors: *S. FARRIS[1], O. STEWARD[2];
[1]Anat. & Neurobio., [2]Reeve Irvine Res. Inst., Univ. of California Irvine, Irvine, CA

Abstract: The mRNA for the immediate early gene Arc/Arg3.1 (Activity-regulated cytoskeleton associated gene) is induced by synaptic activity or learning experiences. Arc is unique among neuronal mRNAs in that its newly synthesized transcripts are rapidly and robustly shipped out to dendrites where they accumulate near recently activated synapses. This points to Arc as a candidate for coupling changes in neuronal activity to synaptic plasticity. Previous studies have revealed hints that the selective localization at active synapses may involve both targeting to active synapses and degradation of Arc mRNA. Other evidence suggests that Arc mRNA degradation may be translation dependent. Here we assess whether there is activity and translation-dependent degradation of Arc mRNA in vivo. We used a paradigm in which Arc transcription and delivery into dendrites is induced by electroconvulsive seizure (ECS) and subsequent targeting to active synapses is achieved by high frequency stimulation of the medial perforant path. Simultaneous with the targeting of activated synapses, Arc mRNA disappears from the non-activated portions of the dendrite. Local blockade of protein synthesis with cycloheximide prevented the activity-dependent decrease in mRNA levels in the inactive dendritic zones, indicating that Arc mRNA decay is translation-dependent. Based on this, we assessed whether the enzymes involved in mRNA degradation were present in the dendritic laminae and whether their levels were altered after stimulation paradigms that induced degradation. Immunostaining for Upf1 and Staufen, proteins that are necessary for nonsense mediated decay (NMD)- a type of translation-dependent mRNA degradation, was diminished in the activated dendritic lamina in which Arc mRNA accumulates. This is consistent with the possibility that Arc mRNA is degraded in the non-activated regions of the dendrite and somehow protected in the activated region. These data indicate that Arc mRNAs are subject to degradation mechanisms, that this degradation is enhanced by strong synaptic activity and is dependent on

active translation. Activity-dependent mRNA degradation occurring in the distal portion of neurons in vivo is a novel finding that suggests a new mechanism to how neurons can selectively modify the spatial and temporal content of mRNA in response to activity.

Disclosures: S. Farris, None; **O. Steward**, None.

Nanosymposium

626. Transcription and Translation in Plasticity III

Location: Room 24A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 626.14

Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant MH082106

Title: Epigenetic mechanisms in the entorhinal cortex regulate hippocampal area CA1 during consolidation of long-term memory

Authors: *S. GUPTA, B. J. WALTERS, R. L. DAVIS, L. E. DOBRUNZ, F. D. LUBIN; Neurobio., Univ. Alabama, BIRMINGHAM, AL

Abstract: Extensive research has established the importance of hippocampal epigenetic marks in long-term memory formation. Additionally, previous studies from our lab implicate a dynamic role for histone methylation in area CA1 of hippocampus during memory consolidation of fear. Because exchange of sensory information between the hippocampus and cortex occurs primarily through the entorhinal cortex (EC), we next sought to investigate the role of histone methylation in the EC following contextual fear conditioning (CFC). In the present study, we found a significant increase in histone H3 lysine 9 dimethylation (H3K9me2), a transcriptional repressive mark in the EC with CFC. We observed an increase in histone H3 lysine 4 trimethylation (H3K4me3) a transcriptional active mark in the EC following context-exposure alone or CFC. Twenty-four hours after CFC H3K9me2 returned to baseline levels whereas the H3K4me3 was significantly reduced in the EC following context-exposure alone or CFC compared to naïve control animals. Together these results suggest that histone methylation is dynamically regulated in the EC following either novel context learning or associative contextual learning of fear. Interestingly, our results suggest that H3K9me2 in the EC maybe an associative-learning-specific signal. Thus, we further investigated the effect of blocking H3K9me2 in the EC on associative long-term memory formation. Remarkably, we observed an enhancement in freezing, 24h following blockade of G9a (H3K9 histone methyltransferase) in the EC. Intriguingly, we found that inhibition of H3K9me2 in area CA1 decreased freezing behavior 24h later on Test day 1.

We also found changes in several memory-related genes including MeCP2, Dnmt3b and mGluR1 within the EC, which were altered with inhibition of H3K9me2. Moreover, inhibition of H3K9me2 in the EC inversely affected histone marks in area CA1, suggesting a molecular mechanism by which the EC inputs to area CA1 in order to regulate fear memory consolidation. Thus, our data suggest molecular connectivity between the EC and area CA1 at the level of the chromatin. Currently we are investigating the effect of H3K9me2 inhibition on the cellular connectivity between the EC and area CA1 by generating both LTD and LTP at either the temporoammonic-CA1 or schaffer collateral-CA1 synapses. As neurological disorders such as Alzheimer's disease and mild cognitive impairments are accompanied with neuronal loss observed foremost in the EC, additional study of the role of EC in cognition are necessary and will contribute to a better understanding of the molecular and cellular dialogue between the EC and hippocampus during long-term memory formation.

Disclosures: S. Gupta, None; **B.J. Walters**, None; **L.E. Dobrunz**, None; **F.D. Lubin**, None; **R.L. Davis**, None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.1

Topic: C.02. Alzheimer's disease and other dementias

Support: F32AG033445

T32AG000260

R01AG030142

Title: Regulation of the beta-secretase by eIF2alpha phosphorylation

Authors: *K. R. SADLEIR[1], P. OSTEN[2], R. VASSAR[1];
[1]Cell and Mol. Biol., Northwestern Univ., CHICAGO, IL; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The β-Secretase, β-site APP Cleaving Enzyme1 (BACE1) is the enzyme that initiates the production of the β-amyloid (Aβ) peptide involved in Alzheimer's disease (AD). Reduction of BACE1 levels by gene targeting or RNA interference inhibits Aβ generation and amyloid plaque formation. Conversely, modest overexpression of BACE1 in transgenic mice increases

Aβ production and amyloid deposition demonstrating that Aβ production and amyloid pathology can be modulated by BACE1 levels. Inhibition of BACE1 should effectively lower Aβ levels in AD, but therapeutically useful BACE1 inhibitors have proved challenging to develop, and there are concerns over the side effects of complete inhibition of BACE1 due to behavioral deficits in BACE1 null mice. BACE1 protein and activity are elevated in the brains of Alzheimer's patients. Blocking this increase in BACE1 could be therapeutically useful in slowing or preventing AD without affecting baseline levels of BACE1 and minimizing side effects, but the mechanism of BACE1 upregulation is still not fully understood.
Recently, our lab has generated data indicating that chronic or acute energy deprivation can elevate translation of BACE1 and Aβ generation through an increase in phosphorylation of the eukaryotic initiation factor eIF2α. Using the 5XFAD mouse model that has elevated BACE1 and p-eIF2α similar to human patients, we are currently investigating the role of eIF2α phosphorylation in AD-associated BACE1 increase. Adeno-associated virus is injected into the brains of neonatal mice to express a constitutively active form of GADD34 (GADD34CA), the regulatory subunit of protein phosphatase 1c, which dephosphorylates eIF2α. Neuron-specific expression of GADD34CA is maintained for at least six months, and results in decreased eIF2α phosphorylation. We are currently working to determine the effect of eIF2α phosphorylation on AD phenotypes such as BACE1 elevation, $A\beta_{42}$ generation, plaque formation and neuronal degeneration.

Disclosures: K.R. Sadleir: None. **P. Osten:** None. **R. Vassar:** None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.2

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH 1R21AG031483

NIH 5P01AG15379

NIH 5R01AG014713

Title: Alteration of voltage-gated sodium channel α-subunit levels and surface expression in BACE1-null mice

Authors: *D. KIM, M. T. GERSBACHER, D. M. KOVACS;

Genet. and Aging Res. Unit, MIND, Massachusetts Gen. Hospital/ Harvard Med. Sch., Charlestown, MA

Abstract: BACE1 cleaves various neuronal substrates, with mostly unknown physiological consequences. We and others have previously reported that β-subunits of the voltage-gated sodium channel are substrates of BACE1 and presenilin/γ-secretase. In follow-up studies, we found that elevated BACE1 activity increases processing of the β2-subunit ($Na_v\beta_2$) and modulates total and surface levels of the pore-forming sodium channel α-subunit $Na_v1.1$ in neuroblastoma cells and brains of mice (Kim et al., *Nat. Cell Biol.* 2007). Here, we investigate physiological changes of sodium channel metabolism in BACE1-null mice. First, we analyzed sodium channel α-subunit levels in brains of BACE1-null mice at one and three months of age. At both ages, we found ~40-50% decrease in $Na_v1.1$ protein and mRNA levels in BACE1-null versus wild-type mouse brains. In the hippocampus of BACE1-null mice, we found a robust 57% decrease of $Na_v1.1$ levels and also decreased $Na_v\beta_2$ C-terminal fragments. Brain levels of the $Na_v1.2$ α-channel subunit also decreased, but only in three months-old mice. $Na_v1.1$ levels were remained unchanged in BACE1-heterozygous mouse brains. This suggests that 50% decrease of BACE1 activity is not sufficient to alter $Na_v1.1$ levels in mouse brains. Finally, we performed surface biotinylation studies in acutely dissociated hippocampal slices from BACE1-null mice. Hippocampal surface $Na_v1.1$ levels were significantly decreased, but interestingly, $Na_v1.2$ surface levels were increased in BACE1-null mice perhaps as a compensatory mechanism for reduced surface $Na_v1.1$. In an attempt to dissect molecular mechanisms, we tested whether endogenous BACE1 requires its substrate $Na_v\beta_2$ to regulate levels of the $Na_v1.1$. Using cultured $Na_v\beta_2$-null and wild-type cortical/ hippocampal neurons as a model system, we confirmed that $Na_v\beta_2$ is required for BACE1 activity dependent modulation of $Na_v1.1$. Together, our data show that endogenous BACE1 activity regulates total and surface levels of voltage-gated sodium channels in mouse brains. Both decreased $Na_v1.1$ and elevated surface $Na_v1.2$ may result in a seizure phenotype since $Na_v1.1$ mainly regulates sodium currents in hippocampal GABAergic inhibitory neurons while $Na_v1.2$ and other α-subunits are highly expressed in pyramidal excitatory neurons. However, our data on BACE1-heterozygous mice also imply the existence of a therapeutic window for inhibiting BACE1 activity to block Aβ burden without affecting sodium channel metabolism.

Disclosures: D. Kim, None; **M.T. Gersbacher**, None; **D.M. Kovacs**, None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.3

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH/NIA

American Health Assistance Foundation

Title: Characterization of dynamic BACE1 endosomal transport by live cell imaging

Authors: *V. BUGGIA-PREVOT, X. MECKLER, K. S. VETRIVEL, G. THINAKARAN; Neurobio., Univ. Chicago, CHICAGO, IL

Abstract: Alzheimer's disease-associated β-amyloid peptides (Aβ) are produced by the sequential cleavage of amyloid precursor protein (APP) by β-secretase BACE1 and γ-secretase complex. Because BACE1 activity is increased in the brains of patients with Alzheimer disease and its cleavage is the first step of Aβ production, BACE1 is thought to be a promising therapeutic target. The type I transmembrane protein BACE1 is transported through the secretory and endocytic pathways, and APP cleavage is thought to occur in endosomes. Internalized BACE1 can be recycled to the plasma membrane, retrogradely transported to the TGN, or degraded in lysosomes. The C-terminal tail seems to be important for the regulation of BACE1 trafficking although only the GGA family of adaptors, which interact with the C-terminal dileucine motif of BACE1, is well documented in the literature for regulating BACE1 trafficking. In order to characterize the dynamics of BACE1 transport, we expressed fluorescent protein-tagged BACE1 in different cell types including COS, HeLa, and primary hippocampal neurons and performed live cell imaging. We found that in non-neuronal cells BACE1 is localized near the perinuclear region and in highly motile tubular-vesicular structures dispersed throughout the cell. In primary hippocampal neurons, BACE1 localizes in somato-dendritic compartments and is enriched in axons. We then characterized the identity of the dynamic membrane structures containing BACE1 in non-neuronal cells and primary cultured hippocampal neurons by immunofluorescence labelling with antibodies against a number of organelle markers. We also expressed constitutively active and dominant-negative mutants of several candidate proteins involved in intracellular transport to determine the modulation of BACE1 dynamics in this system. Our goal is to characterize the multiple steps of BACE1 trafficking order to develop a better understanding of BACE1 processing of APP in various organelles.

Disclosures: V. Buggia-Prevot: Research Grant; NIH/NIA, American Health Assistance Foundation. **X. Meckler:** None. **K.S. Vetrivel:** None. **G. Thinakaran:** NIH/NIA, American Health Assistance Foundation.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.4

Topic: C.02. Alzheimer's disease and other dementias

Support: CIHR

Title: Regulation of APP processing and Aβ production by BACE1

Authors: *Y. DENG, X. ZHANG, R. WANG, K. BROMLEY-BRITS, W. SONG;
Townsend Family Laboratories, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Alzheimer's disease is the most common neurodegenerative disorder leading to dementia. Deposition of amyloid β protein (Aβ) in the brains is one of the hallmarks of AD pathogenesis. Aβ is generated from a larger β-amyloid precursor protein (APP) by sequential cleavages of β-secretase and γ-secretase. Beta-site APP cleaving enzyme 1 (BACE1), the β-secretase *in vivo*, is essential for Aβ production. Despite of robust expression of APP gene resulting in high level of APP protein *in vivo*, Aβ production through the amyloidogenic pathway of APP processing is a rare occurrence under normal condition. To further examine how APP processing and Aβ production are regulated by BACE1 and its implication in AD pathogenesis and drug development, several cell lines stably expressing BACE1, wildtype APP and Swedish mutant APP were established. Generation of APP CTFs and Aβ species from the stable cell lines, AD transgenic mouse tissues and postmortem brain tissues from AD patients were analyzed. We found that BACE1 differentially processed APP proteins and BACE cleavage APP at Asp1 site of Aβ was upregulated in some AD sporadic cases. The results suggest that the preferential cleavage site by BACE1 may play an important role in AD pathogenesis under curtain pathological conditions.

Disclosures: Y. Deng, None; **X. Zhang**, None; **R. Wang**, None; **K. Bromley-Brits**, None; **W. Song**, None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.5

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH/NIA 1R01AG033016-01A1

Cure Alzheimer's Fund (CAF)

Title: GGA3 happloinsufficiency results in persistent elevation of BACE1 and Abeta levels following experimental head trauma

Authors: *K. R. WALKER[1], E. L. KANG[1], A. N. CAMERON[1], R. E. TANZI[2], M. J. WHALEN[3], G. TESCO[1];
[1]Neurosci., Alzheimer's Dis. Res. Laboratory, Tufts Univ. Sch. of Med., Boston, MA; [2]Genet. and Aging Res. Unit, MIND, Massachusetts Gen. Hosp., Charlestown, MA; [3]Dept. of Critical Care Medicine, Harvard Med. School, Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Traumatic brain injury (TBI) is the strongest environmental risk factor for Alzheimer's disease (AD). Clinical and experimental TBI is associated with accelerated beta-amyloid (Abeta) deposition, a hallmark of AD pathology. However, the molecular mechanisms linking TBI and AD remain unknown. We reported that BACE and beta-secretase activity increase following cerebral ischemia *in vivo* and caspase activation *in vitro* due to post-translational stabilization of BACE1 protein. We demonstrated that the impaired degradation of BACE1 is due to caspase-mediated cleavage of GGA3, an adaptor protein involved in BACE1 trafficking. Additionally, we showed that GGA3 levels are decreased while BACE1 levels are elevated in the brains of AD subjects (Tesco et al., 2007). We report here that BACE1 is elevated while GGA3 is depleted following experimental TBI in C57Bl6/J mice in a pattern similar to that observed following experimental cerebral ischemia (Tesco et al. 2007). Additionally, Abeta40 levels are significantly elevated (40%) in the injured hemisphere of C57Bl6/J mice 48hrs post-injury. In order to determine the role of GGA3 in the regulation of endogenous BACE1 under normal conditions and post-TBI, heterozygous GGA3+/- mice (129.C57Bl6/J background, Bay Genomics) were used to generate a GGA3 knockout mouse model. Young adult GGA3 deficient mice are healthy, fertile and appear normal at 6mths of age. We demonstrate that endogenous BACE1 levels are elevated (~ 30%) in the brains of GGA3 deficient mice indicating that GGA3 regulates BACE1 levels *in vivo*. Yet, despite elevated endogenous BACE1 levels Abeta40 and APPC99 levels are not significantly increased in GGA3 deficient mice at least at 6mths of age. When GGA3 null and heterozygous mice were subjected to experimental TBI, BACE1 and Abeta40 levels were similarly increased in the injured hemisphere of GGA3-/-, GGA+/- and GGA+/+ mice at 48 hrs post-injury. However, we have found that while BACE1 and Abeta40 return to normal levels in the sub-acute phase (7 days post-injury) of TBI in GGA+/+ mice, GGA3 haploinsufficiency results in a persistent elevation of BACE1 and Abeta levels. These new findings provide a potential mechanism linking TBI and AD and support the hypothesis that subjects with lower levels of GGA3 may be at increased risk of developing AD because of a persistent elevation of BACE1 and Abeta after injury.

Disclosures: K.R. walker: None. **E.L. Kang:** None. **A.N. Cameron:** None. **R.E. Tanzi:** None. **M.J. Whalen:** None. **G. Tesco:** None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.6

Topic: C.02. Alzheimer's disease and other dementias

Support: 1R01AG025952-04

Title: Endogenous BACE1 is mono- and K63-linked polyubiquitinated at lysine 501

Authors: *G. TESCO, E. L. KANG, K. R. WALKER, F. PIAZZA;
Neurosci., Tufts Univ. Sch. of Med., BOSTON, MA

Abstract: Ubiquitination is a reversible post-translation modification of cellular proteins. Ubiquitin (Ub) is covalently attached to the epsilon-amino group of lysine residues of the target protein. We report here that both endogenous and ectopically expressed BACE1 is ubiquitinated upon overexpression of Ub in different cell types (human neuroglioma H4, murine neuroglioma N2A, and primary neuronal cell cultures). The carboxyl terminus of BACE1 contains a lysine at the amino acid position 501. To test whether lysine 501 is a site of ubiquitination, we substituted lysine 501 to arginine (K501R) in a V5-tagged BACE1 expression vector and found that amino substitution K501R greatly reduced BACE1 ubiquitination in N2A cells as well as in H4 cells. Consistent with a role of ubiquitination on BACE1 degradation, levels of BACE1K501R are increased compared to that of BACE1 wild type during time course experiments in N2A cells. Ubiquitination at one (monoubiquitination) or multiple lysines (multiubiquitination) of a target protein regulates its endocytosis and sorting to the lysosomes for degradation. Given that Ub contains 7 lysine residues, once a molecule of Ub is attached to the target protein, additional Ub molecules can be linked resulting in the formation of polyubiquitin chains. Elongation in polyUb chains can occur at any of the 7 lysine residues present in Ub. K48-linked ubiquitination mainly targets proteins for proteasomal degradation. In contrast K63-linked polyubiquitination plays a role in endocytosis and represents a specific signal for protein sorting into the multivesicular body (MVB) pathway. In order to determine whether both ectopically expressed and endogenous BACE1 is mono-, K48- and/or K63-linked polyubiquitinated, mutant HA-tagged Ub expression vectors were transfected in H4 or N2A cells. The overexpression of a mutant Ub in which all 7 lysine residues are substituted with arginines (Ub-KO) and thus it is unable to form any Ub chains resulted in monoubiquitination of BACE1. Moreover, the overexpression of a mutant Ub having all lysines but K63 substituted by arginines, thus allowing only K63-linked ubiquitination, also resulted in BACE1 ubiquitination. Finally, BACE1 was ubiquitinated following the overexpression of a mutant Ub in which K48 has been substituted to arginine and thus, is unable to form polyUb chains at K48 (UbK48R). Our present findings showing that

BACE1 is monoubiquitinated and polyubiquitinated via K63-linked Ub chains further support our previous observations that BACE1 is degraded via the lysosomal pathway and a role for GGA3/Ub interaction in the delivery of BACE1 to the MVB/lysosomes pathway.

Disclosures: G. Tesco, None; **F. Piazza**, None; **E.L. Kang**, None; **K.R. Walker**, None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.7

Topic: C.02. Alzheimer's disease and other dementias

Support: Memosad, EU

FWO flanders

Methusalem grant Flanders

IUAP Belgium

Stichting Alzheimer onderzoek (SAO)

Title: Novel research horizons for presenilins and γ-secretases in Alzheimer's disease

Authors: *B. G. DE STROOPER[1,2];
[1]K.u.Leuven, Leuven, Belgium; [2]VIB, Leuven, Belgium

Abstract: Presenilins are the catalytic subunits of larger tetrameric γ-secretase complexes. The degradome of these aspartyl proteases consists of at least 60 different substrates. γ-Secretase is key to regulated intramembrane proteolysis, releasing protein fragments which potentially transduce signals at both sides of the cell membrane. Characteristic for this novel form of cellular signaling is its irreversible nature, providing direction to biological processes. We discuss recent insights in structure-function and assembly of the γ-secretase complexes and emerging insights in the regulation of the activity of these enzymes. This novel knowledge will help to develop better drugs for Alzheimer's disease and cancer. .

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Disclosures: B.G. De Strooper: Research Grant; Johnson en Johnson, Movetis. Consultant/Advisory Board; Johnson en Johnson, Envivo.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.8

Topic: C.02. Alzheimer's disease and other dementias

Support: Grants-in-Aid for Young Scientists (S) from Japan Society for the Promotion of Science

Targeted Proteins Research Program of the Japan Science and Technology Corporation

Title: Structure and function relationship analysis of γ-secretase modulator GSM-1

Authors: *T. TOMITA[1,3], Y. OHKI[1], S. YOKOSHIMA[2], T. HIGO[2], N. SHIMADA[2], H. KOIZUMI[2], S. OSAWA[1], T. FUKUYAMA[2], T. IWATSUBO[1,3];
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Abstract: Genetic and biological studies provide strong evidence that the production and deposition of amyloid-β peptides (Aβ) contribute to the pathogenesis of Alzheimer's disease (AD). Thus, β- and γ-secretases, that are responsible proteases for the Aβ generation, are plausible molecular targets for AD treatment. γ-Secretase is an unusual aspartic protease that cleaves the scissile bond within the transmembrane domain. This unusual enzyme is composed of a high molecular weight membrane protein complex containing presenilin, nicastrin, Aph-1 and Pen-2. Drugs that regulate the production of Aβ by regulating the γ-secretase activity could provide a disease-modifying effect on AD, although recent studies suggest that the γ-secretase plays important roles in cellular signaling including Notch pathway. Recently, much attention

has been focused on γ-secretase modulators (GSMs), which selectively decrease or increase the generation of Aβ42, the most aggregable and predominantly deposited species in AD brains, without affecting Notch signaling. Among GSMs, GSM-1 is a potent phenylpiperidine compound that reduces the secretion of Aβ42 and increases that of Aβ38, from cultured cells. We found that GSM-1 simultaneously decreased the secretion of Aβ37 and Aβ39 without affecting Aβ40, AICD as well as NICD generation. Furthermore, GSM-1 derivatives that were modified at the carboxylic acid increased Aβ42 secretion, suggesting that the Aβ42-lowering activity of GSM-1 is dependent on its carboxylic acid moiety. GSM-1 lowered the Aβ42 generation in an in vitro assay using recombinant substrate and reconstituted γ-secretase complex, suggesting the direct action of GSM-1 on the γ-cleavage. Finally, we generated a GSM-1-based photoprobe equipped with a benzophenone and a biotin moieties, GSM-1-BpB, preserving the Aβ42-lowering activity. Photoaffinity labeling experiments revealed that GSM-1-BpB biotinylated the N-terminal fragment of PS1. These data suggest a direct mode of action of GSM-1 on the γ-secretase.

Disclosures: T. Tomita, None; **Y. Ohki**, None; **S. Yokoshima**, None; **T. Higo**, None; **N. Shimada**, None; **H. Koizumi**, None; **S. Osawa**, None; **T. Fukuyama**, None; **T. Iwatsubo**, None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.9

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant EY014227

NIH Grant RR022570

NIH Grant AG010485

NIH Grant AG022550

NIH Grant AG027956

Felix and Carmen Sabates Missouri Endowed Chair in Vision Research

Title: Presenilin potentiates ryanodine receptor-mediated calcium release from intracellular

stores

Authors: *A. PAYNE[1,2], S. KAJA[1,2], S.-Y. HWANG[1,2], P. KOULEN[1,2];
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Abstract: Ryanodine Receptors (RyRs) are calcium channels of intracellular organelles such as the endoplasmic reticulum (ER). Disruption of RyR function has been described in several pathologies of dysregulated calcium, including but not limited to, Alzheimers disease (AD), age-related macular degeneration, and glaucoma. Presenilins (PS) are ubiquitously distributed throughout many tissues, mediating processes such as calcium homeostasis, Notch signaling, and Amyloid Precursor Protein (APP) processing. Both Presenilin proteins (PS1 and PS2) are nine transmembrane spanning proteins targeted to the ER. We hypothesized that a novel interaction between the N-terminus of PS and the RyR leads to increased calcium release from intracellular stores.
Using immunochemistry and electrophysiology we have previously shown that soluble N-terminal fragment of both PS (PS1-NTF or PS2-NTF) function as allosteric agonists at the RyR that increase its open probability (Hayrapetyan *et al*., Cell Calcium 2008, 44:507-18; Rybalchenko *et al*., Int J Biochem Cell Biol 2008, 40:84-97). Binding of the PS1-NTF or PS2-NTF fragment to RyR was concentration dependent, potentiated the channel open probability, and increased the mean calcium current. Also, the PS1-NTF increased RyR open probability at activating cytosolic calcium concentrations (1μM) while the PS2-NTF extended RyR functional range by facilitation of channel gating at inhibitory calcium concentrations (1mM).
Here, we studied the effects of the PS-RyR interaction on intracellular calcium release at the cellular level by using optical recordings of intracellular calcium concentrations. Neuronal cells treated with increasing cytosolic concentrations of recombinant PS-NTF stimulated with caffeine displayed a marked, RyR-specific increase of calcium release compared to untreated cells. Other recombinant functional domains of PS had no effect on calcium release, indicating a specific interaction between the N-terminus of PSs and RyRs. PS increased the amplitude, duration, and area under the curve of RyR-mediated calcium transients. PS had no effect on the frequency or rate of calcium release.
We here report a functional interaction between PS and RyRs that occurs under physiologically relevant conditions at the cellular level. Our results propose a novel mechanism of neuronal calcium regulation with the inherent potential to prompt novel neuroprotective stategies that address dysregulated intracellular calcium in chronic neurodegenerative diseases such as AD, age-related macular degeneration, and glaucoma.

Disclosures: A. Payne, None; **S. Kaja**, None; **S. Hwang**, None; **P. Koulen**, None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.10

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant 5RO1NS055161

ADDF

Title: Small synthetic peptides from PS1 amino-terminal domain significantly inhibit the production of Aß in vitro and in brains of APP transgenic mice

Authors: *N. N. DEWJI[1], E. MASLIAH[2], E. ROCKENSTEIN[3], S. SINGER[4], M. ARASOGHLI[5];
[1]Dept Med., Univ. California San Diego, LA JOLLA, CA; [2]Neurosciences and Pathology, [3]Neurosciences, [4]Biol., [5]Med., UCSD, La Jolla, CA

Abstract: Our earlier published studies showed that a cell-cell interaction, mediated by the specific binding of the β-amyloid precursor protein (β-APP) on one cell surface with presenilin (PS) on the other cell surface, is a required initial step in the ultimate production of at least the major part of β-amyloid (Aβ) from β-APP. In these experiments, a cell-based assay was used in which some cells were modified to express surface β-APP but no PS, and other cells to express surface PS but no β-APP. These two cell populations were co-cultured for 24h at densities that ensured cell-cell contact. Aβ production in such co-cultures could only occur if the two cell types interacted with one another to provide the β-APP and the PS required for the generation of Aβ. We also demonstrated that if the entire N-terminal domain of the PS was first added to the culture, the amount of Aβ produced was significantly reduced.
We now report the identification of two lead peptides, P8 (PS-1 residues 66-73) and P4 (PS-1 residues 41-55), synthesized to sequences in the N-terminal domain of PS-1, that are active inhibitors of Aß production both *in vitro* and *in vivo*.
In *in-vitro* experiments, ß-APP-only and PS-1-only cells were co-cultured as described above, in the presence and absence of 0-3µM P4, P8 and control peptides. The production of Aß40 was analyzed by ELISA and shown to decrease in a dose-dependent manner, with complete inhibition of Aß40 production with 3 µM P8, and 60% inhibition with 3µM P4, over co-cultures without the peptides or those with control peptides.
Inhibition of Aß production by P4 and P8 was next studied in the brains of Bl6/DBA/SW APP transgenic mice. Peptide solutions were delivered directly into lateral ventricles of six month old, age-matched, male and female mice by cannula implanted into the frontal cortex and connected to an osmotic minipump. P4, P8 and control peptides (10µM) or vehicle only were delivered for two weeks, after which the mice were maintained for an additional two weeks before being sacrificed.
Immunohistochemical analysis of Aβ deposits in the hippocampus and cortex was carried out on sections immunolabeled with antibodies against Aβ 1-16, followed by (FITC)-conjugated anti-mouse IgG. Total Aß production decreased by about 40% in both the hippocampus and cortex by P4 treatment, and by about 50% by P8.

ELISA analysis of Aß40 and 42 levels in the cortex showed an inhibition of the production of both Aß 40 and 42 by the two peptides. P8 inhibited Aß40 production by about 75% and Aß 42 by about 40%. P4 inhibited Aß40 production by about 60% and Aß 42 production by about 40%. P4 and P8 are being further developed as potential peptide drugs to inhibit the production of Aß in Alzheimers disease.

Disclosures: **N.N. Dewji**, Cenna Biosciences Inc, Consultant/Advisory Board; **E. Masliah**, None; **E. Rockenstein**, None; **S. Singer**, Cenna Biosciences Inc., Ownership Interest; **M. Arasoghli**, None.

Nanosymposium

627. Alzheimer's Disease: Presenilins, BACE, Beta and Gamma Secretase

Location: Room 31C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 627.11

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH-NIA AG-032180

Title: Amyloid precursor protein and presenilin might regulate synapse plasticity by modulating neurotrypsin expression and concomitant agrin cleavage

Authors: *A. ALMENAR-QUERALT[1], K. SCHIMMELPFENG HENTHORN[2], S. GUNAWARDENA[2], C. HERRERA[2], S. N. KIM[2], I. GARCIA-BASSETS[2], L. S. GOLDSTEIN[2];
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Abstract: Disturbed synaptic plasticity and function are believed to lead to progressive memory and cognitive decline in Alzheimer’s disease (AD). Although amyloid precursor protein (APP) and presenilin (PS) mutations cause familial AD, how they lead to synaptic dysfunction is not clearly understood. To identify new synaptic pathways regulated by APP and PS dysfunction we turned to Drosophila where, unlike mammals, a single gene for APP, APP like (Appl), and PS (Psn) exist. By expression profiling Appl and Psn mutants in Drosophila larval brains, we found that a serine protease, Tequila (Teq), reported to regulate long-term memory, is negatively regulated by Appl and Psn. Supporting a role of Teq in synaptic events we found altered neuromuscular junction (NMJ) synaptic morphology in Teq mutants. The proposed mammalian homolog of Teq, neurotrypsin, is a synaptically secreted serine protease that causes mental retardation in humans when mutant. We also find APP and PS to negatively regulate

neurotrypsin expression in several mammalian systems suggesting a conserved regulatory pathway across species. In particular, we find that reduction of PS switches the transcriptional state of the neurotrypsin promoter by promoting histone acetylation and recruitment of PolII, the transcription factor CREB and the co-activator CBP. These PS-induced transcriptional changes of the neurotrypsin promoter correlate with changes in neurotrypsin-dependent cleavage of the heparan sulfate agrin, recently proposed to induce formation of dendritic filopodia. More interestingly, we find that familial AD associated PS and APP mutations alter neurotrypsin expression, and, consequently neurotrypsin-dependent cleavage of agrin in mouse embryonic fibroblasts (MEFs) and in mouse hippocampus. In conclusion, our findings reveal a new function for APP and PS as upstream modulators of neurotrypsin expression and its agrin cleavage activity, and suggest that impairments of this pathway caused by APP and PS FAD associated mutations might contribute to compromised synaptic plasticity leading to impaired learning and memory processes in AD.

Disclosures: A. Almenar-Queralt, None; **K. Schimmelpfeng Henthorn**, None; **S. Gunawardena**, None; **C. Herrera**, None; **S.N. Kim**, None; **I. Garcia-Bassets**, None; **L.S. Goldstein**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.1

Topic: C.02. Alzheimer's disease and other dementias

Support: Australian Research Council

National Health & Medical Research Council

Title: Parkinsonism in aged tau knockout mice and decreased tau in Parkinson’s disease and MPTP-treated mice

Authors: *P. LEI[1,2], S. AYTON[1,3], Y. HUNG[1,3], J. DUCE[1,3], G. D. CICCOTOSTO[1,2], R. CAPPAI[1,2], D. I. FINKELSTEIN[1], A. I. BUSH[1,2];
[1]Mental Hlth. Res. Inst., Parkville, Australia; [2]Dept. of Pathology, [3]Ctr. for Neurosci., the Univ. of Melbourne, Carlton, Australia

Abstract: Tau protein, the key component of neurofibrillary tangles involved in Alzheimer’s disease, has been recently genetically linked to Parkinson’s disease (PD). Transgenic mice

expressing mutant tau develop a PD phenotype by an uncertain mechanism. Tau knockout mice up to 7 months of age were previously reported to lack morphological abnormalities of the central or peripheral nervous systems, or behavioral deficits. Here we report that older $tau^{-/-}$ mice (12 months of age) develop Parkinsonism accompanied with brain neurodegeneration. We show that the wet brain weight of $tau^{-/-}$ mice (n = 15) is significantly decreased compared to normal background-matched mice (C57BL6/SV129) (n = 13), while the body weight remains the same. Further, we found that continual loss of tau with aging resulted in cortical shrinkage and enlargement of the lateral ventricles, but no change in corpus callosum thickness. Tyrosine Hydroxylase staining suggests substania nigra (SN) neuronal loss in $tau^{-/-}$ mice. Accordingly, we found that $tau^{-/-}$ mice exhibit impaired motor behaviors evidenced by significantly reduced locomotion distance in the open field test, significantly less time maintained in the rotarod test, and significantly more time to turn in the pole test. Results from Y maze testing do not indicate cognitive impairment. Tau protein levels were significantly decreased in SN of the brains of PD patients. Similarly, MPTP-treated mice, an animal model of PD, exhibited lower SN tau levels as early as the third day post treatment. Collectively, these results indicate potential importance of tau in PD pathogenesis.

Disclosures: P. Lei: None. **S. Ayton:** Other Research Support; Victorian Brain Bank Network. **Y. Hung:** None. **J. Duce:** None. **G.D. Ciccotosto:** None. **R. Cappai:** None. **D.I. Finkelstein:** Consultant/Advisory Board; Prana Biotechnology Ltd. **A.I. Bush:** Research Grant; Australian Research Council, National Health & Medical Research Council. Consultant/Advisory Board; Prana Biotechnology Ltd.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.2

Topic: C.02. Alzheimer's disease and other dementias

Support: The Alzheimer's Association (Chicago, USA)

Title: Multifunctional neuroprotective iron chelating drugs as a novel therapeutic strategy for Alzheimer's disease

Authors: *O. WEINREB, T. AMIT, L. KUPERSHMIDT, M. B. H. YOUDIM;
Technion-Israel Inst. of Technology, Medicine, Pharmacology, Eve Topf Ctr., Haifa, Israel

Abstract: The concept of iron chelators, which can cross the blood brain barrier and possess iron

chelating ability for removal of excess of iron in the neurodegenerative brain, lead our group to develop novel non-toxic, lipophilic brain permeable multifunctional drugs. Based on a multimodal drug design paradigm, we deliberately incorporated the neuroprotective propargylamine moiety of the anti-Parkinson drug rasagiline (Azilect, Teva Inc.) into the antioxidant-iron chelator moiety of an 8-hydroxyquinoline derivative of the neuroprotective brain permeable iron chelator, VK28**.** Among these compounds, M30 and HLA20 were found to possess neuroprotective effects against a variety of neurotoxins in neuronal cell cultures and in vivo models of neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease (AD). M30 and HLA20 were demonstrated to possess a wide range of pharmacological activities, including regulatory effects on neuronal differentiation, neurite outgrowth and hypoxia inducible factor-1 (HIF-1) alpha and HIF-1 related neuroprotective target genes**.** Regarding AD**,** we demonstrated that M30 induced a significant down-regulation of membrane-associated holo-amyloid precursor protein (APP) levels in mouse hippocampus and human SH-SY5Y neuroblastoma cells. In addition, M30 was found to suppress the translation of luciferase reporter mRNA via the APP 5'UTR sequence and to markedly reduce the amyloid beta (Abeta) levels in the medium of CHO cells, stably transfected with the APP "Swedish" mutation. In accordance, M30 was also shown to protect cultured cortical neurons against Abeta25-35 toxicity. Recently, we have demonstrated that chronic M30 (5 mg/kg) administration attenuated behavioral deficits in APPswe/PS1 transgenic mouse model of AD. Additionally, M30 decreased APP and Abeta levels and plaques in the frontal cortex, hippocampus and parietal cortex of APPswe/PS1 transgenic mice. These observations are appearing to offer M30 for potential therapeutic compound for AD.

Disclosures: O. Weinreb, None; **T. Amit**, None; **L. Kupershmidt**, None; **M.B.H. Youdim**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.3

Topic: C.02. Alzheimer's disease and other dementias

Support: Allon Therapeutics Inc.

Title: Translational neuroscience in tauopathy: From activity-dependent neuroprotective protein to the drug candidate, davunetide

Authors: *I. GOZES, Y. SCHIRER;

Sackler Sch. Med/Tel Aviv Univ., Tel Aviv, Israel

Abstract: Activity-dependent neuroprotective protein (ADNP) is essential for brain formation, neuronal plasticity, and neuroprotection. Partial knockdown of ADNP results in tau pathology (hyperphosphorylation, tangle like pathology) and neuronal death as well as cognitive impairments. Tau related-neurotoxicity and neurodegeneration has been implicated in the pathogenesis of Alzheimer's disease (AD) and related tauopathies. The rTg4510 tau transgenic mouse model of frontotemporal dementia (FTD) expresses doxycycline-repressible human P301L mutant tau (4 repeat tau-tubulin binding isotype) and exhibits progressive neuronal loss and behavioral impairments. To establish a potential reciprocal connection between tau pathology and ADNP expression we used this mutated tau mouse model. Analysis of ADNP mRNA (real time quantitative polymerase chain reaction) and protein expression (western) in the cerebral cortex of 1, 3, 5.5, 9-month-old Tg and non-Tg mice showed a significant increase in ADNP expression in young Tg mice compared to non-Tg mice and a decline with progressing pathology. The expression of the dynamic mouse tau (3 repeat tubulin binding domains) was also monitored in this model and results showed an expression pattern paralleling that of ADNP. The correlation between tau expression and ADNP expression suggest a potential compensatory mechanism in trying to protect against tauopathy. In this respect, the ADNP-derived peptide drug candidate NAP (davunetide) protected in part against ADNP-associated dysfunction, and against tau hyperphosphorylation and tangle-like deposits in relevant models of tau pathology. It is our working hypothesis that NAP (davunetide) replaces the paucity/reduced activity of ADNP under pathological conditions.
Importantly, in clinical trials, davunetide improved cognitive scores in patients suffering from amnestic mild cognitive impairment (a precursor to Alzheimer's disease) known to exhibit neurofibrillary tangles. Based on these results, davuentide is currently advancing to late stage clinical trials in progressive supranucelar palsy, exhibiting tauopathy and rapid degeneration (www.allontherapeutics.com).

Disclosures: I. Gozes: Other Research Support; Allon Therapeutics Inc.. Consultant/Advisory Board; Allon Therapeutics Inc.. **Y. Schirer:** None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.4

Topic: C.02. Alzheimer's disease and other dementias

Support: CIHR

NSERC

MSFHR

Title: Glycogen synthase kinase regulate amyloid precursor processing independent of gamma secretase activity

Authors: *P. T. LY, H. ZOU, W. ZHOU, W. SONG;
Univ. British Columbia, Vancouver, BC, Canada

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder leading to dementia. Neuritic plaques, neurofibrillary tangles, and neuronal loss represent the main histological hallmarks observed in AD brains. Particularly, Amyloid β-protein (Aβ) accumulation, which is the central component of neuritic plaques, appears to play a critical role in AD pathogenesis. Aβ is generated from sequential cleavages of the β-amyloid precursor protein (APP) by the β- and γ-secretases. Therefore, inhibition of the pathways that lead to Aβ generation will have therapeutic implications for the treatment of AD. Previous research indicated that glycogen synthase kinase 3 (GSK3) facilitates Aβ production by positively modulating γ-secretase activity. Lithium chloride (LiCl) and valproic acid (VPA) are well known inhibitors of GSK3. We found that LiCl and VPA treatment lead to an accumulation of APP C-terminal fragments (CTF) in vitro and in vivo, indicating inhibition of γ-secretase activity. Although LiCl and VPA are GSK3 inhibitors, these compounds also activate a plethora of signaling cascades that may differentially regulate APP processing. Using a GSK3 specific inhibitor, ARA014418, we observed reduced CTF levels in a dose-dependent manner. This indicated that GSK3 inhibition by ARA014418 inhibited APP processing via a γ-secretase-independent mechanism. We also found that ARA014418 treatment to a mouse model of Alzheimer's disease reduced plaque formation and rescued cognitive deficits. Our work suggests that GSK3 regulates amyloid processing at different levels and that GSK3 remains a valid target for treating AD pathology.

Disclosures: P.T. Ly, None; **H. Zou**, None; **W. Zhou**, None; **W. Song**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.5

Topic: C.02. Alzheimer's disease and other dementias

Title: Pharmacological enhancement of PP2A activity by SIG1012 as an Alzheimer's disease therapeutic

Authors: *S. P. BRAITHWAITE[1], J. R. FERNANDEZ[1], Y. CHAO[1], X. FENG[1], M. VORONKOV[1], M. STOCK[1], J. B. STOCK[1], E. PLANEL[2];
[1]Signum Biosci., Monmouth Junction, NJ; [2]Neurosciences, Ctr. Hospitalier de l'Université Laval, Quebec City, QC, Canada

Abstract: Deficits in PP2A levels, activity and methylation status have been reported in Alzheimer's disease patients. Furthermore, PP2A is a major tau phosphatase and central regulator of metabolic processes. Therefore it is hypothesized that restoration of PP2A activity in Alzheimer's disease may be of therapeutic benefit and modify the course of disease progression. We have identified a small molecule potentiator of PP2A, SIG1012, that inhibits PP2A demethylation, therefore promoting assembly of PP2A subunits into functional holoenzymes and increasing its enzymatic activity. The efficacy of pharmacological enhancement of PP2A by SIG1012 has been tested in cellular and in vivo models relevant for Alzheimer's Disease. SIG1012 treatment reduced PP2A demethylation in cultured hippocampal neurons, furthermore, in cellular models of PP2A inhibition and subsequent tau hyperphopshorylation, SIG1012 treatment reduces tau phosphorylation levels. In vivo, SIG1012, administered orally, inhibits anesthesia induced changes in tau phosphorylation. This effect is primarily through the direct effect of PP2A on phosphorylated tau as it does not influence the phosphorylation state of a number of relevant kinases. SIG1012 is also efficacious in reversing deficits in Tau overexpressing transgenic models of Alzheimer's Disease.
Together these studies indicate that PP2A enhancement by SIG1012 is safe and efficacious in reversing Alzheimer's Disease relevant endpoints in vivo. Thus PP2A modulation provides a novel, disease modifying, therapeutic mechanism that can be pharmacologically targeted.

Disclosures: **S.P. Braithwaite**, Signum Biosciences, Employment; **J.R. Fernandez**, Signum Biosciences, Employment; **Y. Chao**, Signum Biosciences, Employment; **X. Feng**, Signum Biosciences, Employment; **M. Voronkov**, Signum Biosciences, Employment; **M. Stock**, Signum Biosciences, Employment; Signum Biosciences, Ownership Interest; **J.B. Stock**, Signum Biosciences, Ownership Interest; **E. Planel**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.6

Topic: C.02. Alzheimer's disease and other dementias

Support: NINDS, NIH

Title: Quantitative phosphoproteomics of neuronal cytoskeletal [neurofilament (NF-M/H), Tau] and synaptic proteins in Alzheimer's disease. Implication for therapeutics in neurodegeneration

Authors: *P. RUDRABHATLA[1], H. JAFFE[2], H. C. PANT[2];
[1]NINDS, BETHESDA, MD; [2]NINDS, NIH, Bethesda, MD

Abstract:
Aberrant hyperphosphorylation of proline directed serine/threonine (pSer/Thr-Pro) residues in neuronal cytoskeletal proteins (neurofilament and Tau) is one of the major pathological hallmarks of neurodegenerative disorders such as Alzheimer Disease (AD), Amyotrophic Lateral Sclerosis (ALS) and Parkinson's Disease (PD). Human neurofilament (NF-H/M) and Tau comprises of multiple SP repeats. Here, we used quantitative phosphoproteomics, iTRAQ (isobaric tag for relative and absolute quantitation) and analyzed the phosphorylation sites of NF-M/H and Tau from AD cortex. We identified 14 hyperphosphorylated sites of NF-M, 9 Lys-Ser-Pro (KSP) sites, two variant motifs, Glu-Ser-Pro (ESP) Ser736 and Leu-Ser-Pro (LSP) Ser837, and 3 non-S/TP motifs, Ser783, Ser788 and Thr750. All the Ser/Thr residues are phosphorylated at significantly greater abundance in AD brain (4-10 fold) compared to normal brain. Eleven hyper phosphorylated sites have been identified in NF-H, with greater abundance of phosphorylation in AD compared to normal brain, comprising 10 KSP sites and one non-SP site, Thr642. Our data provide the direct evidence that NF-M/H are hyperphosphorylated in AD compared to normal brain and suggest the role of both proline-directed and non proline-directed protein kinases in AD. We also analyzed the phosphorylation sites of Tau (isoform 1) in AD and report that Y711, S713, T720, S721 are abundantly phosphorylated in AD compared to normal brain. The Y711 in Tau is phosphorylated 15-fold higher in AD compared to normal brain, suggesting that Tyr phosphorylation is involved in AD brain. The same AD brain patients showed the down regulation of synaptic protein phosphorylation. We demonstrate that Ser14 and Thr5, CKII phosphorylation sites of syntaxin 1A are down regulated in AD brain. Furthermore, the phosphorylation of other synaptic proteins, syntaxin B1 (Ser 14), septin 5 (Ser 226, Y218), septin 2 (Ser 248), vesicle transport-related protein (Thr403) are down regulated in AD brain. This study represents the first comprehensive analyses of iTRAQ quantification of phosphorylation sites of human neuronal cytoskeletal [neurofilament (NF-M/H), Tau] and synaptic proteins from AD brain. Unraveling the phosphorylation sites of neuronal cytoskeletal and synaptic proteins would lead to a greater understanding of not only normal brain functioning but also successful kinase and/or phosphatase-based therapeutics.

Disclosures: P. Rudrabhatla, None; **H. Jaffe**, None; **H.C. Pant**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.7

Topic: C.02. Alzheimer's disease and other dementias

Support: Rosalinde and Arthur Gilbert Foundation/American Federation for Aging Research

CurePSP

NIA grant AG031291

Title: Rapid association of Hsc70 with tau after microtubule destabilization

Authors: *J. O'LEARY[1], U. K. JINWAL[1], S. I. BORYSOV[1], J. R. JONES[1], Q. LI[1], J. KOREN, III[1], J. F. ABISAMBRA[1], G. D. VESTAL[1], L. Y. LAWSON[1], A. G. JOHNSON[1], L. J. BLAIR[1], Y. JIN[1], Y. MIYATA[2], J. E. GESTWICKI[2], C. A. DICKEY[1];
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Abstract: The microtubule associated protein tau is an important protein in neuron biology as it aids in the regulation of the dynamic stability of microtubules, synaptic transmission and axonal transport. However, in a group of neurodegenerative diseases, such as Alzheimer's disease (AD) and other tauopathies, conformational changes in tau are associated with the initial stages of disease pathology. In addition, recent work by our group and others suggests that members of the heat shock protein (Hsp) 70 family play a significant role in tau regulation. We have found that pharmacological manipulation of the ATPase activity of the two cytosolic Hsp70 proteins regulates tau levels. Clinically, this is of great importance due to the close relationship between the accumulation of tau and cognitive dysfunction in AD. One of the earliest pathological alterations of tau in AD is the folding of tau into the MC1 conformation, where amino acids at residues 7 - 9 interact with residues 312 - 342. The mechanism of this conformational change in tau and the subsequent effect on function and association to microtubules is largely unknown. Furthermore, how the formation of MC1 relates to the initial loss of function of tau at microtubules is also unknown. Our new findings suggest that heat shock cognate (Hsc) 70 facilitates tau-mediated microtubule polymerization. The association of Hsc70 with tau was rapidly enhanced following treatment with microtubule destabilizing agents. The fate of tau released from the microtubule was found to be dependent on ATPase activity of Hsc70. Microtubule destabilization also rapidly increased the MC1 folded conformation of tau. An in vitro assay suggests that Hsc70 facilitates formation of MC1 tau. However, in a hyper-phosphorylating environment the formation of MC1 was abrogated, but Hsc70 binding to tau was enhanced. Thus, under normal circumstances, MC1 formation may be a protective conformation facilitated by Hsc70. However in a diseased environment, Hsc70 may preserve tau in a more unstructured state, perhaps facilitating its pathogenicity.

Disclosures: J. O'Leary, None; **U.K. Jinwal**, None; **S.I. Borysov**, None; **J.R. Jones**, None; **Q. Li**, None; **J. Koren, III**, None; **J.F. Abisambra**, None; **G.D. Vestal**, None; **L.Y. Lawson**, None; **A.G. Johnson**, None; **L.J. Blair**, None; **Y. Jin**, None; **Y. Miyata**, None; **J.E. Gestwicki**, None; **C.A. Dickey**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.8

Topic: C.02. Alzheimer's disease and other dementias

Support: Alzheimer's Association Zenith Award

T32 AG20506

Title: Neuronal vulnerability in Alzheimer’s disease: Age-related loss of calbindin predisposes cholinergic neurons to tangle formation and degeneration

Authors: *A. T. BAKER-NIGH, D. RIASCOS, C. GEULA;
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Abstract: The presence and toxicity of pathologic proteins in neurodegenerative disorders has been the subject of extensive experimental attention. However, the reason(s) for selective neuronal vulnerability in these disorders is unknown. We used the human basal forebrain cholinergic system as a model to address this issue. The cholinergic neurons of the basal forebrain (BFCN) are characterized by early, selective and severe loss in Alzheimer’s Disease (AD). Age is the primary risk factor for AD and other neurodegenerative disorders of the elderly, in which loss of BFCN is also a common finding. We have hypothesized that age-related changes make a major contribution to neuronal vulnerability, including that of BFCN, in the above disorders. We have demonstrated the exclusive presence of calbindin-D28k (CB) in the primate BFCN. We have also shown a significant and selective age-related loss of CB from the BFCN in the human and in non-human primates. . We have suggested that the age-related loss of CB, which is involved in regulation of intracellular calcium levels, deprives the BFCN of the capacity to buffer intracellular calcium levels and leaves them vulnerable to calcium toxicity due to increased intracellular calcium. Here we report that tangle formation and degeneration occur nearly exclusively in BFCN that have lost their CB in the course of normal aging. PHF-1 immunoreactive tangles and pre-tangles were present in normal aged and AD BFCN. However,

double stained sections revealed that only 1-3% of these were in CB-positive BFCN; the remaining 97-99% were in CB-negative BFCN. Similarly, only 0.4-1% of CB-positive neurons in the normal elderly and 4-10% in AD contained tangles and pre-tangles. Thioflavin S stained tangles showed an identical pattern of distribution. The total number of CB-positive BFCN in AD was similar to that in normal elderly. However, the percentage of all BFCN that contained CB in AD was significantly higher, due to degeneration of CB-negative BFCN. We conclude that the presence of CB protects the BFCN against the neurodegenerative process in AD and perhaps also in other neurodegenerative disorders. Our findings suggest that development of therapeutic strategies aimed at preventing calcium dysregulation will be of potential value in AD.

Disclosures: A.T. Baker-Nigh, None; **D. Riascos**, None; **C. Geula**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.9

Topic: C.02. Alzheimer's disease and other dementias

Support: CurePSP Grant 472-09 to KB

NIH Grant AG023012 to BTL

Title: Regulation of tau pathology by the microglial fractalkine receptor

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Abstract: Aggregates of the hyperphosphorylated microtubule associated protein tau (MAPT) are an invariant neuropathological feature of Alzheimer’s Disease (AD) and related tauopathies. While increasing evidence suggests that alterations in microglial activation is a common feature of tauopathies, the exact pathogenic role of microglia in disease pathogenesis remains unclear. Here we show that neuroinflammation mediated by toll-like receptor (TLR4) or genetic deletion of the microglial fractalkine receptor, CX3CR1, promotes MAPT phosphorylation and aggregation through a mechanism that is mediated via the p38 mitogen activated protein kinase (MAPK) signaling pathway. First, we demonstrate that lipopolysaccharide (LPS)-mediated

microglial activation induces hyperphosphorylation of endogenous mouse MAPT in non-transgenic mice that is further enhanced in mice lacking CX3CR1 and is dependent upon functional toll-like receptor 4 and interleukin 1 receptors. Second, the humanized MAPT transgenic (hTau) mouse model of tauopathies lacking CX3CR1 exhibits enhanced MAPT hyperphosphorylation and aggregation, formation of silver positive neurofibrillary tangles as well as behavioral impairments that correlated with increased levels of active p38 MAPK and its downstream target, activation transcription factor 2 (ATF2). Finally, in vitro experiments utilizing primary microglia and cortical neurons demonstrate that microglial activation elevates the level of active p38 MAPK and enhances MAPT hyperphosphorylation within neurons that can be blocked by administration of an interleukin 1 receptor antagonist and a specific p38 MAPK inhibitor. Taken together, our results demonstrate that fractalkine-receptor deficiency in microglia directly induces MAPT pathology via activation of TLR4/IL1/p38 MAPK pathway and suggests that CX3CR1 and/or p38 MAPK signaling pathways may provide novel therapeutic targets for human tauopathies.

Disclosures: K. Bhaskar, None; **M. Konerth**, None; **O. Kokiko-Cochran**, None; **A. Cardona**, None; **R.M. Ransohoff**, None; **B.T. Lamb**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.10

Topic: C.02. Alzheimer's disease and other dementias

Support: Consortium for Frontotemporal Dementia

Title: The communicome of a disease: Application and potential of human plasma proteomics to study frontotemporal dementia

Authors: *P. A. JAEGER[1,2], M. BRITSCHGI[1], C.-H. SUN[1], H. JOHNS[1], B. BURKHOLDER[3], S. PRADHAN[1], R. C. PETERSEN[4], D. S. KNOPMAN[4], B. F. BOEVE[4], A. L. BOXER[6], A. KARYDAS[6], B. L. MILLER[6], R. RADEMAKERS[5], D. W. DICKSON[5], N. GRAFF-RADFORD[5], T. WYSS-CORAY[1,7];
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Abstract: Diagnostics and treatment of neurodegenerative proteopathies such as Alzheimer Disease (AD) or Fronto-temporal dementia (FTLD) have been hindered by the lack of reliable biochemical markers for disease identification and limited understanding of the underlying pathological mechanisms. While major advances have been made in the last decades to help understand hereditary (familial) forms of these diseases, the vast majority of cases is of the sporadic type and not associated with an identifiable mutation. Yet the common neurological and pathological representation of the diseases in both familial and sporadic cases indicates the involvement of shared pathological pathways. Increased markers of inflammation, altered neurotrophic support, or changes in immune system modulating compounds have been reported, but it is still unclear how these factors link together into a pathological network. To help identify these complex pathways we employed medium scale plasma proteomics by measuring more than 700 soluble cellular communication factors which we collectively termed the "communicome" in plasma from control (N=100), AD (N=60), and FTLD (N=103) patients, as well as from FTLD patients with familial Progranulin mutations (N=27). By using an antibody microarray based approach in combination with statistical and computational tools we were able to identify altered pathways, even when the absolute changes in plasma protein levels were only moderate (0.5 to 2.5 fold) and the background levels variable. Applying this approach we discovered several new pathways that appear deregulated in sporadic AD and FTLD. After confirming our findings through a variety of independent experiments, we believe that these pathways could represent interesting new targets for therapeutic intervention in neurodegenerative diseases. While our approach is not initially designed to discover diagnostic markers, our findings underscore that while single molecular markers might be unreliable predictors of the diseases, looking at members of complete pathways can be a promising way to increase diagnostic specificity and sensitivity.
The study was supported by the Consortium for Frontotemporal Dementia.

Disclosures: P.A. Jaeger, None; **M. Britschgi**, None; **C. Sun**, None; **S. Pradhan**, None; **H. Johns**, None; **R.C. Petersen**, None; **D.S. Knopman**, None; **B.F. Boeve**, None; **A.L. Boxer**, None; **A. Karydas**, None; **B.L. Miller**, None; **R. Rademakers**, None; **D.W. Dickson**, None; **N. Graff-Radford**, None; **T. Wyss-Coray**, None; **B. Burkholder**, RayBiotech, Inc., Employment.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.11

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH P01-AG017586

NIH T32-AG00255

Koller Foundation for ALS Research Grant

Gift from the Podolin family

Burroughs Wellcome Fund

Benaroya Fund

Title: Dysregulation of endogenous TDP-43 in conditional transgenic mice expressing human TDP-43

Authors: *E. B. LEE, L. M. IGAZ, L. K. KWONG, A. CHEN-PLOTKIN, E. SWANSON, T. UNGER, J. MALUNDA, Y. XU, M. J. WINTON, J. Q. TROJANOWSKI, V. M.-Y. LEE; Ctr. for Neurodegenerative Dis. Res., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Transgenic mice conditionally expressing human wild type TDP-43 (hTDP-43-WT) and hTDP-43 with a defective nuclear localization signal (hTDP-43-ΔNLS) directed by the CaMKIIα promoter were generated to elucidate mechanisms of neurodegeneration in TDP-43 proteinopathies. Expression of hTDP-43-WT or hTDP-43-ΔNLS led to phosphorylated and ubiquitinated TDP-43 inclusions, neuron loss in selectively vulnerable forebrain regions, corticospinal tract degeneration and motor spasticity recapitulating key aspects of frontotemporal lobar degeneration and primary lateral sclerosis. Remarkably, neurodegeneration was linked to a dramatic downregulation of endogenous mouse TDP-43 in nuclei of affected neurons associated with changes in gene expression, especially up regulation of genes involved in chromatin assembly. Our data suggest that perturbation of highly regulated endogenous nuclear TDP-43 results in loss of functions and changes in downstream gene regulatory pathways that trigger degeneration of selectively vulnerable neurons.

Disclosures: E.B. Lee, None; **L.M. Igaz**, None; **L.K. Kwong**, None; **A. Chen-Plotkin**, None; **E. Swanson**, None; **T. Unger**, None; **J. Malunda**, None; **Y. Xu**, None; **M.J. Winton**, None; **J.Q. Trojanowski**, None; **V.M. Lee**, None.

Nanosymposium

628. Tau, Alzheimer's, FTD, TDP-43, and Neurodegeneration

Location: Room 23A

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 628.12

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant T32 NS007205

NIH Grant P50 AG005681

NIH Grant P30 NS057105

Charles and Joanne Knight Alzheimer Research Initiative

Title: Core features of human frontotemporal dementia recapitulated in progranulin knockout mice

Authors: *N. GHOSHAL[1], J. T. DEARBORN[2], D. F. WOZNIAK[2], N. J. CAIRNS[3]; [1]Dept. of Neurol., Washington Univ., SAINT LOUIS, MO; [2]Dept. of Psychiatry, [3]Depts. of Neurol. and Pathology & Immunol., Washington Univ., Saint Louis, MO

Abstract: Frontotemporal dementia (FTD) represents 10-20% of all dementia cases. FTDs are characterized by changes in behavior, personality, and language with dementia appearing late in the disease. Behavioral changes include altered social comportment, lack of motivation, withdrawal, and apathy. Memory deficits are manifested by impaired social learning and memory performance. Dominantly inherited FTD comprises 5-10% of all cases. Mutations in the progranulin gene (*GRN*) co-segregate with affected individuals in a subset of these kindreds. To date, 68 *GRN* mutations have been identified (http://www.molgen.ua.ac.be/ADMutations) many of which introduce a premature termination codon with resultant absence of the mutant *GRN* transcript. This loss of functional progranulin implicates a haploinsufficiency mechanism for neurodegeneration.

To develop an FTD mouse model, we are studying consequences of *GRN* mutations using progranulin knockout mice ($GRN^{-/-}$) previously generated on C57BL/6 background by homologous recombination (Kayasuga et al., 2007). Previously, we reported that 9-12 month-old knockout ($GRN^{-/-}$; n=8) male mice did not demonstrate heightened aggression compared to age-matched wild type ($GRN^{+/+}$; n=7) male controls on the resident-intruder test as has been previously reported for these mice at much younger ages (Kayasuga et al., 2007). Consistent with these findings, we report here that $GRN^{-/-}$ mice showed significantly reduced reactivity to handling relative to that of $GRN^{+/+}$ controls (p= 0.009) across 3 test days. In addition, we reported previously that acquisition (spatial learning) performance of $GRN^{-/-}$ mice was significantly impaired during place trials in the Morris water maze and that there was evidence of mild retention deficits as indexed by the $GRN^{-/-}$ mice showing reduced platform crossings. Here we report that $GRN^{-/-}$ mice (n=12) did not show significant improvements in performance from trial 1 to trial 4 within a learning set water maze protocol when the platform was moved daily to a new location over 5 days. In contrast, $GRN^{+/+}$ controls (n=11) did show a significant "savings" from trial 1 to trial 4 across days (p=0.047). Now we also report new pathological analyses that reveal increased ubiquitin-positive lesions, astrocytosis, and microgliosis characteristic of FTD in elder *GRN*-/- as compared to *GRN*+/+ mice. These lesions were found in key anatomical

regions subserving the behavioral findings such as the hippocampus, cortex, and thalamus. Our data suggest that the *in vivo GRN*$^{-/-}$ mouse model reproduces some analogous behavioral and neuropathological aspects of the FTD phenotype and thus may serve as a valuable *in vivo* model of FTD.

Disclosures: **N. Ghoshal**, N. Ghoshal, has participated or is currently participating in clinical trials of antidementia drugs sponsored by: Elan/Janssen, Eli Lilly and Company, Wyeth, Novartis, Pfizer, and Bristol-Myers Squibb, Other; **J.T. Dearborn**, None; **D.F. Wozniak**, None; **N.J. Cairns**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.1

Topic: C.03. Parkinson’s disease

Support: NIH NINDS NS057795, NS05427, NS04826, NS038377

National Parkinson Foundation

Michael J. Fox Foundation for Parkinson's Research

the American Parkinson Disease Association

the EPFL and the Swiss National Science Foundation

Title: GTPase activity plays a key role in the pathobiology of LRRK2

Authors: *Y. XIONG[1], C. E. COOMBES[2], X. LI[1], A. KILARU[3], A. D. GITLER[3], W. J. BOWERS[4], D. J. MOORE[5], V. L. DAWSON[1], T. M. DAWSON[1];
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Abstract: Parkinson’s disease (PD) is the second most common neurodegenerative disease and mutations in several genes have been linked to rare familial forms of PD. Mutations in the

leucine-rich repeat kinase 2 (LRRK2) gene have been identified as a unambiguous cause of late-onset, autosomal dominant familial PD and also sporadic PD. The LRRK2 gene encodes an extremely large protein containing multiple enzymatic and protein-protein interaction domains, including Roc-GTPase and protein kinase domains. Point mutations have been found in almost all of the identified domains of LRRK2. LRRK2 exhibits kinase activity and this activity is dependent on GTP-binding activity via the GTPase domain, whereas GTP-binding activity does not require kinase activity. However, the protein domains and/or enzymatic activities that are important for LRRK2-induced neurodegeneration and the molecular mechanism and/or pathways by which LRRK2 variants induce neuronal toxicity are poorly understood. To dissect the pathways and mechanisms involved in LRRK2-induced toxicity, we have developed a model of LRRK2-induced cytotoxicity in the budding yeast, Saccharomyces cerevisiae. Remarkably, expression of GTPase domain-containing fragments of human LRRK2 is sufficient to elicit the toxicity, which can be modulated by altering GTPase activity and is associated with defects in endocytic vesicular trafficking and autophagy. These LRRK2 variants induce similar toxicity and vesicular defects in both yeast and primary neuronal models. LRRK2-induced toxicity in yeast acts through pathways distinct from α-synuclein-induced toxicity. A genome-wide genetic screen identified modifiers of LRRK2-induced toxicity in yeast including components of vesicular trafficking pathways, which can also modulate LRRK2-caused trafficking defects. Our results provide novel insight into the basic pathobiology of LRRK2 and suggest that the GTPase domain contributes to LRRK2-induced toxicity and reveal potential treatment options for LRRK2-associated PD.

Disclosures: Y. Xiong, None; **C.E. Coombes**, None; **X. Li**, None; **A. Kilaru**, None; **A.D. Gitler**, None; **W.J. Bowers**, None; **D.J. Moore**, None; **V.L. Dawson**, None; **T.M. Dawson**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.2

Topic: C.03. Parkinson’s disease

Support: National Parkinson Foundation

American Parkinson Disease Association

Swedish Research Council

NIH Grant NS060885

NIH Grant NS062165

NIH Grant NS03877

Swiss National Science Foundation

Title: Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2

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Abstract: Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene cause late-onset, autosomal dominant familial Parkinson's disease (PD) and also contribute to idiopathic PD. *LRRK2* mutations represent the most common cause of PD with clinical and neurochemical features that are largely indistinguishable from idiopathic disease. The molecular mechanisms underlying LRRK2-linked disease are not yet clear. Currently, transgenic mice expressing wild-type (WT) or disease-causing mutants of LRRK2 have largely failed to produce frank neurodegeneration, although abnormalities in nigrostriatal dopaminergic neurotransmission have been observed. To model LRRK2-linked disease in vivo, we have developed and characterized transgenic mice expressing human LRRK2 bearing the familial PD mutations, R1441C or G2019S, driven by a hybrid CMV-enhanced human PDGFβ promoter. The G2019S LRRK2 mice exhibit widespread transgene expression throughout the brain, including within the nigrostriatal dopaminergic pathway. Our model provides the first demonstration that expression of G2019S mutant LRRK2 induces a modest yet significant loss of nigrostriatal pathway dopaminergic neurons in an age-dependent manner (~20% loss; $p < 0.005$). Dopaminergic neuronal loss occurs in the absence of classic neuropathological protein aggregates or inclusions. However, electron microscopic analysis reveals various autophagic abnormalities in the brains of aged G2019S LRRK2 mice. Furthermore, the neurite complexity of midbrain dopaminergic neurons is markedly reduced in primary cultures derived from G2019S LRRK2 mice. These new LRRK2 transgenic mice will provide important tools for understanding the mechanism(s) through which familial mutations precipitate neuronal degeneration and PD.

Disclosures: D.J. Moore, None; **J.L. Daher**, None; **D. Ramonet**, None; **L. Glauser**, None; **B.M. Lin**, None; **T.M. Dawson**, None; **V.L. Dawson**, None; **O. Pletnikova**, None; **J.C.**

Troncoso, None; **M.K. Lee**, None; **J. Kim**, None; **Y. Liu**, None; **B. Thomas**, None; **R. Banerjee**, None; **M.F. Beal**, None; **L. Yang**, None; **M. Westerlund**, None; **D. Galter**, None; **J.M. McCaffery**, None; **N.A. Jenkins**, None; **N.G. Copeland**, None; **D.A. Swing**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.3

Topic: C.03. Parkinson’s disease

Title: An emerging role of LRRK2 as a key regulator of actin dynamics in neurons

Authors: *L. PARISIADOU, C. XIE, H. CAI;
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Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder that is pathologically characterized by the loss of nigrostriatal dopaminergic neurons and the intraneuronal accumulation of α-synuclein positive inclusions, named Lewy bodies and Lewy neurites. Mutations in the *LRRK2* gene cause phenotypes with a strong overlap to idiopathic late onset form of the disease. Therefore, the detailed functional characterization of the *LRRK2* gene product could shed more light on the molecular events underlying the disease pathophysiology. However, until now the physiological role of LRRK2 protein remains elusive. Here, we describe our findings that assign an emerging role of LRRK2 in actin dynamics. Towards this direction, we showed that LRRK2 regulates neurite outgrowth of developing neurons by modulating F-actin remodeling. The underlying mechanism is the LRRK2-dependent phosphorylation of an F-actin -binding family of proteins collectively known as ERM (ezrin radixin moesin) proteins. To a further step, we evaluated the role of LRRK2 on more mature neurons. Golgi staining of striatal neurons derived from *LRRK2* knockout mice showed multiple changes in spine morphology and soma diameter. These alterations were very likely attributed to the impaired F-actin dynamics. Furthermore, in an attempt to identify the mechanism by which LRRK2 affects the dynamics of F-actin, we are actively investigating actin remodeling related proteins which display significant alteration of activities in the absence of LRRK2. These potential new findings in combination with additional data from our lab showing an involvement of LRRK2 on microtubule dynamics, clearly demonstrate an essential role of LRRK2 on cytoskeleton. The selective vulnerability of dopaminergic neurons possibly attributes to the great complexity of these neurons; therefore, even a subtle perturbation on cytoskeletal dynamics could distract the delicate organization of these neurons.

Disclosures: L. Parisiadou, None; **C. Xie**, None; **H. Cai**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.4

Topic: C.03. Parkinson’s disease

Support: NIH Grant AG028797

National Parkinson Foundation

American Parkinson Disease Association

Michael J. Fox Foundation for Parkinson’s Research

Johnson & Johnson Corporate Office of Science and Technology

Title: C. elegans model of LRRK2-linked Parkinson's disease

Authors: *S. G. CHEN[1], C. YAO[1], Y. GAO[1], W. WANG[1], A. L. WILSON-DELFOSSE[2]; [1]Pathology, [2]Pharmacol., Case Western Reserve Univ., Cleveland, OH

Abstract: Mutations in the gene encoding leucine-rich repeat kinase 2 (LRRK2) are thus far the most frequent known cause of autosomal dominant and idiopathic Parkinson’s disease (PD). The pathogenic mutations R1441C and G2019S occur within the GTPase and kinase domains of LRRK2, respectively. To study the pathogenesis of LRRK2-linked PD, we have generated transgenic *C. elegans* overexpressing human LRRK2 wild-type, R1441C and G2019S in dopaminergic (DA) neurons and other neuronal populations. Overexpression of LRRK2 proteins in DA neurons (using a DA neuron-specific promoter) causes age-dependent neurodegeneration, behavioral deficits, and locomotor dysfunction that are accompanied by a reduction of dopamine levels *in vivo*. In comparison, R1441C and G2019S mutants cause more severe phenotypes than the wild type protein. Interestingly, treatment with exogenous dopamine rescues the LRRK2-induced behavioral and locomotor phenotypes. In contrast, expression of GTPase/kinase-defective LRRK2 mutants or knockout of the *C. elegans* LRRK2 homolog, LRK-1, prevents the LRRK2-induced neurodegeneration and behavioral abnormalities. Hence, our transgenic LRRK2 *C. elegans* models recapitulate key features of PD including progressive neurodegeneration, impairment of dopamine-dependent behavior and locomotor function, and reduction in dopamine

levels. Furthermore, our findings provide strong support for the critical role of GTPase/kinase activity in LRRK2-linked pathologies. We have also examined neuronal and behavioral phenotypes of *C. elegans* overexpressing LRRK2 wild-type and mutants in all neurons driven by a pan-neuronal promoter. These invertebrate models will be useful for studying the molecular mechanisms underlying mutant LRRK2-induced neurodegeneration and for developing novel strategies toward the treatment of PD.

Disclosures: S.G. Chen, None; **C. Yao**, None; **Y. Gao**, None; **W. Wang**, None; **A.L. Wilson-Delfosse**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.5

Topic: C.03. Parkinson’s disease

Support: NIH/NINDS

M. J. Fox Foundation for Parkinson’s Research

Title: The pathological study of BAC transgenic mice brain expressing PD related G2019S mutation of LRRK2

Authors: *J. WANG[1], X. LI[1], J. C. PATEL[2], M. E. RICE[3], Z. YUE[1,4];
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Abstract: G2019S mutation of the leucine-rich repeat kinase 2 (LRRK2) has now been recognized as the most common cause of familial and some sporadic forms of Parkinson disease (PD). Although it has been suggested that the enhanced kinase activity associated with G2019S mutation is attributable to the pathogenesis of PD, the pathological role of LRRK2 in the process of diseases remains unknown. Using BAC (bacterial artificial chromosome) technique, we have previously developed FLAG-tagged wild-type LRRK2 (LRRK2-Wt) transgenic mice and G2019S (LRRK2-G2019S) mutant mice. These two LRRK2 transgenic mice express the LRRK2 protein at a similar level (~ 6-8 folds over the endogenous). We first found a significant increase of LRRK2-G2019S kinase activity over that of LRRK2-Wt in the proteins purified from the brain. We then showed that dopamine transmission and striatal dopamine content is significantly

reduced in LRRK2-G2019S mice. In contrast, LRRK2-Wt mice have elevated dopamine release, accompanied with enhanced motor function. However, TH staining in midbrain did not display obvious dopaminergic terminal degeneration in the striatum or neuronal loss in substantia nigra pars compacta (SNpc) up to 18 months old. Our results thus reveal a pivotal role for LRRK2 in regulating striatal DA transmission and consequent control of motor function. The G2019S may exert pathogenic effects by impairing these functions of LRRK2. Our recent observation indicated a significant accumulation of LRRK2-staining in the glial cells at cerebral cortex as well as cerebellum of LRRK2-G2019S mice, as compared to LRRK2-Wt or non-transgenic mice. Glial fibrillary acidic protein (GFAP) staining demonstrated increased astrocyte activation in LRRK2-G2019S mice over that of non-transgenic control, whereas over-expression LRRK2-Wt mice shows effective protection to astrocyte gliosis. Thus, our observation may suggest additional pathogenic role of LRRK2-G2019S in PD that involves gliosis. This project is funded by M. J. Fox Foundation for Parkinson's Research and NIH/NINDS.

Disclosures: J. Wang, None; **X. Li**, None; **J.C. Patel**, None; **M.E. Rice**, None; **Z. Yue**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.6

Topic: C.03. Parkinson's disease

Support: National Institute of Neurological Disorders and Stroke

Michael J. Fox Foundation

National Institute of Diabetes and Digestive and Kidney Diseases

Title: Loss of LRRK2 causes impairment of protein degradation pathways, accumulation of α-synuclein, and apoptotic cell death in aged mice

Authors: *Y. TONG[1], H. YAMAGUCHI[1], E. GIAIME[1], S. BOYLE[2], R. KOPAN[2], R. J. KELLEHER, III[3], J. SHEN[1];
[1]Brigham & Women's Hosp Harvard Med. Sch., BOSTON, MA; [2]Washington Univ. Sch. of Med., St. Louis, MO; [3]Massachusetts Gen. Hosp. & Harvard Med. Sch., Boston, MA

Abstract: Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common genetic cause of Parkinson's disease. LRRK2 is a large protein containing a small GTPase domain

and a kinase domain, but its physiological role is unknown. To identify the normal function of LRRK2 *in vivo*, we generated two independent lines of germline deletion mice. The dopaminergic system of *LRRK2*$^{-/-}$ mice appears normal, and numbers of dopaminergic neurons and levels of striatal dopamine are unchanged. However, *LRRK2*$^{-/-}$ kidneys, which suffer the biggest loss of LRRK compared with other organs, develop striking accumulation and aggregation of α-synuclein and ubiquitinated proteins at 20 months of age. The autophagy-lysosomal pathway is also impaired in the absence of LRRK2, as indicated by accumulation of lipofuscin granules as well as altered levels of LC3-II and p62. Furthermore, loss of LRRK2 dramatically increases apoptotic cell death, inflammatory responses and oxidative damage. Collectively, our findings show that LRRK2 plays an essential and unexpected role in the regulation of protein homeostasis during aging, and suggest that LRRK2 mutations may cause Parkinson's disease and cell death *via* impairment of protein degradation pathways, leading to α-synuclein accumulation and aggregation over time.

Disclosures: Y. Tong, None; **H. Yamaguchi**, None; **E. Giaime**, None; **J. Shen**, None; **S. Boyle**, None; **R. Kopan**, None; **R.J. Kelleher**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.7

Topic: C.03. Parkinson's disease

Title: Lrrk2 transgenic drosophila: implications in lrrk2-linked Parkinson 's disease

Authors: *W. SMITH;
Univ. of Maryland Sch. of Pharm., BALTIMORE, MD

Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative disorders, characterized by selective loss of dopaminergic neurons, and the presence of Lewy bodies. The pathogenesis of PD is not well understood. Mutations in the leucine-rich repeat kinase 2 (LRRK2) cause familial PD with pleomorphic pathology, and constitute the most common known cause of PD. We recently generated a LRRK2 drosophila model that resembles some key features of human Parkinsonism. In this model, expression of the human LRRK2 variant (the most common PD-linked mutation) G2019S-LRRK2 in flies results in higher protein kinase activity and causes a more severe dopaminergic neurodegeneration and motor dysfunction than expression of equivalent levels of wild type LRRK2. To explore LRRK2 modifiers in developing

PD-like phenotypes, we conduct a small scale fly genetic RNAi screen. We found that knockdown of *hemipterous (*hep, or JNKK) increased survival and improved locomotor activity in G2019S-LRRK2 transgenic flies. Furthermore, knockdown of hep in dopaminergic neurons in G2019S-LRRK2 transgenic flies significantly reduced loss of dopaminergic neurons. Co-expression of dominate-negative allele of hep-DN with G2019S-LRRK2 also significantly increased survival, and improved locomotor dysfunction and reduced dopaminergic neuron degeneration. Treatment with a JNK inhibitor, SP600125, also reduced G2019S-LRRK2-induced loss of dopaminergic neurons. These results indicate that hep (JNKK) pathway plays an important role in LRRK2-linked dopaminergic neuron degeneration and PD-like phenotypes. These studies provide new insight into the molecular mechanisms underlying LRRK2-linked PD pathogenesis, and a unique fly model for screening of genetic modifiers and rational therapeutics.

Disclosures:

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.8

Topic: C.03. Parkinson’s disease

Support: NIH AG000945-01

NIH AG000944-01

Title: G2019S LRRK2 specifically expressing in midbrain dopaminergic neuron perturbs dopamine homeostasis in mice

Authors: *H. CAI[1], X.-L. GU[2], C.-X. LONG[2], X. LIN[2], L. PARISIADOU[2];
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Abstract: Parkinson disease (PD), the most common degenerative movement disorder, is pathologically characterized by a relatively selective loss of midbrain dopaminergic neurons and the presence of a-synuclein-positive protein aggregates within neurons. Although most cases of PD are sporadic, increasing number of genetic mutations has been linked to familial PD, including autosomal dominant mutations in Leucine-rich Repeat Kinase 2 (LRRK2). The G2019S missense mutation in LRRK2 represents one of the most frequent mutations in both sporadic and familial PD. To study the pathogenic mechanism of G2019S LRRK2 in vivo, we

generated and characterized human LRRK2 wild-type (wt-LRRK2) and G2019S LRRK2 (G2019S) inducible transgenic (Tg) mice using the “tet-off” system. Previously, we have shown that LRRK2 regulates the progression of PD-related A53T alpha-synuclein-induced neuropathology when LRRK2 is mainly expressed in the forebrain regions (Lin, Parisiadou et al. 2009). To further investigate the pathogenic role of LRRK2 in the midbrain dopaminergic (DA) neurons, we selectively LRRK2 is specifically expressed in DA neurons under the control of paired-like homeodomain transcription factor 3 (Pitx3) promoter. The spontaneous activities of mutant mice are normal and no obvious difference in DA neuronal neurodegeneration as well as reactive glial cells is detected in the brain of Pitx3-tTA/G2019S Tg mice up to 18 months of age. Interestingly, dopamine metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC) and 3, 4-dihydroxyphenylethanol (DOPET) are significantly decreased in the striatum of Pitx3-tTA/G2019S Tg mice. Furthermore, enhanced tyrosine hydroxylase (TH) and aldehyde dehydrogenase 1 family, member 1 (ALDH1A1) protein levels are markedly up-regulated. These results indicate that LRRK2 may play an important role in regulating dopamine homeostasis in the midbrain DA neuron.

Disclosures: H. Cai, None; **X. Gu**, None; **C. Long**, None; **X. Lin**, None; **L. Parisiadou**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.9

Topic: C.03. Parkinson’s disease

Support: NIH/NINDS P50NS38377

Michael J. Fox Foundation

NIEHS R01ES014470

NIH R01-AG023593

NIH/NINDS R00-NS058111

NIH R01 NS36420

DAMD17-02-1-0695

Title: Inhibitors of leucine rich repeat kinase 2 (lrrk2) protect against lrrk2-models of Parkinson's disease

Authors: *B. LEE[1], J.-H. SHIN[1], J. VANKAMPEN[2], S. HAMAMICHI[3], L. PETRUCELLI[2], G. A. CALDWELL[3], K. A. CALDWELL[3], A. B. WEST[4], H. KO[1], Y.-I. LEE[1], K. A. MAGUIRE-ZEISS[5], W. J. BOWERS[7], H. FEDEROFF[6], V. L. DAWSON[1], T. M. DAWSON[1];
[1]Johns Hopkins Univ., BALTIMORE, MD; [2]Dept. of Neurosci., Mayo Clin. Col. of Med., Jacksonville, FL; [3]Dept. of Biol. Sci., The Univ. Alabama, Tuscaloosa, AL; [4]Univ. of Alabama, Birmingham, AL; [5]Dept. of Neurosci., [6]Georgetown Univ. Med. Ctr., Washington DC, DC; [7]Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Leucine rich repeat kinase 2 (LRRK2) mutations are a common cause of Parkinson's disease (PD). Disease segregating mutations in LRRK2 lead to neurotoxcity *in vitro* and loss of dopamine neurons in PD patients. LRRK2 toxicity is linked to kinase activity, since in context mutations in LRRK2 that interfere with kinase activity inhibits LRRK2 toxicity *in vitro*. Whether LRRK2 toxcity requires kinase activity *in vivo* and whether pharmacologic inhibition could protect against LRRK2 toxicity is not known. To identify potential LRRK2 kinase inhibitors, we screened the Biomol kinase and phosphatase inhibitor library by measuring LRRK2 autophosphorylation and LRRK2-mediated myelin basic protein (MBP) phosphorylation. Eight potential LRRK2 kinase inhibitors were identified and two of them, GW5074 and indirubin-3-monooxime, which are the most potent inhibitors, were applied to verify their protective effect on LRRK2-induced neuronal toxicity *in vitro* and *in vivo* models. GW5074 and indirubin-3-monooxime attenuated LRRK2 G2019S overexpression-induced primary neuronal toxicity, loss of dopaminergic (DA) neurons of LRRK2 G2019S overexpressed transgenic *C. elegans*, and degeneration of DA neurons in substantia nigra after stereotactical injection of herpes simplex virus (HSV)-LRRK2 G2019S. Here we show that pharmacologic inhibition of LRRK2 kinase activity protects against LRRK2 toxicity both *in vitro* and *in vivo.*

Disclosures: B. Lee, None; **T.M. Dawson**, None; **V.L. Dawson**, None; **J. Shin**, None; **H. Ko**, None; **Y. Lee**, None; **J. VanKampen**, None; **L. Petrucelli**, None; **S. Hamamichi**, None; **K.A. Caldwell**, None; **G.A. Caldwell**, None; **A.B. West**, None; **K.A. Maguire-Zeiss**, None; **W.J. Bowers**, None; **H. Federoff**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.10

Topic: C.03. Parkinson's disease

Support: Edward R. and Anne G. Lefler Postdoctoral Fellowship

NIH grant AG023094

The Brigham and Women's Hospital Udall Center of Excellence for Parkinson's Disease Research (NS038375)

Title: LRRK2 kinase activity, dimer formation and phosphorylation are influenced by its subcellular localization

Authors: *Z. BERGER[1,2], K. A. SMITH[1], M. J. LAVOIE[1,2];
[1]Neurol., Brigham and Women's Hosp., BOSTON, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: Autosomal dominant mutations in the leucine rich repeat kinase 2 (LRRK2) are the most common genetic cause of inherited and sporadic Parkinson's disease (PD). Alterations in LRRK2 kinase activity are thought to underlie the pathogenesis of its PD-linked mutations; however, many questions regarding basic aspects of LRRK2 function remain unclear, including the cellular mechanisms of LRRK2 regulation. In this study, we have explored the importance of subcellular localization on various biochemical properties of LRRK2.
To analyze the potential of LRRK2 to form a dimer, we used glycerol velocity gradients as a separation technique. We observed LRRK2 in two distinct pools in whole cell lysates, corresponding to a monomer and a less abundant dimer. This was confirmed by both heterologous co-immunoprecipitation and Blue Native PAGE analyses. Using size exclusion chromatography and Blue Native PAGE we found that the LRRK2 dimer was observed experimentally at its theoretical molecular weight (~500 kDa). Surprisingly, however, the LRRK2 monomer eluted at an abnormally high MW (above 1 MDa) on size exclusion chromatography but not on Blue-Native PAGE. In order to determine the functional implications of LRRK2 dimer formation, LRRK2 was separated by glycerol gradients and the relative kinase activity of monomer and dimer was analyzed. Wild-type LRRK2 dimer exhibited ~8-fold greater kinase activity compared to LRRK2 monomer. This suggests that dimer formation represents a crucial step in regulating LRRK2 kinase activity, a model consistent with other MAPKKKs.
To determine if the LRRK2 dimer is localized preferentially to certain cellular compartments, we analyzed LRRK2 from cytosol and membrane fractions. We observed substantial enrichment of the LRRK2 dimer at the membrane using glycerol gradients, heterologous co-immunoprecipitation, and crosslinking. As predicted, increased LRRK2 kinase activity was also observed from the membrane associated pool compared to the cytosolic LRRK2. Additional biochemical differences include increased binding to GTP and lower levels of phosphorylation of membrane-associated LRRK2 compared to the cytosolic form.
In summary, we demonstrate for the first time that the subcellular localization of wild-type LRRK2 is associated with changes in four distinct biochemical properties likely crucial for LRRK2 function. We propose a novel mechanism of regulating LRRK2 function through its membrane localization, dimerization and potentially its phosphorylation. These findings may have implications for the identification of substrates, the biochemical composition and nature of active LRRK2, and LRRK2 function within the cell.

Disclosures: Z. Berger, None; **K.A. Smith**, None; **M.J. LaVoie**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.11

Topic: C.03. Parkinson’s disease

Support: NIH Grant R00 NS058111

Title: Autophosphorylation of the LRRK2 GTPase domain enhances kinase activity and dimerization

Authors: *P. J. WEBBER[1], A. D. SMITH[2], S. SEN[3], M. B. RENFROW[4], J. A. MOBLEY[2], A. B. WEST[5];
[1]Neurol., [2]Biochem. and Mol. Genetics, Surgery, UAB Lab. for Biomed. FT-ICR Mass Spectrometry, [3]Neurology, Ctr. for Neurodegeneration and Exptl. Therapeutics, Neurol., [4]Biochem. and Mol. Genetics, UAB Lab. for Biomed. FT-ICR Mass Spectrometry, [5]Neurology, Ctr. for Neurodegeneration and Exptl. Therapeut., Univ. Alabama Birmingham, BIRMINGHAM, AL

Abstract: Mutations in the LRRK2 gene are the most prevalent known cause of genetic Parkinson’s disease (PD), and a majority of the disease associated mutations occur in the kinase and GTPase domains of LRRK2 protein. Several PD-linked mutations enhance LRRK2 autophosphorylation and kinase activity in vitro. To identify the sites of LRRK2 autophosphorylation, collision induced dissociation (CID) and electron transfer dissociation (ETD) mass spectrometry were applied in tandem with LRRK2 peptide digests with trypsin, chymotrypsin and Lys C enzymes. We and others describe LRRK2 autophosphorylation of the GTPase domain at multiple residues localized to the GTP binding pocket suggesting reciprocal regulation between the kinase and GTPase domains. We study the effects of alterations within the GTPase domain on kinase activity through the use of PD-associated mutations and phosphomimetic substitutions of autophosphorylation sites. Many of these changes in the GTPase domain result in enhanced kinase activity and dimerization, whereas mutations that disrupt formation of the GTP-binding pocket ablate kinase activity. These data suggest that autophosphorylation is a significant event for LRRK2 functional regulation, and complete characterization may provide further insight to pathology caused by LRRK2 PD-associated mutations.

Disclosures: P.J. Webber, None; **A.B. West**, None; **A.D. Smith**, None; **S. Sen**, None; **M.B. Renfrow**, None; **J.A. Mobley**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.12

Topic: C.03. Parkinson's disease

Support: Wellcome Trust 088145

Title: Characterisation of LRRK2-Tubulin interactions in Parkinson's disease

Authors: *D. C. BERWICK[1], B. M. H. LAW[1], R. M. SANCHO[2], K. HARVEY[1];
[1]Dept. of Pharmacol., The Sch. of Pharm., London, United Kingdom; [2]MRC Ctr. for Neurodegeneration Res., King's Col., London, United Kingdom

Abstract: Leucine-Rich Repeat Kinase-2 (LRRK2) encoded by *PARK8* is a large multi-domain protein containing both serine/threonine kinase and GTPase activities. Mutations in the kinase and RocCOR GTPase domains of LRRK2 are believed to affect enzymatic activity, thereby eliciting neuronal cell death, although the pathways involved remain unknown. The study of LRRK2 is particularly important as *PARK8* mutations are by far the most common known cause of familial PD, accounting for up to 40% of all cases in some populations. Interestingly *PARK8* mutations cause a heterogeneous range of brain pathologies that is remarkably similar to idiopathic PD but also give rise to pathologies more typical for Alzheimer's disease or amylotrophic lateral sclerosis. This observation led to the idea that LRRK2 has an upstream role in the pathogenesis of neurodegeneration.
To identify novel interactors of the RocCOR domain of LRRK2 we performed a yeast two hybrid (YTH) screen of a human embryonic whole-brain cDNA library and found an interaction with β-tubulins, an observation that has been confirmed by other laboratories. This association with components of the microtubule (MT) cytoskeleton suggests LRRK2 may be involved in certain MT-dependent processes, or indeed, the regulation of the dynamic instability of MTs. Intriguingly numerous MT-dependent events are affected in neurodegeneration leading to PD, not least the transport of vesicles and mitochondria along axons.
Here we have systematically investigated the requirements for interaction between LRRK2 and β-tubulin. Using quantitative YTH and co-immunoprecipitation studies in mammalian cells we have found that binding requires the Roc domain of LRRK2 and is specific for the TUBB and TUBB4 isoforms of β-tubulin via their unique C-terminal sequences. Interestingly, interactions

are weakened by the PD-causing Roc domain mutations R1441G and R1441H, suggesting a potential pathogenic mechanism for these mutations. In addition, we have investigated the association of LRRK2 with the MT cytoskeleton and microtubule associated proteins. Our data suggest a model whereby pathogenic *PARK8* mutations may elicit neurodegeneration via the dysregulation of MT-dependent processes.

Disclosures: D.C. Berwick, None; **B.M.H. Law**, None; **K. Harvey**, None; **R.M. Sancho**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.13

Topic: C.03. Parkinson’s disease

Support: MEXT

Elan Pharmaceuticals

Title: Biochemical characterization of the dimerization of LRRK2

Authors: *G. ITO, T. IWATSUBO;
Univ. of Tokyo, Tokyo, Japan

Abstract: Leucine-rich repeat kinase 2 (LRRK2) is a causative gene for an adult-onset, autosomal-dominant form of familial Parkinson disease (FPD; PARK8). Since some FPD mutations of LRRK2 cause upregulation of the kinase activity, abnormal activation of LRRK2 is hypothesized to have a major role in the neurodegenerative process, although little is known about the regulatory mechanism of LRRK2 activity. The observations that a large number of kinases are activated upon dimerization, and that crystallographic analysis of the GTP binding domain of LRRK2 suggested dimerization of LRRK2 led us to hypothesize that dimerization is key to LRRK2 activity. Firstly, we confirmed that a small fraction of 3xFLAG-LRRK2 is co-immunoprecipitated with LRRK2-2xmyc upon overexpression in HEK293 cells. We then subjected the overexpressed 3xFLAG-LRRK2 to blue-native polyacrylamide gel electrophoresis (BN-PAGE) and found that LRRK2, with a calculated mass of ~280 kDa, predominantly migrated at ~600 kDa position (p600) associated with smearing substances in higher molecular weight (HMW) ranges as well as faint bands at ~200 and 300 kDa position. These results suggested that a fraction of LRRK2 forms a dimer in cells. Two dimensional BN/SDS-PAGE analysis revealed that both the p600 and the HMW substances consist of full-length LRRK2. To

investigate into the functional significance of dimerization of LRRK2, we employed a chemical-induced dimerization (CID) system, in which LRRK2 is amino-terminally fused with a domain of FKBP12 harboring some mutations (Fv). Upon treatment of HEK293 cells overexpressing Fv-LRRK2 with a bifunctional small compound (AP20187), the Fv region, and eventually fused LRRK2, are forced to dimerize in cells. We confirmed that the band corresponding to Fv-LRRK2 migrating at ~600 kDa position is shifted to a HMW range upon treatment with AP20187 on a BN-PAGE gel probably because of the induced dimerization of the spontaneously existing dimer. We also found that autophosphorylation of Fv-LRRK2 (1326-2527), which is not phosphorylated by other kinases in cells, is up-regulated in the presence of AP20187 upon [32P] metabolic labeling. These results suggest that induced dimerization/oligomerization of LRRK2 facilitates in trans autophosphorylation in cells. The specific kinase activity of LRRK2 in the presence or absence of AP20187 will also be examined by an in vitro kinase assay to clarify whether dimerization is involved in the activation of the kinase activity of LRRK2.

Disclosures: G. Ito, None; **T. Iwatsubo**, None.

Nanosymposium

629. LRRK2 Parkinson's Disease

Location: Room 32B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 629.14

Topic: C.03. Parkinson’s disease

Title: Regulation of endogenous lrrk2 by ifn-g in human pbmc sub-population

Authors: *Y. J. SAGOT[1], J. THEVENET[2], R. PESCINI-GOBERT[2], J. E. FABREGUE[2], C. WALTZINGER[3], A. FAZIO[2], R. HOOFT VAN HUIJSDUIJNEN[2], C. WIESSNER[2]; [2]NDD-TA, [3]AIID-TA, [1]Merck Serono Geneva SA, Geneva, Switzerland

Abstract: Leucine-rich repeat kinase 2 (LRRK2) has been identified as a causal gene for Parkinson disease, the second most common human neurodegenerative disorder. Despite major efforts, LRRK2 biological function and LRRK2 regulation remain largely unknown. LRRK2 expression is not restricted to neural tissue, but also found in other organs such as liver and lung. Notably expression has been reported also in spleen-derived B-cells implicating a possible function of LRRK2 in immune responses. In the present study, we have undertaken a detailed analysis of LRRK2 expression and regulation in human Peripheral Blood Mononuclear Cells (hPBMC) preparations. LRRK2 expression was confirmed to be mostly restricted to CD19+ B cells, whereas CD4+ and CD8+ T cells were devoid of LRRK2 messenger RNA. In addition the $CD14^{+}/CD16^{+}$ sub-population of monocytes, representing activated monocytes expressed very

high level of LRRK2, whereas $CD14^+/CD16^-$ cells had only low level of LRRK2. We then tested whether various stress factors known to induce monocyte activation increased LRRK2 levels in hPBMC. The most robust factor increasing LRRK2 expression was found to be IFN-γ. Current investigations aim to identify functional consequences of increased LRRK2 expression in IFN-γ treated hPBMC. Of note, hPBMC are easily accessible in patients and effects of LRRK2 inhibitors in such cells might be developed as surrogate markers for drug development.

Disclosures: Y.J. Sagot, None; **J. Thevenet**, None; **R. Pescini-Gobert**, None; **J.E. Fabregue**, None; **C. Waltzinger**, None; **A. Fazio**, None; **R. Hooft van Huijsduijnen**, None; **C. Wiessner**, None.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.1

Topic: C.17. Drugs of Abuse and Addiction

Support: Deutsche Forschungsgemeinschaft

Bundesministerium für Bildung und Forschung

Title: Prevention of inflammatory pain and analgesic tolerance by immune cell-derived opioid peptides

Authors: *C. STEIN;
Anesthesiol., Freie Univ. Berlin - Charité, Berlin, Germany

Abstract: Opioids can inhibit pain by activating opioid receptors outside the central nervous system. We aim at the discovery of novel molecules and therapeutic approaches devoid of central side effects such as addiction, tolerance, respiratory depression or sedation. Opioid receptors are present and upregulated on peripheral sensory neurons, and opioid peptides are expressed in immune cells within injured tissue. Environmental stress and releasing agents can lead to secretion of these peptides and to local analgesia by inhibiting the excitability of peripheral sensory neurons. We have examined G-protein coupling and signaling of opioid receptors in sensory neurons, opioid peptide processing, release and extracellular degradation by immune cells, and adhesion molecules, chemokines and growth factors governing the migration of opioid containing cells to inflamed tissue. As a result of the interaction between immune cell-derived opioid peptides and opioid receptors on peripheral sensory neurons, tolerance does not

develop to the analgesic effects of locally applied exogenous opioids in inflammatory pain. Clinical data will be presented demonstrating that peripherally active or locally administered opioids can potently inhibit acute and chronic inflammatory pain associated with surgery or arthritis.

Disclosures: C. Stein: None.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.2

Topic: C.17. Drugs of Abuse and Addiction

Support: R37-DA11672

KO5-DA20570

K99-DA25182

Title: Mechanisms of long-acting opioid antagonists

Authors: *C. I. CHAVKIN, E. J. MELIEF, M. MIYATAKE, M. R. BRUCHAS;
Univ. Washington Sch. Med., SEATTLE, WA

Abstract: Three classes of kappa opioid ligands have been distinguished: typical agonists that activate G-protein signaling to produce analgesia and other G-protein-mediated actions, conventional antagonists (e.g. naloxone and buprenorphine) that competitively inhibit agonist binding, and a third class of highly selective KOR ligands (e.g. norBNI, JDTic and GNTI) that activate the JNK family of Mitogen-activated protein kinases (MAPK) that produce long-lasting inactivation of KOR signaling that persists weeks after the drug is cleared. How JNK activation disrupts KOR signaling is not yet known, but we asked if this form of ligand-directed signaling might also be responsible for effects at the mu opioid receptor (MOR), and we predicted that MOR inactivation by this JNK mechanism might resemble receptor desensitization. Although many of the mu selective opioid agonists desensitize MOR through G-protein receptor kinase (GRK) and beta-arrestin-dependent internalization, prior studies have noted that the prototypical mu opioid morphine fails to efficiently internalize MOR, yet still produces acute analgesic tolerance. In the current study, we found that acute analgesic tolerance to morphine and related opioids (morphine-6-glucuronide and buprenorphine) was blocked by JNK inhibition, but not by

G protein receptor kinase 3 (GRK3) knockout. In contrast, a second class of mu opioids including fentanyl, methadone, and oxycodone produced acute analgesic tolerance that was blocked by G protein receptor kinase 3 knockout, but not by JNK inhibition. Acute mu opioid receptor desensitization, demonstrated by reduced DAMGO-stimulated [35S]GTPyS binding to spinal cord membranes from morphine pretreated mice, was also blocked by JNK inhibition; however desensitization of DAMGO-stimulated [35S]GTPyS binding following fentanyl pretreatment was not blocked by JNK inhibition. JNK-mediated receptor inactivation of the kappa opioid receptor was evident in both agonist-stimulated [35S]GTPyS binding and opioid analgesic assays; however, gene knockout of JNK 1 selectively blocked kappa receptor inactivation, whereas deletion of JNK 2 selectively blocked mu opioid receptor inactivation. These findings suggest that ligand-directed activation of c-Jun N-terminal kinases generally provides a novel mode of G protein-coupled receptor regulation.

Disclosures: C.I. Chavkin, None; **E.J. Melief**, None; **M. Miyatake**, None; **M.R. Bruchas**, None.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.3

Topic: C.17. Drugs of Abuse and Addiction

Title: The novel opioid receptor modulator RDC-0313 (ALKS 33) reduces olanzapine-induced weight gain in female rats

Authors: *M. S. TODTENKOPF, K. S. O'NEILL, S. M. KELLY, K. A. RICHIE, R. L. DEAN, III, L. J. DAHM, D. R. DEAVER;
Alkermes, Waltham, MA

Abstract: Antipsychotics (ATAP) can cause metabolic dysfunction and weight gain. Female patients have a 3.6-fold increased risk of weight gain than male patients (Hakko et al., 2006) and increases in BMI can be observed in as little as 1 week post initiation of treatment (Kluge et. al., 2009). Weight gain has been reported with risperidone, clozapine, sertraline and olanzapine. A rat model exists using olanzapine (OLZ) administration to female rats (Davoodi, et al. 2006) to study mechanisms associated with ATAP-induced weight gain and evaluate potential treatment options.
Endogenous opioids may be involved in the regulation of some types of weight gain based on nonclinical data (Yuan, et al., 2009). RDC-0313 is a new chemical entity that acts as an

antagonist at μ opioid receptors, with mixed agonist/antagonist activity at κ and δ receptors. We have demonstrated key differences in the pharmacology of RDC-0313 when compared to naltrexone using rodent models and RDC-0313 is currently under development for Central Nervous System-related disorders. The major objective of this study was to examine potential effects of RDC-0313 on OLZ-induced weight gain and compare to naltrexone (NTX). Four groups of female rats (n = 8/group) were used for this study: 1) OLZ only; 2) OLZ with NTX; 3) OLZ with RDC-0313; and 4) vehicle control. Rats were assigned to treatment groups using a random block design based on initial body weight. The OLZ group was given PO twice daily (6 hours between doses) at a dose of 1 mg/kg (in 1% methylcellulose, for 10 consecutive days). NTX and RDC-0313 were both administered at doses of 2 mg/kg (SC) concurrent with the afternoon administration of OLZ. There was an effect of Treatment ($F_{(3,28)} = 9.7$, $p < 0.001$), Day ($F_{(3,9)} = 359.8$, $p < 0.001$) and a Treatment x Day interaction ($F_{(27, 252)} = 10.2$, $p < 0.001$) on body weight. While all rats gained weight during the study, OLZ alone administration caused greater increases in weight gain and the increased gain was apparent by day 5. Co-administration of NTX with OLZ did not affect OLZ-induced weight gain. In contrast, weight gain in rats receiving RDC-0313 was similar to those in vehicle-controls rats, demonstrating the ability of RDC-0313 to block OLZ-induced weight gain.
Based on these data, unlike naltrexone which had no effect, RDC-0313 may be able to reduce OLZ-associated weight gain and potentially offer an adjunct therapy to patients with antipsychotic-related weight gain.

Disclosures: **M.S. Todtenkopf**, Alkermes, Inc., Employment; **K.S. O'Neill**, Alkermes, Inc., Employment; **S.M. Kelly**, Alkermes, Inc., Employment; **K.A. Richie**, Alkermes, Inc., Employment; **R.L. Dean**, Alkermes, Inc., Employment; **L.J. Dahm**, Alkermes, Inc., Employment; **D.R. Deaver**, Alkermes, Inc., Employment.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.4

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA13429

Title: Kappa opioid agonists as potential antipruritics

Authors: ***A. COWAN**, S. INAN;
Temple Univ. Sch. Med., Philadelphia, PA

Abstract: Over the past 35 years, preclinical psychopharmacologists have evaluated the behavioral effects of a variety of benzomorphan, arylacetamide and morphinan kappa agonists in mice, rats and monkeys. Across the chronological time line between the advent of ethylketazocine and the marketing of nalfurafine, several of these compounds were screened against agents that induce compulsive scratching in rodents. The documented anti-scratch activities of U50488 against bombesin, enadoline against compound 48/80, and nalfurafine against GNTI (the kappa antagonist), for example, provided a basic rationale for the commercial development of nalfurafine as an antipruritic in clinical medicine. Current research is focusing on the relationship between peripherally restricted kappa agonists (e.g., CR845) and the suppression of scratch in animals. We call attention to the anti-scratch properties of asimadoline, an arylacetamide kappa agonist with limited CNS penetration, that is being developed by Tioga/Ono against diarrhea-predominant irritable bowel syndrome. This agent possesses dose-related anti-scratch activity (0.10-5 mg/kg, s.c.) against compound 48/80 (2 mg/kg, s.c. behind the neck) and against GNTI (0.3 mg/kg, s.c. behind the neck) models of itch in male SW mice (25-30 g). These promising results may hasten the formulation of asimadoline as a skin-directed antipruritic.

Disclosures: A. Cowan, None; **S. Inan**, None.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.5

Topic: C.17. Drugs of Abuse and Addiction

Title: Binge eating disorder: A target for opioid antagonists

Authors: *S. MCELROY;
Lindner Ctr. of HOPE, Mason, OH

Abstract: Binge eating disorder (BED) is characterized by recurrent, distressing, and uncontrollable bouts of ingestion of large amounts of food (binge eating) without the inappropriate compensatory weight loss behaviors of bulimia nervosa. The lifetime prevalence of BED in the general population of the United States is estimated to be 3% and it is associated with psychiatric comorbidity, obesity, impaired quality of life, and disability. Several lines of evidence suggest opiate antagonists might be useful treatments for BED. First, the opioid antagonist naltrexone is effective in alcohol and opiate dependendence, and BED is related to

substance use disorders. Second, the endogenous opioid system is involved in eating behavior and possibly binge eating. Opioid peptides from all classes (endorphins, dynorphins, and enkephalins) stimulate food intake in laboratory animals, while opioid antagonists suppress food intake, including binge eating behavior. For example, nalmefene, a μ and κ opioid antagonist, significantly attenuated binge eating in rats conditioned to binge eat a high sugar diet. Third, preliminary data suggest opioid antagonists may suppress binge eating in patients with eating disorders. Intravenous administration of naloxone selectively suppressed the consumption of sweet, high fat foods in obese and lean subjects with bulimia nervosa, but not in control subjects. Studies in bulimia nervosa and case reports in BED have found that supratherapeutic doses of naltrexone (e.g., 200 to 400 mg per day) suppress binge eating (though placebo-controlled studies of naltrexone at standard doses in bulimia nervosa have largely been negative). RDC-0313, a new chemical entity in clinical development by Alkermes, binds non-selectively to μ, κ and δ opioid receptors, primarily as an antagonist at μ opioid receptors, with mixed agonist/antagonist activity at κ and δ receptors. Compared with naltrexone, RDC-0313 has a 5-fold greater affinity for the μ opioid receptor and much greater bioavailability after oral ingestion. Our group is presently conducting a proof-of-concept randomized, double-blind, placebo-controlled study of RDC-0313 in 60 adults with BED and obesity. In this presentation, the above information will be synthesized, including: a brief overview of BED, particularly its relationship to substance abuse and obesity; the role opioids are thought to play in binge eating; an overview of the response of binge eating to opioid antagonists in preclinical and clinical studies; and the design for the ongoing proof-of-concept study of RDC-0313 in patients with BED and obesity.

Disclosures: S. McElroy: Research Grant; Alkermes, Cephalon, Forest Labs, Jazz Pharmaceuticals, Inc, Orexigen Therapeutics, Inc, Shire. Consultant/Advisory Board; Eli Lilly and Company. Other; Inventor on US Patent No. 6,323,236 B2, Use of Sulfamate Derivatives for Treating Impulse Control Disorders, patent's assignee, University of Cincinnati, received payments from J&J Pharmaceutical R&D.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.6

Topic: C.17. Drugs of Abuse and Addiction

Support: 1 R25 RR024231-03

Title: Treatment of self-biting behavior in adult rhesus macaques with oral and long acting

naltrexone (VIVITROL®)

Authors: D. KEMPF[1], *R. L. DEAN[2], R. P. BOHM[1], K. C. BAKER[1];
[1]Tulane Natl. Primate Res. Ctr., Covington, LA; [2]Life Sci/Toxicol, Alkermes, WALTHAM, MA

Abstract: Self-injurious behavior (SIB), a multifactorial abnormal behavior with a poorly understood etiology, may be defined as any self-directed behavior that results in tissue injury. The condition is a significant human health problem and also spontaneously affects the captive rhesus macaque (*Macaca mulatta*) population with self-biting being the most common expression and suggests that nonhuman primates may serve as a promising model for the human condition.
Currently, there are no widely accepted treatments for SIB. However, studies with human and nonhuman primates have shown that therapeutic drugs such as oral naltrexone hydrochloride (NTX) are effective in reducing its occurrence. The proposed mechanism of action for attenuation of the behavior by NTX is through competitive inhibition of endogenous opioid receptors, which limits the resultant euphoria or analgesia that is experienced following a SIB episode. The reduction of positive feedback prevents the abnormal behavior from serving as motivation for maintenance of the behavior, thereby reducing incidence. Studies in the literature, pertaining to oral NTX treatment for SIB in humans, reported that 80% of human patients responded positively to treatment and 47% showed an improvement response of 50% or more. Similar results were obtained in a preliminary study at the Tulane National Primate Research Center which showed that 85% of rhesus macaques exhibiting self-wounding prior to therapeutic intervention reduced SIB episodes after initiating oral NTX treatment. Of the animals that responded positively, 38% showed a rate reduction of at least 50%.
The current study examines the effect of VIVITROL, a long acting thirty day injectable NTX, for the treatment of SIB in eight, otherwise healthy, adult, singly housed, rhesus macaques. During the four week pre- treatment and four week post-treatment phases no therapeutic intervention is administered so that each animal may serve as its own control. In the eight week treatment phase, animals receive two consecutive long acting NTX administrations. Data collection for all phases of the study consists of clinical monitoring and six hours per phase of videotaped data to evaluate frequencies of self-biting and other behaviors. Weekly blood samples measuring serum levels of long acting injectable NTX and the major metabolite 6-β-naltrexol are obtained and compared to the results of a concurrent long acting injectable NTX pharmacokinetic study in nonhuman primates to validate therapeutic range in study subjects. This study will help determine the efficacy of long acting injectable NTX for self-injurious behavior in nonhuman primates.

Disclosures: **D. Kempf**, drug product provided, no financial support, Other; **R.L. Dean**, Alkermes, Inc., Employment; **R.P. Bohm**, drug product provided, no financial support, Other; **K.C. Baker**, drug product provided, no financial support, Other.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.7

Topic: C.17. Drugs of Abuse and Addiction

Title: Comparison of RDC-0313 (ALKS 33), a novel potent opioid receptor modulator, and naltrexone on nucleus accumbens dopamine release following ethanol and amphetamine administration

Authors: *D. J. EYERMAN, R. L. DEAN, M. S. TODTENKOPF, K. A. RICHIE, D. MUEHLENBEIN, D. R. DEAVER;
Alkermes Inc, CAMBRIDGE, MA

Abstract: Opioids play an important role in modulating mesolimbic reward circuitry which participates in the rewarding effects of drugs of abuse. Oral administration of Naltrexone (NTX) is used for treatment of alcohol and opioid dependence. In rodent models of alcohol drinking, NTX is more effective when given by parenteral routes than when given orally. RDC-0313, a novel opioid receptor modulator, has been shown to have distinct *in vitro* and *in vivo* pharmacological properties when compared to naltrexone. The purpose of this study was to extend our initial findings by determining the ability of RDC-0313 and NTX, given by either the oral or subcutaneous (SC) route, to modify dopamine (DA) release within the shell of the nucleus accumbens (NAc-sh) induced by the administration of ethanol (EtOH) and d-amphetamine (AMPH). Doses used of NTX and RDC-0313 were chosen based on historical literature and previous internal *in vivo* pharmacology and pharmacokinetic studies.
Administration of EtOH (2 g/kg, IP) or AMPH (0.5 mg/kg, IP) following a baseline sampling period, produced increases ($P<.05$) in extracellular NAc-sh DA with maximal percentage increases from baseline of 35% and 340% respectively. SC administration of NTX (1mg/kg) 30 minutes prior to psychostimulant challenge ($P<.05$) inhibited both EtOH and AMPH induced increases in NAc-sh DA. However, oral administration of NTX (10mg/kg), which has been shown to produce similar plasma concentrations of NTX to 1 mg/kg SC, was unable to inhibit either EtOH or AMPH-stimulated DA release in the NAc-sh. In fact, oral administration of NTX resulted in a significant increase in the peak concentrations of dopamine measured after AMPH and increased the AUC of dopamine release following AMPH treatment. In contrast to NTX, RDC-0313 (1 mg/kg, SC or 10 mg/kg, PO) ($P<.05$) reduced both EtOH and AMPH induced DA release regardless of the route of administration when given 30 minutes prior to psychostimulant challenge.
Previously we have shown that oral RDC-0313 was more effective than oral NTX in reducing EtOH consumption in rats using a FR2 drinking paradigm. In this series of experiments, we have further extended these findings by demonstrating that in contrast to the SC route, oral NTX is unable to attenuate dopamine release in the NAc-sh following administration of either EtOH or AMPH. Importantly, RDC-0313, a novel opioid receptor modulator, appears to have the same efficacy in blocking pyschostimulant induced DA release, regardless of the route of

administration. Based on these data, RDC-0313 may be a superior opioid receptor modulator for treating reward-related disorders.

Disclosures: **D.J. Eyerman**, Alkermes, Inc, Employment; **R.L. Dean**, Alkermes, Inc, Employment; **M.S. Todtenkopf**, Alkermes, Inc, Employment; **D.R. Deaver**, Alkermes, Inc, Employment; **K.A. Richie**, Alkermes, Inc, Employment; **D. Muehlenbein**, Alkermes, Inc, Employment.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.8

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA024044

NIH DA023132

NIH DE017782

Title: Toll-like receptor 4 (TLR4) as a glial "opioid" receptor for suppressing analgesia, and driving tolerance, dependence, reward, and respiratory depression

Authors: *L. R. WATKINS;
Dept Psychology & Neurosci., Univ. Colorado At Boulder, BOULDER, CO

Abstract: Glial toll-like receptor 4 (TLR4) as a novel, non-opioid receptor for glial activation by drugs of abuse
Presenter: Linda R. Watkins, University of Colorado-Boulder
We have recently documented that opioids activate non-neuronal cells called glia in a non-stereoselective manner via a classical immune receptor called toll-like receptor 4 (TLR4). Our studies have documented that opioids induce proinflammatory responses by glia (and, intriguingly, CNS endothelial cells through which blood-borne opioid have to pass to reach the CNS) via TLR4 rather than through classical opioid receptors. This opioid-induced glial (and likely endothelial cell) activation of TLR4 compromises the clinical utility of opioids by compromising acute opioid analgesia, and increasing opioid tolerance, dependence, reward, and respiratory depression. Blockade of glial activation, blockade of TLR4 and/or TLR4 knockout all provide converging lines of evidence supporting this conclusion across endpoints. Notably,

TLR4 is the same glial activation receptor also implicated in driving chronic pain from neuropathy and now implicated in enhancing the ataxic and sedative effects of alcohol as well (M. Hutchinson et al., in prep). We and our collaborators (M. Hutchinson, Univ. Adelaide; K. Rice, NIDA; T. Sammakia and H. Yin, CU-Boulder) are in pursuit of new chemical entities that will specifically block TLR4 with drugs that are orally available and blood-brain barrier permeable so to increase the clinical utility of such therapeutics.

Disclosures: L.R. Watkins, None.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.9

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA00254

T32 GM07767

T32 DA07267

Title: Delta-opioid receptors and the modulation of emotion

Authors: *E. M. JUTKIEWICZ;
Pharmacol., Univ. of Michigan, Ann Arbor, MI

Abstract: Activation of the delta-opioid receptor system engenders an interesting profile of behavioral responses somewhat distinct from traditional opioid activity. Delta-opioid agonists produce antidepressant-like and anti-anxiety effects in a number of preclinical assays, including the forced swim, tail suspension, learned helplessness, olfactory bulbectomization, novelty-induced hypophagia, and elevated plus maze tests. These effects are mediated by the delta-opioid receptor as they are blocked by the delta-opioid antagonist naltrindole and are independent of agonist-induced seizures and locomotor stimulation. In addition, these effects are observed following acute and chronic administration of delta-opioid agonists with little evidence of tolerance development. Conversely, delta-opioid receptor blockade and knockout produces pro-depressant-like effects in animal models. Although little is known about the mood-regulating mechanisms of delta-opioid receptors, they have been shown modulate the release of multiple neurotransmitters, such as serotonin, dopamine, GABA, and glutamate, proposed to play a role in

antidepressant actions. Additionally, there is evidence that delta-opioid agonists increase brain-derived neurotrophic factor and neurogenesis, popular theories for the actions of antidepressant medications. In conclusion, these findings demonstrate that the delta-opioid receptor system may be involved in the regulation of mood and emotion and is a potential target for the development of novel antidepressant and anxiolytic drugs. Supported by USPHS grants DA00254, T32 GM07767, T32 DA07267.

Disclosures:

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.10

Topic: C.17. Drugs of Abuse and Addiction

Title: Development of a six-month implantable formulation of buprenorphine for the treatment of opioid addiction

Authors: *K. L. BEEBE[1], S. SREEDHARAN[1], R. PATEL[1], A. PATKAR[2];
[1]Titan Pharmaceuticals, Inc., S SAN FRAN, CA; [2]Psychiatry, Duke Univ. Med. Ctr., Durham, NC

Abstract: Background:
Sublingual buprenorphine (SL BPN) is an effective and well-tolerated treatment for opioid addiction. However, the daily dosing associated with sublingual administration hinders treatment compliance, increases the risk of misuse and diversion, and potentially contributes to patient relapse and treatment failure. Probuphine (buprenorphine hydrochloride [HCL]/ethylene vinyl acetate [EVA]) is a matchstick-sized subcutaneous implant that delivers a low, continuous level of BPN for 6 months with a single treatment, thus insuring compliance and greatly reducing the risk of misuse and diversion. Safety, efficacy, and BPN plasma concentration results from a six month, Phase 3 study are presented.
Methods:
Following induction with sublingual BPN (12-16 mg/day), opioid-dependent (DSM-IV-TR) outpatients at 18 clinical sites in the U.S. were randomized (2:1) to receive either 4 Probuphine (n=108) or 4 placebo (n=55) implants. Urine samples were collected 3 times weekly and analyzed for illicit opioids; monthly blood samples were collected to assess plasma buprenorphine.
Results:

The in-office implant insertion and removal procedures were generally well-tolerated, and Probuphine was associated with minimal adverse events consistent with the known safety profile of buprenorphine. The most common adverse events in the Probuphine group were headache, insomnia, nasopharyngitis, nausea, and constipation. Probuphine was superior to placebo in the proportion of opioid negative urine samples (p=.0117), retention in treatment (p=.001), and control of opioid withdrawal (p=.0008) and cravings (p=.0006) over the full 6 month period. Seventy-one (66%) of the Probuphine group and 17 (31%) of the placebo group completed six months of treatment. There was no evidence of unscheduled implant removal or attempted removal. Consistent with findings of a Phase 2 study, pharmacokinetic results in Probuphine-treated patients showed an early, brief pulse of BPN release, followed by steady-state plasma levels achieved within 3-4 weeks and maintained for 6 months. The mean steady-state plasma BPN level post-implant was 0.72 ng/mL (n=51).
Discussion:
Results indicate that Probuphine is an effective and well-tolerated treatment option for patients with opioid addiction. A confirmatory, active and placebo-controlled Phase 3 study is currently being conducted.

Disclosures: **K.L. Beebe**, Titan Pharmaceuticals, Inc., Employment; **S. Sreedharan**, Titan Pharmaceuticals, Inc., Employment; **R. Patel**, Titan Pharmaceuticals, Inc., Employment; **A. Patkar**, Duke University Medical Center, Employment; National Institutes of Health (NIDA, NIAAA, NIMH) SAMHSA, AstraZeneca, Bristol-Myers Squibb, Cephalon, Forest, GlaxoSmithKline, J & J, Jazz Pharmaceuticals, Lundbeck, McNeil Inc, Organon, Pfizer, Research Grant; SAMHSA, Research Grant; AstraZeneca, Research Grant; Bristol-Myers Squibb, Research Grant; Cephalon, Research Grant; Forest, Research Grant; Pfizer, Research Grant; GlaxoSmithKline, Research Grant; J & J, Research Grant; Jazz Pharmaceuticals, Research Grant; Lundbeck, Research Grant; McNeil Inc, Research Grant; Organon, Research Grant; Bristol-Myers Squibb, Speakers Bureau/Honoraria; Cephalon/Alkermeser, Speakers Bureau/Honoraria; Merck, Speakers Bureau/Honoraria; PfizerReckitt Benckiser, Speakers Bureau/Honoraria; Reckitt Benckiser, Speakers Bureau/Honoraria; Bristol-Myers Squibb, GlaxoSmithKline, Cephalon/Alkermes, and Reckitt Benckiser;, Consultant/Advisory Board; GlaxoSmithKline, Cephalon/Alkermes, and Reckitt Benckiser;, Consultant/Advisory Board; GlaxoSmithKline, Consultant/Advisory Board; Cephalon/Alkermes, Consultant/Advisory Board; Reckitt Benckiser, Consultant/Advisory Board.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.11

Topic: C.17. Drugs of Abuse and Addiction

Title: PTI-609, a novel anti-inflammatory/analgesic agent that binds filamin A

Authors: *L. H. BURNS[1], A. BLASKO[1], H.-Y. WANG[2];
[1]Pain Therapeutics, Inc., San Mateo, CA; [2]CUNY Med. Sch., New York, NY

Abstract: We present here PTI-609, a new chemical entity with strong anti-inflammatory and analgesic properties in a single molecule. PTI-609 binds the scaffolding protein filamin A (FLNA) with pM affinity. Its second function is activation of the mu opioid receptor (MOR) via a binding site distinct from that of current opioids. We previously showed that FLNA interacts with MOR to regulate a Gi/o-to-Gs switch in MOR - G protein coupling that is immediate and very transient after acute administration of opioids but persistent after chronic administration. The persistent Gs coupling is associated with opioid tolerance and dependence, and the acute but transient Gs coupling after acute administration may contribute to reward, as it activates the transcription factor CREB. By binding to a pentapeptide region on FLNA with pM affinity, PTI-609 can suppress MOR - Gs coupling, thus providing strong analgesia without the classical problems associated with opioid drugs. Antinociceptive efficacy by oral administration is similar to morphine in the mouse tailflick assay. PTI-609 has the added benefit of providing strong anti-inflammatory activity. We have shown that PTI-609 suppresses inflammatory cytokine release by 75% from LPS-stimulated human astrocytes at 100 fM, and this suppression of cytokine release remains robust as concentrations increase up to 100 nM. In the rat collagen-induced arthritis model, PTI-609 administered orally twice daily significantly reduced paw swelling. These data show PTI-609 to be a promising new drug candidate with both analgesic and anti-inflammatory properties in a single molecule.

Disclosures: **L.H. Burns**, Pain Therapeutics, Inc., Employment; **A. Blasko**, Pain Therapeutics, Inc., Employment; **H. Wang**, Pain Therapeutics, Inc., Other Research Support.

Nanosymposium

630. Translational Studies of Opioid Systems in Addiction

Location: Room 2

Time: Tuesday, November 16, 2010, 1:00 pm - 4:00 pm

Program Number: 630.12

Topic: F.03. Motivation and Emotion

Support: National Institute of Drug Abuse Grant R21DA024430

Title: Persuasion neuroscience? Functional MRI of effective and ineffective anti-drug messages

in adolescents

Authors: *A. W. MACDONALD, III[1], M. J. STEEN[2], R. J. FABER[3], M. LUCIANA[2], K. D. VOHS[4], M. C. YZER[3];
[1]Dep Psychol, Univ. Minnesota, MINNEAPOLIS, MN; [2]Dept. of Psychology, [3]Sch. of Journalism and Mass Communications, [4]Carlson Sch. of Mgmt., Univ. Minnesota, Minneapolis, MN

Abstract: RATIONALE: The effectiveness of anti-drug media campaigns, such as those employed by the U.S. Office of National Drug Control Policy is controversial; drug use in markets exposed to such campaigns are often unchanged or worse. The current study builds on work that identified actual anti-drug public service announcements (PSA's) perceived persuasive strength among an adolescent sample. The extent to which PSA's were arousing was strongly predictive of their strength, or perceived message effectiveness.
METHOD: The current, on-going study recruited 15-19 year-olds of both genders. Participants watched 10 strong and 10 weak anti-drug PSA's, and 10 non-drug advertisements while undergoing functional MRI. They also provided second-by-second arousal ratings and convergent effectiveness information for each 30-second ad. PSA's and non-drug ads used various combinations of vicarious, or observational, operant (or so-called "model-based") conditioning and classical (or "model-free") conditioning. PSA's in the corpus conveyed the negative consequences of drug use.
RESULTS: Among the 37 participants available there were no differences in brain activity between strong and weak PSA's in amygdala, insula or nucleus accumbens, three regions commonly associated with arousal, valence and learning. Strong PSA's showed reliably greater arousal-related activity in ventromedial prefrontal cortex (vmPFC) as well as in bilateral regions of frontopolar cortex and dorsolateral prefrontal cortex (DLPFC) and left ventrolateral prefrontal cortex (VLPFC). These differences were not moderated by individual's previous use of drugs.
DISCUSSION: Perceived persuasive strength did not result in differential arousal-related activity in a number of common arousal and valence networks, despite sufficient power to detect such activity. The vmPFC, a region associated with emotion regulation or establishing associations between stimuli and emotional states for the purpose of decision-making, was differentially active. Large differences in lateral regions of PFC, such as DLPFC and left VLPFC, may represent activity related to vicariously representing the reward and punishment structure of the stimuli in real-time.

Disclosures: A.W. Macdonald, None; **M.J. Steen**, None; **R.J. Faber**, None; **M. Luciana**, None; **K.D. Vohs**, None; **M.C. Yzer**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.2

Topic: D.04. Vision

Support: NIH Grant F31MH087077

NSF Grant BCS0642584

Title: Visual object processing interferes with feature-based selective attention

Authors: *A. C. SNYDER[1,2,3], J. J. FOXE[3,2];
[1]The Grad. Ctr. of CUNY, New York, NY; [2]Program in Cognitive Neurosci., The City Col. of New York, New York, NY; [3]Albert Einstein Col. of Med., Bronx, NY

Abstract: It has often been observed that when one feature of an object is attended, other behaviorally irrelevant features of that object show enhanced processing as well. This finding has been linked to feature-binding processes that underlie the perception of a coherent object. Thus, it seems that object-processing interacts with feature-based attention. Recently, our laboratory has demonstrated anticipatory suppression of visual features as indexed by EEG alpha-band power increases. For that study, we used random dot stimuli with incoherent motion expressly to reduce Gestalt object percepts. We hypothesized that if the features of the stimulus were bound together by object-related processing, the ability to bias attention between the mutually bound features would be reduced. In the current study, we directly tested this prediction by using both coherent and incoherent dot stimuli in a within-subjects cued attention behavioral paradigm. When incoherent stimuli were used, subjects showed a response time benefit for valid cues and a response time cost for invalid cues, as compared to non-informative neutral cues. Preliminary results indicate that no response time benefit exists for valid cues when coherent dot stimuli are used, consistent with a disruptive interaction of object-related feature-binding with anticipatory feature-based attentional biasing.

Disclosures: A.C. Snyder, None; **J.J. Foxe**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.3

Topic: D.04. Vision

Support: Bundesministerium für Bildung und Forschung DIP-METACOMP

Bundesministerium für Bildung und Forschung 01 EZ 0867 (Innovationswettbewerb Medizintechnik)

Bundesministerium für Bildung und Forschung 01GQ0705 (Bernstein Group for Computational Neuroscience Bremen)

Deutsche Forschungsgemeinschaft SFB 517

Center of Advanced Imaging Bremen

Zentrum fur Kognitionswissenschaften Bremen

Title: Discriminability of direction of attention with and without stimuli based on V4 epidural recordings: A perspective for high-performance brain-computer interfaces

Authors: *U. A. ERNST, D. ROTERMUND, K. TAYLOR, S. MANDON, Y. SMIYUKHA, S. D. NEITZEL, A. K. KREITER, K. R. PAWELZIK;
Univ. Bremen, Bremen, Germany

Abstract: Epidural local field potentials (LFP's) have been shown to be particularly promising signal sources for constructing high-performance brain-computer interfaces (BCI's). Previously we have shown that they allow for discrimination between different visual stimuli, which is further enhanced by attention. Furthermore, the LFP's also permit to extract information about different cognitive states such as different directions of attention, which might be used as control signals in a multitude of BCI applications. This raises the question, how well direction of attention can be discriminated for different target-distracter distances and whether the direction of attention can be distinguished even in absence of the stimuli.
Two macaque monkeys were trained to perform delayed-match-to-sample tasks, in which the animals had to direct attention to one of two sequences of shapes presented simultaneously on a computer screen. In one setting, distracter and target had a size of 4x4 degrees (visual angle) and were located in different visual hemifields with a distance of 5.8 degrees. In the other setting, distracter and target had a size of 1x1 degrees, and were located in the same visual hemifield separated by a gap of less than one degree. The animals had to signal the reoccurrence of the initial shape of the attended sequence. Recordings of epidural LFPs were performed with a chronically implanted electrode array, covering parts of areas V1 and V4.
The LFPs were split into their frequency components by applying a Morlet wavelet transform. Using a support vector machine (SVM) we identified signatures of the direction of attention contained in the power spectral amplitudes in different frequency bands, and quantified discrimination performance for the attentional state.
Surprisingly, we achieved a higher classification performance of up to 99.9% correct for nearby stimuli, compared to a performance of up to 95% correct for distant stimuli. This suggest that even small differences in the direction of attention can be distinguished very well, thus allowing

to build reliable BCIs with high spatial resolution. Even without an actual visual stimulus, we were able to classify the attended location.
For investigating the putative mechanisms underlying the large power spectral amplitude differences between the attentional conditions, we also analysed the phase information from the complex-valued wavelet spectra. We found that phase progression is more stable under attention, suggesting that the coherent oscillations underlying the LFP signals are more regular, or comprise a larger number of participating synchronized neurons.

Disclosures: U.A. Ernst, None; **D. Rotermund**, None; **K. Taylor**, None; **S. Mandon**, None; **Y. Smiyukha**, None; **S.D. Neitzel**, None; **A.K. Kreiter**, None; **K.R. Pawelzik**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.4

Topic: D.04. Vision

Support: NIH Grant EY017292

NIH Grant EY017921

Title: Feature-based synchrony between prefrontal cortex and V4 during visual attention

Authors: *R. RAJIMEHR[1], G. GREGORIOU[2], H. ZHOU[1], R. DESIMONE[1];
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Abstract: Selective visual attention in humans and monkeys is mediated by a widespread network of brain areas. This network includes areas in frontal and parietal cortex, which process task-relevant visual information, and send ‘top-down’ attentional signals to earlier cortical areas to modulate ‘bottom-up’ visual representations. Neural synchrony has been proposed as a possible mechanism for linking top-down and bottom-up signals during visual attention. However, it is not clear how visual features of attended stimuli contribute to such synchronous interactions between distinct brain areas. Here we addressed this question by simultaneous recording from ventrolateral prefrontal cortex (PFC) and area V4 in macaque monkeys during a visual attention task. In this task, the monkeys were instructed to attend to one of three colored gratings, depending on the color of a central cue. Cells in PFC had large, bilateral receptive fields with a stronger response at the fovea. About 50 % of the recording sites in PFC showed a strong color selectivity in both spiking activity and local field potential (LFP). The latency of

color-selective response in PFC was earlier than the latency of color-based attentional modulation in V4. The spike-LFP coherence analysis between the two areas revealed theta-band synchronization and alpha/beta-band desynchronization when the preferred color of PFC cells was the attended color and this color appeared inside the receptive field of V4 cells. The results suggest that the information about the central attentional cue and the attended target stimulus are linked through an effective oscillatory coupling between PFC and V4.

Disclosures: R. Rajimehr, None; **G. Gregoriou**, None; **H. Zhou**, None; **R. Desimone**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.5

Topic: D.04. Vision

Title: Feature-based attentional modulation for binocular disparity in macaque middle temporal visual area (MT)

Authors: *D. A. RUFF[1], R. T. BORN[2];
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Abstract: Feature-based attention has been shown to selectively alter the gain of sensory neurons in a manner that is dependent upon the relationship between the stimulus properties of an attended stimulus and the tuning preferences of a neuron. Treue and Martinez-Trujillo (Current Biology, 2004) have proposed the feature similarity gain model to describe these effects; however, it is currently unknown whether this model can explain effects during conditions when attention is directed to stimuli that contain multiple feature dimensions to which a neuron is sensitive.
To evaluate this question we trained a rhesus monkey to perform a speed change detection task at an attended location while an unattended stimulus was presented in the receptive field of an MT neuron. The attended stimulus could vary both in its direction of motion and its binocular disparity. This experimental design allowed us to test for feature attention effects for both binocular disparity -- which has not previously been demonstrated -- and direction of motion. We found that attention to the preferred binocular disparity significantly increased the response of a population of 110 neurons relative to attention to the null disparity. The amount of attentional modulation for binocular disparity was slightly, but not significantly, smaller both for individual neurons and the population average, than it was for direction of motion (average ~4% modulation for binocular disparity vs. ~5% for direction). Additionally, for a population of 25

neurons we were able to confirm that feature-based attentional modulation to binocular disparity was not due to the attended stimulus matching the binocular disparity of the stimulus presented in the receptive field of an MT neuron, but was instead due to the match between the attended stimulus and the tuning preferences of that neuron.
Our results demonstrate that there are feature-based attention effects for binocular disparity in area MT in addition to those previously shown for direction of motion. We also show that attention can modulate the activity of sensory neurons as a function of their tuning preferences to multiple stimulus features in accordance with the feature similarity gain model.

Disclosures: D.A. Ruff, None; **R.T. Born**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.6

Topic: D.04. Vision

Support: Wellcome Trust

Royal Society

National Institutes of Health

Exeter College (Oxford)

Title: Expected reward size alters the effect of electrical microstimulation on perceptual decisions about a structure-from-motion figure in cortical area V5/MT of rhesus macaque

Authors: N. CICMIL[1], B. G. CUMMING[2], A. J. PARKER[1], *K. KRUG[1];
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Abstract: Electrical microstimulation in V5/MT of the rhesus macaque biases the monkey's reports of the rotation of a bistable cylinder that is portrayed by structure-from-motion (SFM) with random-dots. The monkey's perceptual reports are shifted in favor of the motion and disparity tuning preference of the neurons at the stimulated site. Perceptual reports of this kind are based upon information about both sensory stimulus and reward value but it is not known how these two factors are combined.

We investigated how the size of the expected pay-off for a correct report at the end of the trial interacts with an additional electrical signal introduced by microstimulation during the trial. Two rhesus macaques were trained to report the direction of rotation of a SFM cylinder with an eye-movement. Cylinders' direction of rotation could be disambiguated by applying binocular disparity to separate front and back surfaces. Pay-off for a correct response was a fluid reward. As the number of consecutive correct choices increased, the size of the available fluid reward increased in two steps up to a maximum. The maximum reward size was twice the average sub-maximal reward size. 2s trials with and without electrical microstimulation were pseudo-randomly interleaved. The stimulation train consisted of 20μA biphasic pulses throughout stimulus presentation. The proportion of reports in one direction as a function of disparity (psychometric functions) were fitted for stimulated and non-stimulated trials with a pair of cumulative Gaussian curves separated by a horizontal offset. As previously reported, in a majority of sites (27/48), microstimulation shifted the psychometric function towards the site's tuning preference (chi-square $p<0.05$). At each stimulation site, trials were split into two conditions, maximal and sub-maximal expected reward size. The shift induced by microstimulation was significantly smaller for the maximal reward size (Wilcoxon sign-rank test, $p < 0.001$). This difference was significant for each monkey individually ($p < 0.05$). There was however no difference in psychometric threshold between the two conditions.
Our findings are consistent with the proposal that monkeys are carrying out a covert reaction time task involving 'drift diffusion' towards a decision bound (Kiani et al 2008; Rorie et al 2010). In this model, large expected reward sizes increase the starting position of the integration process for sensory information, leaving less time during which electrical microstimulation is integrated before the bound is reached. Consequently, when the pay-off is high, integration times should be shorter and the effect of microstimulation should be smaller.

Disclosures: N. Cicmil, None; **K. Krug**, None; **A.J. Parker**, None; **B.G. Cumming**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.7

Topic: D.04. Vision

Support: NWO 400-05-134

Title: Action preparation increases sensitivity to relevant features

Authors: *T. P. GUTTELING[1], S. Y. PARK[1], R. S. KAHN[1], J. L. KENEMANS[2], S. F. W.

NEGGERS[1];
[1]Rudolf Magnus Inst., Utrecht, Netherlands; [2]Dept. of Exptl. Psychology, Utrecht Univ., Utrecht, Netherlands

Abstract: In order to successfully execute an action, such as picking up a cup of coffee, one needs to be aware of many things. Some features are more important than others, such as the orientation or the size of the object to be grasped. Previous research has shown that the preparation or intention to execute a certain action engages an attentional process to facilitate the successful execution of that action. In this way, perception is 'primed' for the upcoming action. This has been shown for eye movements and spatial attention, where perception at the saccade endpoint is enhanced before eye movement execution. This interaction has also been suggested to exist for the skeletomotor system, although only through indirect measures. In the current study we therefore show this effect using a direct measure of visual perception.
Methods: Subjects (n=16) were instructed to either grasp or point to a bar that appeared on the display. After target presentation, but before the grasping or pointing action could be initiated, the bar could change orientation. Subjects were asked to report whether a change occurred. An identical experiment was also performed using a change of luminance. Hit rates and false alarm rates were used to calculate the sensitivity d' for the grasping and pointing conditions. Hand movements were recorded using a magnetic motion tracker to ensure proper grasping and pointing actions were made.
Results: The first experiment was performed using a feature that is relevant for grasping, but not for pointing (orientation). As expected, a significant increase in sensitivity (d') was observed for orientation detection when subjects were grasping relative to pointing. In the second experiment, a feature was used that is not relevant for either grasping or pointing (luminance). Here, no differences in visual sensitivity between grasping and pointing were found.
Discussion: We found direct evidence for increased perceptual sensitivity to a visual feature that is relevant for a certain action in the 'movement preparation phase' before the action was executed. The sensitivity to detect a change in orientation was higher when preparing to grasp an object, than when preparing to point to that object. Likewise, the sensitivity for a feature that is irrelevant for the action in preparation did not change.
As we expect the cortical areas involved in motor preparation to be involved in the top-down modulation of visual perception, we are currently engaged in a transcranial magnetic stimulation (TMS) study of the motor areas involved in preparation of grasping movements, using the same paradigm.

Disclosures: T.P. Gutteling, None; **S.Y. Park**, None; **R.S. Kahn**, None; **J.L. Kenemans**, None; **S.F.W. Neggers**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.8

Topic: D.04. Vision

Support: NIH Intramural Program

Title: A top-down component of decision-related activity in monkey MT

Authors: *A. MOEENY[1], B. G. CUMMING[2];
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Abstract: The activity of neurons in many sensory cortices is correlated with subject's decisions in threshold tasks, often thought to reflect a causal effect of noise in the sensory neurons on the decision. However, the possibility that the upcoming decision affects the activity of sensory neurons (a top-down component) remains open. Here we attempt to demonstrate this top-down component directly. Two monkeys were trained to report the perceived direction of rotation of a transparent cylinder. Binocular disparity rendered the stimulus unambiguous on some trials. On trials with zero binocular disparity, the stimulus was ambiguous. We biased the perceived rotation in the ambiguous trials, to see if this changes neuronal activity. For the first 500ms of each trial binocular disparity defined the direction of rotation. For the remaining 1500ms the disparity was zero. In these trials monkeys were rewarded at random. Nonetheless, they tended to report the direction of rotation defined in the first 500ms. For 34 MT neurons, an initial disparity biasing the animal to report the neuron's preferred rotation direction produced higher rates than the opposite bias. We used the area under the ROC curve to compare spike counts for the final 1000ms (mean 0.60, SD 0.26, $p<0.001$). This result could arise if the response to the initial disparity persisted throughout the trial. In one monkey a second manipulation biased reports without any change in initial firing rate. Stationary dots were presented for 500ms with disparity defining the cylinder. Then the disparity was set to zero and the dots were set in motion, so that only motion defined the cylinder for the final 1500ms. The animal's reports indicated that dots that were on the front surface of the stationary cylinder tended subsequently to be seen on the front surface of the rotating cylinder. Again, biasing the animal to report a neuron's preferred direction of rotation produced higher firing rates (ROC for the final 1000ms of 0.56, SD 0.08, $p < 0.001$, 42 neurons). These stimulus manipulations bias animals' reports of rotation direction, but should not directly activate MT neurons during the second half of each trial. We conclude that the observed firing rate differences are top down effect of the perceived stimulus upon activity in MT.

Disclosures: A. Moeeny, None; **B.G. Cumming**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.9

Topic: D.04. Vision

Support: Ministerio de Ciencia e Innovación (MICINN), Spain

Dirección Xeral de Investigación, Desenvolvemento e Innovación, Xunta de Galicia (INCITE), Spain

Title: Trial-to-trial covariation between perceptual decisions and neuronal activity in the premotor ventral cortex

Authors: *J. L. PARDO-VAZQUEZ, I. PADRON, C. ACUÑA;
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Abstract: The neuronal representation of the different stages of decision-making is an important topic in neuroscience. There are several brain cortical areas involved in representing the components of the decision. However, in these brain areas the correlation between the choice and the neural activity of individual neurons trial-to-trial was low. The ventral premotor cortex (PMv) is known to participate in perceptual decisions and therefore it is a good candidate to encode the choice. We recorded from single neurons in the PMv while trained monkeys performed in a visual discrimination task. In this task, the monkeys report a decision based on the comparison of the orientation of two lines (S1 and S2) shown sequentially and separated by a delay. The first stimulus (S1) was maintained in working memory and its memory trace was used to compare and decide the orientation of S2 to S1. We found that, during the comparison period, the neuronal sensitivity closely matches the behavioral sensitivity. Moreover, the trial-to-trial correlation between the choice and the neuronal activity was high. These results suggest that the PMv neurons represent the choice of the monkey trial-to-trial.

Disclosures: J.L. Pardo-Vazquez, None; **I. Padron**, None; **C. Acuña**, None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.10

Topic: D.04. Vision

Support: NSF grant no. 0622252

ONR

Packard Foundation

Title: Decision making under uncertainty: A neural model based on partially observable Markov decision processes (POMDPs)

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Abstract: A fundamental problem faced by animals is learning to select appropriate actions based on noisy sensory information and incomplete knowledge of the world. It has been suggested that the brain engages in probabilistic (Bayesian) inference during perception but how such probabilistic representations are utilized to select actions has remained unclear.
Here we propose a neural model of action selection and decision making based on the theory of partially observable Markov decision processes (POMDPs). Actions are selected based on not a single "optimal" estimate of state but an entire posterior distribution over states (the "belief" state). We show how such a model provides a unified framework for explaining experimental results in decision making that involve both information gathering actions and overt actions. For learning the appropriate actions, the model utilizes temporal difference (TD) learning for maximizing expected reward. The resulting neural architecture posits an active role for the neocortex in probabilistic computation of beliefs while ascribing a functional role for the basal ganglia in belief representation, value computation, and action selection.
When applied to the well-known random dots motion discrimination task, model neurons representing belief state exhibit responses similar to those of LIP neurons in primate neocortex. More importantly, the appropriate threshold for switching from information gathering to overt actions emerges naturally as part of the reward maximization process during TD learning. Furthermore, in the case where stimulus uncertainty is small, reward prediction errors in the model mimic previously reported dopaminergic responses in the basal ganglia for simple instrumental conditioning tasks.
The model provides a new framework for understanding neural decision making under uncertainty and suggests an important role for interactions between the neocortex and the basal ganglia in learning the mapping between probabilistic sensory representations and actions that maximize rewards.

Disclosures: R.P. Rao: None.

Nanosymposium

631. Visual Cognition: Attention and Decision Making II

Location: Room 5B

Time: Tuesday, November 16, 2010, 1:00 pm - 3:45 pm

Program Number: 631.11

Topic: D.04. Vision

Support: NIH grant 2-R01-MH062349

the Kavli Foundation

Title: Choices under uncertainty: Confidence representation in a neural network of decision making

Authors: *Z. WEI[1], X.-J. WANG[2];
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Abstract: Confidence estimation is important for decision-making under risk, and low confidence about a choice can lead to a change of mind. A recent experiment examined the brain mechanism of confidence representation, using neural recording in the lateral intraparietal cortex (LIP) of behaving monkeys (Kiani and Shadlen 2009). After the subject was presented an ambiguous sensory stimulus in favor of either of two alternative choice targets, a 'sure target' (T_s) with a small reward was introduced during a delay period. It was found that the subjects opted to choose T_s more often when the sensory evidence was weaker, and that LIP neurons reflect not only the monkey's decision and reaction time, but also the choice confidence.
To elucidate the underlying neural circuit mechanism, we implemented the same experimental protocol in a line-attractor spiking network model of decision making (Furman and Wang 2008). In this model, the presentation of two choice targets T_A and T_B activate two competing groups of neurons. A sensory stimulus provides evidence (in favor of one of the two alternatives) with a strength that can be parametrically varied, and the decision is made through winner-take-all attractor dynamics. The introduction of the sure target activates a third group of neurons which has a chance of winning the competition when the sensory evidence is weak and neither of the other two has emerged yet as the winner.
We found that our model accounts for the main behavioral observations from the monkey experiment, such as the probability of choosing the sure target as a function of the stimulus duration and strength. It also predicts how this probability depends on the sure target onset time. Moreover, the model reproduces salient LIP neural data, especially the differential activity when the choice is T_{in} (in the response field), T_{opp} (opposite from it) or T_s. Our results indicate that the difference between the activities r_A and r_B of neurons selective for T_A and T_B can serve as a measure of confidence. The 'confidence landscape' in the (r_A,r_B) phase plane is analyzed using

the theory of attractor dynamics.
This work was supported by the NIH grant 2-R01-MH062349 and the Kavli Foundation. We are thankful to Da-Hui Wang for technical support in cluster computing systems. Simulations are mainly done at the HSCC of Beijing Normal University.

Disclosures: Z. Wei, None; **X. Wang**, None.

Nanosymposium

632. Seeing and Feeling Pain

Location: Room 33C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:00 pm

Program Number: 632.1

Topic: D.08. Pain

Support: NSF 0631637

NIH MH076136

NIH RC1DA028608

NIH R01DA027794

2005 Mind and Life Summer Research Institute Francisco J. Varela Memorial Grant Award

Title: Both physical pain and viewing aversive images activate periaqueductal gray, but with different cortical-brainstem connectivity

Authors: *J. BUHLE[1], H. KOBER[3], K. N. OCHSNER[2], P. MENDE-SIEDLECKI[4], J. WEBER[2], B. HUGHES[5], E. KROSS[6], T. D. WAGER[7];
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Abstract: Periaqueductal gray (PAG) receives direct projections from ascending nociceptive pathways in the spinal cord, and human neuroimaging studies reliably report pain-related increases in PAG activity. Animal studies indicate PAG also participates in the coordination of behavioral and physiological responses to threat, including aggression, fear, and learned helplessness. Consistent with this literature, a recent neuroimaging meta-analysis found reliable

PAG activity during negative emotional processing unrelated to nociception (Kober et al., 2008; Wager et al., 2008). In the present study, we interleaved phasic, noxious heat with presentation of aversive photographs during a single fMRI session to directly compare neural activity associated with pain and appraisal of aversive images. PAG activity was greater during both [high - low] pain and [aversive - neutral] image viewing, and there was no consistent difference in the location of the peak activation in the two conditions. Functional connectivity analyses of individual differences in PAG revealed largely overlapping patterns of correlation during pain and aversive image appraisal, including common PAG connectivity in pre-SMA, lateral prefrontal cortex, and orbitofrontal cortex. However, the anterior medial prefrontal cortex and parietal operculum correlated more strongly with PAG during pain, and the parahippocampal cortex and superior frontal gyrus were more correlated with PAG during aversive image appraisal. Overall, these findings suggest that the PAG may be a critical element of human affective responses to aversive stimuli, as it is in animal models, but that the cortical systems responsible for engaging PAG may be specific to the nature of the stimulus.

Disclosures: J. Buhle, None; **H. Kober**, None; **K.N. Ochsner**, None; **P. Mende-Siedlecki**, None; **J. Weber**, None; **B. Hughes**, None; **E. Kross**, None; **T.D. Wager**, None.

Nanosymposium

632. Seeing and Feeling Pain

Location: Room 33C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:00 pm

Program Number: 632.2

Topic: D.08. Pain

Support: CIHR MOP 53304

CIHR STIHR: Health Applications of Cell Signaling in Mucosal Inflammation & Pain

CIHR STIHR: Pain: Moelcules to Community

Canada Research Chair Program (CRC in Brain and Behaviour)

CIHR Banting and Best Canada Graduate Scholarship

Ontario Graduate Scholarship

Title: Cortical thickness in anterior cingulate cortex is associated with self-reported helplessness in temporomandibular disorder

Authors: *T. V. SALOMONS[1], M. MOAYEDI[2,3], I. WEISSMAN-FOGEL[2], K. S. TAYLOR[2], B. V. FREEDMAN[5], M. B. GOLDBERG[5], H. C. TENENBAUM[5], K. D. DAVIS[2,4]; [2]Brain, Imaging & Behaviour - Systems Neurosci., [1]Toronto Western Res. Inst., Toronto, ON, Canada; [3]Inst. of Med. Sci., [4]Surgery and Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada; [5]Dent., Mount Sinai Hosp., Toronto, ON, Canada

Abstract: Introduction: Helplessness has been associated with poorer treatment outcomes in chronic pain. Reduced helplessness is also a mechanism for improvement in temporomandibular disorder (TMD) symptoms following psychotherapeutic intervention. Therefore, identifying biological correlates of helplessness in chronic pain patients may elucidate mechanisms of pain coping. We hypothesized that helplessness would be associated with cortical thickness (CT) in regions involved in pain processing and cognitive modulation of pain.
Methods: A total of 17 females with TMD and 23 age-matched healthy females provided informed consent for the REB-approved study. Helplessness was assessed from the helplessness subscale of the Pain Catastrophizing Scale. Patient disease characteristics (TMD intensity, unpleasantness, and duration) were also documented. A high-resolution FSPGR anatomical scan (FOV=24, slice thickness=1.5mm, 122 axial slices, 256x256 matrix, flip angle=20°) was obtained in a 3T MRI scanner. For CT analysis, we conducted a vertex-wise correlation within a mask of cortical regions associated with processing of pain and its modulation (frontal lobe, cingulate cortex, insula, postcentral gyrus and sulcus and posterior parietal cortex) using FreeSurfer. Age was included in the model. Threshold was set at $p<0.05$, corrected for multiple comparisons.
Results: Group differences in helplessness approached significance: mean +/- SD in patients = 8.24 +/- /4.48, in controls = 5.74 +/- 4.04; F = 3.41, p=0.07). In the TMD group, CT in the mid and posterior cingulate cortices (MCC, PCC) were correlated negatively with helplessness, whereas supplementary motor area (SMA) correlated positively. These associations remained significant after controlling for disease characteristics. However, there were no overall group differences in CT between patients and controls in any of these three regions and CT was not significantly associated with helplessness in any of the regions in the control group.
Discussion: These results are consistent with functional imaging findings demonstrating the involvement of MCC and SMA in processing perceived controllability, and link brain structure to coping abilities. The lack of group differences in CT and robustness of the findings with respect to disease characteristics suggests a complex association between helplessness and CT that may have a strong pre-existing component. These findings raise the possibility that structural vulnerabilities may predispose individuals to helplessness (and thus poorer treatment outcomes) within the context of chronic pain.

Disclosures: T.V. Salomons, None; **M. Moayedi**, None; **I. Weissman-Fogel**, None; **K.S. Taylor**, None; **B.V. Freedman**, None; **M.B. Goldberg**, None; **H.C. Tenenbaum**, None; **K.D. Davis**, None.

Nanosymposium

632. Seeing and Feeling Pain

Location: Room 33C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:00 pm

Program Number: 632.3

Topic: D.08. Pain

Title: Decoding the neural signature of pain using multivariate fMRI analysis

Authors: *C.-S. LIN[1,2], K. WIECH[1,2,3], K. H. BRODERSEN[1,4,5], I. TRACEY[1,2];
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Abstract: Background: Previous studies have shown that the threat value of pain significantly biases the intensity of nociceptive perception even when stimuli are physically identical. In contrast, very little is known about the neural activity underlying (i) pre-stimulus perception of the threat value of pain and (ii) the decision as to whether a nociceptive stimulus is perceived as painful or not. Here, we constructed a searchlight classifier to investigate the spatial deployment of local clusters of voxels that jointly encode the threat value of pain prior to stimulation as well as the quality of the experience during nociceptive stimulation.
Methods: 16 healthy subjects each received 120 radiant heat pulses applied to the dorsum of the right foot. Stimulus intensities were calibrated to near-pain threshold levels and kept constant throughout the experiment. High and low threat values of pain were induced, respectively, through differential information regarding the context of stimulus to be administered, shown as a visual cue before stimulation. At the end of each trial, subjects indicated whether the stimulus had been perceived as painful or not. We passed a spherical searchlight across whole-brain fMRI data to classify (i) 'high-threat' vs. 'low-threat' trials based on data acquired before subjects received a stimulus and (ii) 'pain' vs. 'no pain' trials based on data acquired during the stimulus and categorised via behavioural ratings.
Results: We found that bilateral anterior, mid and posterior insula and the posterior part of the anterior cingulate cortex reliably predicted the perception of pain during stimulation, and that the bilateral occipital lobes, right anterior insula, right orbitofrontal cortex, and the left hippocampal area (i.e., visual and emotional networks) reliably predicted the trial-specific threat context.
Conclusion: Multivariate pattern analysis provides powerful tools for analysing not only the quality of nociceptive stimulation but also its pre-stimulus threat value.

Disclosures: C. Lin, None; **K. Wiech**, None; **K.H. Brodersen**, None; **I. Tracey**, None.

Nanosymposium

632. Seeing and Feeling Pain

Location: Room 33C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:00 pm

Program Number: 632.4

Topic: D.08. Pain

Support: NIH NINDS NS35115

Anonymous

Title: fMRI in frequency space: Relating spontaneous pain to altered resting state fluctuations in chronic back pain

Authors: *M. N. BALIKI, A. T. BARIA, A. V. APKARIAN;
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Abstract: Chronic pain is maladaptive and its impact on brain function goes beyond pain processing and alters the flow and integration of information between brain regions. Here we investigate the impact of chronic pain using a new approach. We study fMRI in frequency space, subdividing BOLD signal into low (0.01-0.05 Hz), mid (0.05-0.12 Hz) and high (0.12-0.2 Hz) frequency oscillatory bands, which we think reflect the intrinsic features of large-scale neural organization of the brain. We use a power spectral density (PSD) analysis to map and compare the anatomical and functional correlates of the 3 frequency bands in healthy controls and in patients suffering from chronic back pain (CBP).
To investigate the changes in the intrinsic fluctuations of the BOLD signal associated with CBP we scanned 28 patients and 16 healthy subjects during rest. For each subject, we extracted the time series of the BOLD signal from each voxel and computed the PSD for the 3 frequency bands. Group average PSD maps for each group were generated by averaging the power of each voxel across all subjects within each group. In healthy subjects we observe that low frequency is mostly localized to cortical regions including medial prefrontal (MPFC) and posterior cingulate and parietal cortices. Regions such as anterior cingulate cortex (ACC) and insula exhibited power in the mid and high bands. When we performed a t-test to determine the difference in power for each frequency band between the 2 groups, we found that the patients exhibited significant increased high frequency power in the MPFC and to a lesser extent in the ACC and insula. Furthermore the increase in PSD for high frequency showed a correlation with the duration of their pain. In the patients a seed ROI correlation analysis indicated that the brain regions that showed aberrant frequency representation also exhibit increased connectivity to each other when compared to control, and was dependent on the amount of pain the patient reported. These results show that chronic pain is represented as a high frequency signal that disturbs the resting brain. To corroborate these results, we examined spectrograms of the MPFC signal in

patients while rating their spontaneous pain or performing a control visual task. We found that increase in high frequency power of the MPFC was temporally correlated with increased spontaneous pain ratings. No correlation was found for the visual rating task. These observations provide us with novel insight about the nature of CBP, which is associated with increased high frequency events, that signal spontaneous pain and disturbs the resting brain

Disclosures: M.N. Baliki, None; **A.T. Baria**, None; **A.V. Apkarian**, None.

Nanosymposium

632. Seeing and Feeling Pain

Location: Room 33C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:00 pm

Program Number: 632.5

Topic: D.08. Pain

Support: NIMH R01MH076136

Title: Expectancy effects and remifentanil administration: Dissociable contributions of opioid and placebo analgesia

Authors: *L. Y. ATLAS[1], R. A. WHITTINGTON[2], N. SONTY[3], T. D. WAGER[4,5]; [1]Columbia Univ., NEW YORK, NY; [2]Anesthesiol., [3]Anesthesiology, Psychiatry, [4]Psychology, Columbia Univ., New York, NY; [5]Psychology, Colorado University-Boulder, Boulder, CO

Abstract: It has long been believed that expectations enhance the effects of active pharmacological treatments. Though common brain regions are influenced by both placebo and opioid treatments, it is unknown whether knowledge of drug delivery influences pain-related brain responses during drug treatment. In two studies, we compared the effects of Open versus Hidden administration of remifentanil, a potent opiate analgesic, on brain and behavioral responses to noxious thermal stimulation. In Study 1, remifentanil was administered during fMRI and crossed with a manipulation of placebo expectancies (Open - Hidden drug delivery context). We assessed whether placebo enhanced drug effects on pain-evoked responses. In Study 2, we used a balanced placebo design to examine the relationship between opioid administration and placebo expectations. We also tested another kind of expectation: Expectations for high vs. low pain, driven by predictive cues presented immediately before painful stimuli.
The results provide evidence for dissociable contributions of expectancy and opioid analgesia, both in behavioral reports and brain activity. Placebo and remifentanil each produced significant effects on pain in both studies. The effects were additive, suggesting that they produce separable effects on pain. In addition, in Study 2, we found additive effects of placebo and predictive cue-

based expectancies on pain, and both effects were additive with drug effects. These results suggest that at least three factors can make separable contributions to pain modulation: opiate drug effects, placebo effects, and expectancies about noxious stimuli. In addition, in Study 1, placebo and drug effects were separable at the brain level. Placebo expectancies modulated later components of the pain-evoked response in pain matrix regions, whereas remifentanil modulated early components of the response.
By crossing placebo expectancies with drug delivery, we were also able to test for interactions between placebo and remifentanil. Though the behavioral effects on pain were additive, we observed a placebo*remifentanil interaction in right anterior insula, a critical region that has been linked to opioid-based modulation in previous studies. Drug-based insula modulation was enhanced when subjects were aware that they were receiving the drug.
These results suggest that expectancies and drug effects may operate through both separable and interacting mechanisms. These findings indicate that drug studies should consider how expectations might interact with drug effects, and that clinicians should consider patient cognitive factors to be important contributors to treatment outcomes.

Disclosures: L.Y. Atlas, None; **R.A. Whittington**, None; **N. Sonty**, None; **T.D. Wager**, None.

Nanosymposium

632. Seeing and Feeling Pain

Location: Room 33C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:00 pm

Program Number: 632.6

Topic: D.08. Pain

Support: NIH NINDS 35115

Title: Depression affects the brain mechanisms of chronic back pain

Authors: *J. A. HASHMI, M. BALIKI, M. L. CHANDA, E. PARKS, A. APKARIAN; Northwestern Univ., Chicago, IL

Abstract: Introduction:
High depression is associated with chronic pain conditions, and is thought to be a significant risk factor for onset of chronic back pain. Our fMRI (functional magnetic resonance imaging) studies show a clear involvement of medial prefrontal cortex (MPFC) in spontaneous pain fluctuations in chronic back pain patients. In addition, there is evidence that MPFC activity relates to levels of depression in chronic rheumatoid arthritis. Here we investigate interaction between depression and chronic pain specifically for MPFC connectivity to the rest of the brain.

Methods:
We obtained fMRI scans from twenty chronic low back pain subjects while rating spontaneous pain (pain runs) or a matched visual-motor rating task. The MPFC signal was regressed with activations in the whole brain before and after adding Beck Depression Inventory (BDI) scores and peak pain ratings to the model.
Results:
The time-course of MPFC activity correlated significantly with posterior insula during pain runs but not during matched visual runs. In addition, peak pain intensity significantly modulated connectivity between MPFC and posterior insula, cingulate and posterior parietal cortex. Furthermore, BDI scores were significantly linked with the correlation between MPFC and the posterior insula. When BDI contribution was covaried out, correlations between peak pain ratings and MPFC connectivity with posterior insula and other regions were no longer significant.
Conclusion:
These findings demonstrate that depression alters connectivity patterns of brain regions that underlie chronic pain.

Disclosures: J.A. Hashmi, None; **E. Parks**, None; **M.L. Chanda**, None; **A. Apkarian**, None; **M. Baliki**, None.

Nanosymposium

632. Seeing and Feeling Pain

Location: Room 33C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:00 pm

Program Number: 632.7

Topic: D.08. Pain

Support: CIHR MOP 53304

CIHR STIHR: Health Applications of Cell Signaling in Mucosal Inflammation & Pain

CIHR STIHR: Pain: Molecules to Community

Ontario Graduate Scholarship

CIHR Banting and Best Canada Graduate Scholarship

Canada Research Chair Program (CRC in Brain and Behaviour)

Title: White matter abnormalities in temporomandibular disorder

Authors: *M. MOAYEDI[1,2], I. WEISSMAN-FOGEL[1], K. S. TAYLOR[1], B. V. FREEMAN[4], M. B. GOLDBERG[4], H. C. TENENBAUM[4], K. D. DAVIS[1,3];
[1]Brain, Imaging & Behaviour - Systems Neurosci., Toronto Western Res. Institute, UHN, Toronto, ON, Canada; [2]Inst. of Med. Sci., [3]Dept. Surgery and Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada; [4]Dent., Mount Sinai Hosp., Toronto, ON, Canada

Abstract: Introduction: Idiopathic temporomandibular disorder (TMD) is a chronic pain condition thought to be caused in part by central dysfunction. We've shown that women with TMD have 1) slower reaction times and attenuated activity in cognitive brain areas during a Stroop task, and 2) gray matter (GM) abnormalities, including increased primary somatosensory cortex and basal ganglia GM, insula thinning, and decreased GM in the primary motor cortex and ventral premotor cortex related to pain intensity (Weissman-Fogel et al., Moayedi et al., HBM 2009). Here, we examined white matter (WM) related to regions with gray matter abnormalities to test whether patients with TMD have abnormal transmission of nociceptive information.
Methods: Seventeen females with TMD (mean age ± SD: 33.1 ± 11.9 years) and 23 healthy females (mean age ± SD: 35.4 ± 10.3 years), all right-handed, consented to procedures approved by the University Health Network and Mount Sinai Hospital REBs. Patients with TMD were diagnosed using TMD-RDC criteria. Two runs of diffusion-weighted MRI were obtained from each subject (b-value = 1000, 23 directions, 2 B0s, FOV = 240 mm, 55 3.0 mm thick slices, TR = 14,500 sec, matrix 128 x 128). Scans were pre-processed for motion and eddy current artefacts in DTiStudio v2.4.01. Corrupted slices on individual scans were discarded. Each subject's runs were averaged to generate a single scan volume. FSL v4.0 - 4.11 was used for image processing to do the following: 1) skull stripping using the Brain Extraction Tool (BET); 2) a diffusion tensor model was fit to the data at each voxel (DTIFIT) to calculate voxelwise fractional anisotropy (FA) values; 3) nonlinear registration and projection onto a standard tract representation (mean FA skeleton) in TBSS . A mask based on brain atlases within FSL focussed the analysis on the brainstem, and motor, somatosensory and prefrontal tracts. Statistical significance was based on two sample t-tests with age as a covariate of no-interest with 5000 permutations using threshold-free cluster enhancement and Bonferroni correction in FSL's Randomise.
Results & Discussion: Patients with TMD had lower FA in tracts associated with motor, cognitive and pain processing regions when compared to controls. This corresponded to changes in the anterior corpus callosum/cingulum, the corticospinal tracts, the internal, external and extreme capsules, the anterior and superior corona radiata, and WM within and adjacent to the MD and anterior nuclei of the thalamus. These WM abnormalities implicate abnormal transmission of information related to pain processing, modulation and motor function in TMD.

Disclosures: M. Moayedi, None; **I. Weissman-Fogel**, None; **K.S. Taylor**, None; **B.V. Freeman**, None; **M.B. Goldberg**, None; **H.C. Tenenbaum**, None; **K.D. Davis**, None.

Nanosymposium

632. Seeing and Feeling Pain

Location: Room 33C

Time: Tuesday, November 16, 2010, 1:00 pm - 3:00 pm

Program Number: 632.8

Topic: D.08. Pain

Support: Mind and Life Institute

The National Institutes of Health NS39426

Title: Brain mechanisms associated with meditation related pain relief

Authors: *F. ZEIDAN[1], K. T. MARTUCCI[2], R. A. KRAFT[2], J. G. MCHAFFIE[2], N. S. GORDON[3], R. C. COGHILL[2];
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Abstract: Mindfulness meditation (MM) has been found to alleviate pain symptoms across clinical and experimental settings. The majority of these findings have been reported in long-term MM interventions that require extensive time and financial commitment. In a recent study, brief MM training was found to reduce pain perception, suggesting that the palliative effects of meditation may be associated with cognitively manipulating appraisals of nociceptive information. However, the neural mechanisms involved in MM related pain relief are unknown. The present study sought to investigate the mechanisms involved in MM and MM related pain relief.
We recruited 15 healthy volunteers, with no prior meditative experience, to participate in a four-day (30 minutes/day) MM training intervention. We assessed brain activation by quantifying cerebral blood flow (CBF) with a pulsed arterial spin labeled MRI technique (pASL, Q2TIPS-FAIR TR=2.5, TE=17.9, TI=1700) appropriate for imaging the cognitive state of meditation. Thermal stimuli were administered to subjects' right calves in alternating patterns of pain (49°C) and rest (neutral 35°C) with 12-second durations at each temperature (6 min total duration per MRI series). General linear modeling analyses were used to identify regional changes in CBF related to MM and pain. A visual analog scale was used to assess pain intensity and unpleasantness after each series.
MM reduced pain unpleasantness ratings by 57% and intensity reports by 40%. Meditation significantly reduced pain related brain activation of the leg representation of the contralateral primary somatosensory cortex. Moreover, meditation related decreases in pain ratings corresponded with widespread activation of pain modulatory brain regions, including the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), putamen, and anterior insula as well as reduced activation in the thalamus. Meditation-induced pain relief may be associated with reframing nociceptive information such that it is appraised in a manner without substantial

influence from its affective meaning and future implications. At a computational level, this process may be accomplished by dynamically re-tuning S1 to minimize its responses to nociceptive information.

Disclosures: F. Zeidan, None; **K.T. Martucci**, None; **R.A. Kraft**, None; **J.G. McHaffie**, None; **N.S. Gordon**, None; **R.C. Coghill**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.1

Topic: E.01. Neuroendocrine

Support: NICHD R00 HD056157

NICHD U54 HD012303

NIH T32 HD007203

Title: Role of BAX-mediated apoptosis in the sexual differentiation of hypothalamic Kiss1 neurons

Authors: S. J. SEMAAN[1], E. K. MURRAY[2], M. C. POLING[1], S. DHAMIJA[1], N. G. FORGER[2], *A. S. KAUFFMAN[1];
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Abstract: The neuropeptide kisspeptin, encoded by the *Kiss1* gene, is a crucial regulator of GnRH neurons, and hence, puberty and reproduction. In rodents, *Kiss1* neurons are found in two regions of the hypothalamus, the arcuate nucleus and the anatomical continuum comprising the anteroventral periventricular (AVPV) and anterior periventricular (PeN) nuclei. Interestingly, the *Kiss1* population in the AVPV/PeN is sexually dimorphic, with females having many more *Kiss1* neurons than males. This *Kiss1* sex difference has been proposed to underlie the ability of females but not males to produce an estrogen-induced GnRH surge. Although we have previously shown that sex steroids during the postnatal critical period guide the sexual differentiation of AVPV/PeN *Kiss1* cells, the mechanism(s) by which sex steroids achieve this effect is unknown. Previous studies found that some sexually-dimorphic traits of the AVPV, such as total cell number, are dependent on BAX-mediated programmed cell death (apoptosis),

whereas other sexually-dimorphic traits, such as tyrosine hydroxylase (TH) neuron number, are not. Here we used adult male and female BAX knockout (KO) mice and their wildtype (WT) littermates to determine if sexually dimorphic AVPV *Kiss1* expression is dependent on BAX signaling. Because *Kiss1* expression is significantly altered by adult sex steroid levels, all mice were gonadectomized in adulthood and treated with the same dose of estradiol before sacrifice. Brains were analyzed for *Kiss1* and *TH* mRNA levels in the AVPV/PeN via *in situ* hybridization. We found that WT males had fewer *Kiss1* neurons in both the AVPV and PeN than WT females, confirming the previously-identified sex difference. Interestingly, BAX KO males also had *Kiss1* levels that were significantly lower than BAX KO females, indicating that sexual differentiation of the *Kiss1* system was not altered in the absence of BAX-mediated apoptosis. Like *Kiss1*, *TH* expression was sexually dimorphic in the AVPV of both WT and BAX KO mice, being higher in females than males. However, unlike *Kiss1*, TH was not significantly different between sexes in the PeN, indicating that in this region, *Kiss1* and *TH* are either two different populations or are under different transcriptional and/or developmental control. Our data suggest that while some sexually-dimorphic traits in the AVPV are induced by BAX-mediated apoptosis, both the *Kiss1* and *TH* sex differences in this region are organized by BAX-independent processes.

Disclosures: S.J. Semaan, None; **E.K. Murray**, None; **M.C. Poling**, None; **S. Dhamija**, None; **N.G. Forger**, None; **A.S. Kauffman**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.2

Topic: E.01. Neuroendocrine

Support: RO1HDO49651

U54HD12629

Title: Dynorphin gene expression in the arcuate nucleus is regulated by testosterone through nonclassical estrogen receptor alpha-dependent pathways in the male mouse

Authors: *V. M. NAVARRO[1], M. GOTTSCH[1], M. JIMENEZ[2], S. HOBBS[1], J. LEVINE[2], D. K. CLIFTON[1], R. STEINER[1];
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Abstract: Dynorphin (Dyn) is coexpressed with kisspeptin (Kiss1) and neurokinin B (NKB) in neurons within the arcuate nucleus (ARC). In the male mouse, testosterone (T) mediates the negative feedback control of gonadotropin secretion, by acting directly on Kiss1/Dyn/NKB neurons in the ARC, which in turn regulate the activity of GnRH neurons. Both the estrogen receptor alpha (ERα) and the androgen receptor (AR) control Kiss1 expression in the male mouse, but the signaling pathway through which T regulates Dyn expression is unknown. Since Kiss1/Dyn/NKB neurons express both ERα and AR, the regulation of Dyn by T could involve either or both of these receptors. To decipher the role of ER pathways in the control of Dyn expression in the male, we studied the effects of T on the expression of Dyn mRNA in the ARC of male mice bearing genetically targeted alterations in ERα signaling pathways (and controls). We found that T inhibited the expression of Dyn in wildtype animals ($p < 0.05$) but had no effect on Dyn expression in animals with targeted deletions of ERα (ERα-/-). However, T inhibited Dyn expression in NERKI mice [ERαAA/-; ($p<0.05$)], which lack estrogen response element (ERE)-dependent signaling (i.e., the classical pathway) but retain ERE-independent ERα signaling pathways (i.e., nonclassical effectors, such as SP-1, AP-1 and MAPkinase). Thus, the inhibitory effect of T on Dyn gene expression in Kiss1/Dyn/NKB neurons of male mice appears to be mediated primarily by ERα through an ERE-independent mechanism. Given the presumed relevance of NKB as cotransmitter with Dyn and kisspeptin in the stimulation of GnRH secretion, we plan to extend this study by determining the mechanism/s through which T modulates NKB expression in the ARC.

Disclosures: V.M. Navarro, None; **M. Gottsch**, None; **R. Steiner**, None; **D.K. Clifton**, None; **S. Hobbs**, None; **J. Levine**, None; **M. Jimenez**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.3

Topic: E.01. Neuroendocrine

Support: NIH U54 HD028138-15

Title: The stimulatory effect of neurokinin B on GnRH/LH release in female mice is dependent on kisspeptin

Authors: *C. S. CALIGIONI, J. J. YANG, S. B. SEMINARA;
Reproductive Endocrine Unit/MGH, Boston, MA

Abstract: Loss-of-function mutations in the genes encoding neurokinin B (NKB) and its receptor have been identified in patients with gonadotropin-releasing hormone (GnRH) deficiency. However, conflicting data exists regarding the precise physiologic role for this neuropeptide in reproduction. NKB has been reported to stimulate, inhibit, or have no effect on luteinizing hormone (LH) secretion. In rodents, sheep, and post-menopausal women, NKB and kisspeptin are co-expressed in neurons of the arcuate/infundibular nucleus, a key region for sex-steroid negative feedback regulation of GnRH secretion across mammalian species. Kisspeptin is a powerful stimulus for GnRH-induced LH secretion, and mutations in the kisspeptin receptor cause GnRH deficiency in both mice and men. Given the genetic data and the expression patterns of the two neuropeptides in the hypothalamus, we hypothesized that NKB acts through the kisspeptin pathway to stimulate GnRH induced LH release. Therefore, we administered the NKB agonist to wild-type (WT) mice and mice with targeted deletions of *Kiss1* or *Kiss1r*.
Adult (6-10 months old) WT, *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ female mice received a subcutaneous injection of the NKB receptor agonist senktide 1mg/kg on the morning of diestrus. After 30 min the animals were killed and blood was collected for LH measurement (RIA).
In WT female mice in diestrus, senktide has a stimulatory effect on LH, raising its levels five times compared to vehicle-injected controls (vehicle, 0.11±0.02 ng/mL; senktide, 0.57±0.12 ng/mL, $P<0.001$). In contrast, in mice with mutations disrupting either kisspeptin or its receptor, senktide did not appreciably alter LH concentrations (*Kiss1r*: vehicle, 0.15±0.05 ng/mL; senktide, 0.12±0.05 ng/mL; *Kiss1*: vehicle, 0.18±0.05 ng/mL; senktide, 0.11 ±0.03 ng/mL).
The NKB receptor agonist stimulates LH secretion in adult wild-type female mice in diestrus, consistent with its being required for normal sexual maturation in humans. This stimulation of LH secretion is dependent on kisspeptin signaling, as senktide does not stimulate LH in mice lacking kisspeptin or its receptor. Our findings suggest that the NKB pathway functions upstream from the kisspeptin pathway and acts through the kisspeptin pathway to stimulate the reproductive endocrine axis.

Disclosures: C.S. Caligioni, None; **S.B. Seminara**, None; **J.J. Yang**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.4

Topic: E.01. Neuroendocrine

Support: NIH R01 HD039916

Title: The actions of neurokinin B in the arcuate nucleus are important for episodic LH secretion

in ewes

Authors: *R. L. GOODMAN[1], C. C. NESTOR[1], J. M. CONNORS[1], I. HOLASKOVA[1], M. N. LEHMAN[2];
[1]West Virginia Univ., MORGANTOWN, WV; [2]Anat. and Cell Biol., Univ. of Western Ontario, London, ON, Canada

Abstract: Recent evidence that mutations in NK3R, the receptor for neurokinin B (NKB), cause infertility in humans has increased interest in the role of NKB in control of GnRH secretion. NKB is found in a set of arcuate (ARC) neurons that also contain kisspeptin and dynorphin. Evidence that these ARC neurons form a neural network, and contain NK3R, has led to the hypothesis that they play an important role in synchronizing the activity of GnRH neurons to produce episodic GnRH and LH secretion. To test this hypothesis, we examined the effects of local administration to the ARC of the NK3R antagonist, SB222200 (SB), on LH pulse patterns in ovariectomized (OVX) ewes. Bilateral chronic guide tubes (18 gauge) were implanted just dorsal to the middle-caudal ARC of OVX ewes (n=7). After a recovery period, LH pulses were monitored in blood samples collected every 12 min for 2 hrs before and 4 hrs after insertion of microimplants (22 gauge tubing containing SB or empty controls) to the tip of the guide tubes. At the end of the 4 hrs of treatments, microimplants were removed. One week later, this protocol was repeated using a cross-over design so that all 7 ewes received both SB and control treatments. At the end of the experiment, ewes were killed and paraformaldehyde-fixed tissue collected for determination of microimplantation sites; five of the seven ewes had appropriately placed sites. In these five ewes, implantation of SB into the ARC produced a hiatus of episodic LH secretion, lasting for 192 ± 74 min (range: 84 to 480 min). Consequently the mean interpulse interval increased from 40.8 ± 2.0 min for the 2 hrs before treatment to 147 ± 83 min for the 4 hrs during SB treatment. Control treatment had no effect on the occurrence of LH pulses, with interpulse intervals averaging 41.4 ± 2.4 min before and 42.2 ± 2.0 min after insertion of empty microimplants. In the two ewes with misplaced microimplants there was also no disruption of LH pulse patterns. These data are consistent with the hypothesis that NKB actions in the ARC are important for normal secretion of GnRH and LH in an episodic pattern.

Disclosures: R.L. Goodman, None; **C.C. Nestor**, None; **J.M. Connors**, None; **I. Holaskova**, None; **M.N. Lehman**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.5

Topic: E.01. Neuroendocrine

Support: U54 HD012303

R01 DK044838

F32 HD058427

T32 HD007203

T32 DK007494

The Hartwell Foundation

Title: The neurokinin 3 receptor agonist, senktide, reduces gonadotropin-releasing hormone synthesis and secretion in the immortalized GT1-7 hypothalamic cell model

Authors: *C. A. GLIDEWELL-KENNEY, A. M. H. GROVE, A. K. IYER, P. L. MELLON; Reproductive Med., Univ. of California San Diego, LA JOLLA, CA

Abstract: The mechanism that triggers puberty has long been a mystery but is generally thought to produce increased hypothalamic gonadotropin-releasing hormone (GnRH) secretion, pituitary luteinizing hormone (LH) secretion and gonadal steroid synthesis. Recently, human genetics of hypogonadotropic hypogonadism identified kisspeptin/GPR54 and neurokinin B/NK3R as two ligand/receptor pairs with key roles in the initiation of puberty. Kisspeptin and neurokinin B mRNA are co-localized in the cell bodies of a population of neurons in the arcuate nucleus whose projections have been shown to come in close proximity to GnRH neuron terminals at the median eminence. Moreover, GnRH terminals express GPR54 and NK3 receptor protein, suggesting a role in regulating GnRH release. Intracerebroventrical (ICV) injection of kisspeptin stimulates, whereas senktide, a potent NK3R agonist, suppresses peripheral LH. These data suggest that NK3R and GPR54 signaling have opposing effects on GnRH release. However, it remains unclear how the loss of an activator or suppressor of GnRH secretion can similarly disrupt puberty. Like GnRH neurons, kisspeptin/neurokinin B neurons also express NK3R. Thus, ICV injection may produce mixed autocrine and paracrine effects. To assess the direct effect of NK3R signaling in GnRH neurons, we employ the immortalized GT1-7 cell line, a model of the differentiated GnRH neuron.
Here, we demonstrate NK3R mRNA expression in GT1-7 cells by RT-PCR. Moreover, preliminary studies show 24 hour treatment of GT1-7 cells in static culture with the NK3R agonist senktide reduces GnRH secretion. GnRH secretion and biosynthesis are often co-regulated, thus we also investigate whether senktide treatment regulates GnRH transcription. Interestingly, the level of NK3R expression decreases with passage in GT1-7 cells. To overcome variability due to changing levels of NK3R, we employ transient transfection of a NK3R expression plasmid in higher passage GT1-7 cells. Using this system, we find that senktide treatment produces a consistent dose- and time-dependent suppression of luciferase reporter gene expression driven by -5 kb of GnRH promoter sequence. We further identify the previously characterized enhancer (-1863 to -1571) and proximal promoter (-173) as sufficient for the

NK3R-mediated suppression. In contrast to the above, previous studies have shown kisspeptin treatment of GT1-7 cells increases GnRH transcription and secretion. Future studies will investigate the effect of co-treatment with senktide and kisspeptin to determine if a hierarchy or cross-talk occurs between NK3R and GPR54 signaling in the GnRH neuron.

Disclosures: C.A. Glidewell-Kenney, None; **A.M.H. Grove**, None; **A.K. Iyer**, None; **P.L. Mellon**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.6

Topic: E.01. Neuroendocrine

Support: NHMRC project grant 606538

JTS is an ARC Future Fellow FT0990986

Title: Kisspeptin appears essential for the full preovulatory LH surge in sheep but GnRH sensitivity to kisspeptin does not change prior to the surge

Authors: *J. T. SMITH[1], M. SHAHAB[2], R. P. MILLAR[3], I. J. CLARKE[1];
[1]Monash Univ., Melbourne, Australia; [2]Qauid-i-Azam Univ., Islamabad, Pakistan; [3]The Queens Med. Res. Inst., Edinburgh, United Kingdom

Abstract: Kisspeptin is the product of the Kiss1 gene, binds to the receptor GPR54, and stimulates gonadotrophin-releasing hormone (GnRH) secretion. Kisspeptin/GPR54 signalling is critical for puberty and normal reproduction. In sheep, Kiss1 mRNA expressing cells are found in the arcuate nucleus and dorsal preoptic area and both appear to mediate the positive feedback effect of estradiol to generate the preovulatory GnRH/luteinizing hormone (LH) surge. To determine the role of kisspeptin on the surge, we administered the kisspeptin antagonist p234-penetratin (8 h continuous intracerebroventricular infusion, 300 µg/h; with an initial 200 µg loading dose) or vehicle control to ewes. Prior to infusions, all ewes were subjected to an estradiol benzoate (EB, 50 µg intramuscular) induced LH surge. In control ewes, EB resulted in LH surges in all animals (n = 6). Kisspeptin antagonist treatment significantly inhibited LH surges in ewes (area under the LH curve analysis revealed LH surges were 56% lower in kisspeptin antagonist treated animals, $P < 0.05$, n = 6), and completely prevented the surge in 2 animals. To further determine the role of kisspeptin on the LH surge, we examined whether the

response to kisspeptin treatment (50 μg iv) varies between the luteal phase of the estrous cycle and the late-follicular phase, just prior to the LH surge (n = 5-6 per group). Hypophysial and jugular blood samples were collected every 10 min for 1 h prior to, and 2 h after kisspeptin treatment and GnRH and LH concentrations were determined. Kisspeptin significantly stimulated GnRH and LH in all animals compared to vehicle treated controls. The LH response to kisspeptin was greater ($P < 0.05$) in ewes during the late-follicular phase. However, the GnRH response to kisspeptin appeared unchanged prior to the surge. These data suggest the GnRH response to kisspeptin (kisspeptin potency) does not appear to change prior to the LH surge. Estrogen priming of the pituitary gland is the most probable explanation for increased LH responses in the late-follicular phase. Despite this, kisspeptin does appear to play an essential role in receiving estrogen stimulatory signals and generating the positive feedback GnRH/LH surge.

Disclosures: J.T. Smith, None; **M. Shahab**, None; **R.P. Millar**, None; **I.J. Clarke**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.7

Topic: E.01. Neuroendocrine

Support: Health Research Council of NZ

NIH

Title: Regulation of gonadotropin-releasing hormone (GnRH) neuron excitability by anteroventral periventricular nucleus (AVPV) neurons

Authors: *A. E. HERBISON[1], X. LIU[1], E. DUCRET[1], S. PETERSEN[2];
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Abstract: The GnRH neurons are embedded within a complex neuronal network that controls fertility. A population of neurons located in the AVPV are thought to provide direct inputs to GnRH neurons and be essential for the induction of the estrogen-dependent preovulatory GnRH/luteinizing hormone surge. The present series of studies has used acute brain slice electrophysiology to examine the electrical characteristics of AVPV neurons and the manner in which they control the activity of GnRH neurons. Cell-attached recordings showed that kisspeptin neurons had a mean firing rate double that of other AVPV cells, and that the firing

rates of AVPV neurons fluctuated over the estrous cycle. Using an angled para-horizontal brain slice preparation from GnRH-GFP transgenic mice, the nature and effects of monosynaptic AVPV inputs to GnRH neurons was examined. These studies showed that stimulation of the AVPV at low frequencies (<1Hz) activated almost exclusive glutamate and GABA inputs to GnRH neurons mediated by $GABA_A$, AMPA and NMDA receptors. No differences were detected in the GABA/glutamate balance of AVPV input to GnRH neurons between males and females, or across the estrous cycle and in ovariectomized, estradiol-treated mice. Most of the AVPV input to GnRH neurons was GABAergic and could either enhance or suppress the electrical excitability of GnRH neurons. Stimulation of the AVPV at higher frequencies (5-10Hz) resulted in additional enhanced electrical firing of GnRH neurons for up to 15 min following the stimulation in ~ 60% GnRH neurons. Because of the similarities of this response to the effects of exogenous kisspeptin on GnRH neurons, the experiments were repeated in GnRH-GFP mice crossed on to a Gpr54 knockout mouse line. This resulted in an almost complete abolition of the post-AVPV stimulus excitation of GnRH neurons in GnRH-GFP-Gpr54 knockout mice. These studies demonstrate that AVPV neurons exhibit fluctuating levels of firing through the estrous cycle and that populations of AVPV neurons utilizing amino acid and neuropeptide transmitters innervate GnRH neurons directly to regulate their electrical excitability. The frequency-dependent release of amino acids and kisspeptin from AVPV neurons innervating GnRH neurons provides a powerful mechanism for controlling their excitability.

Disclosures: A.E. Herbison, None; **S. Petersen**, None; **X. Liu**, None; **E. Ducret**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.8

Topic: E.01. Neuroendocrine

Support: NIH F32 DK085834-01

NIH K08 DK068069-01A2

NIH R01 HD061539

Title: The role of ghrelin in the central integration of metabolism and reproduction

Authors: *H. M. DUNGAN LEMKO, C. E. LEE, D. A. LAUZON, S. A. ROVINSKY, S. OSBORNE-LAWRENCE, J. K. ELMQUIST, J. M. ZIGMAN, C. F. ELIAS;

UT Southwestern Med. Ctr., Dallas, TX

Abstract: Ghrelin, a stomach-derived peptide that stimulates feeding and hyperglycemia, is secreted in response to acute fasting and chronic food restriction. Recent studies suggest that ghrelin suppresses fertility at the level of the hypothalamus, so this hormone may represent a peripheral signal that inhibits reproduction during starvation. Neurons that express the Kiss1 gene integrate peripheral signals to trigger the release of gonadotropins and represent likely candidates for mediating the effects of ghrelin on fertility. Thus, we hypothesized that ghrelin acts as a starvation signal to inhibit Kiss1 expression and suppress fertility. We first predicted that if ghrelin directly affects Kiss1 gene expression, then Kiss1 neurons would express the primary ghrelin receptor, GHSR1a. To test this, we used mice engineered to express the LacZ gene only in Kiss1-expressing cells. We used immunohistochemistry to visualize beta galactosidase protein and in situ hybridization (ISH) to identify GHSR1a mRNA. We observed that about half the Kiss1 neurons in the arcuate nucleus express GHSR1a, suggesting that ghrelin can communicate directly with these neurons. We then theorized that if ghrelin acts as a starvation signal, ghrelin treatment would mimic the inhibitory effect of fasting on Kiss1 gene expression. We used ISH to examine the number of Kiss1-expressing cells in the arcuate nuclei of male fed mice treated with saline, fed mice treated with ghrelin, and fasted mice treated with saline. We observed that mice treated with ghrelin consumed significantly more chow than mice treated with saline, confirming the efficacy of our ghrelin preparation. Both a 24-hr fast and ghrelin treatment significantly decreased the number of cells expressing Kiss1 in the arcuate (46.6 ± 10.9 and 62.4 ± 10.9, respectively) compared to fed, saline-treated mice (104.2 ± 12.9). The estrous cycle is disrupted by starvation in mice and we theorized that rising levels of circulating ghrelin could mediate this effect. We used vaginal smears to track the cycles of intact female mice over time and administered a subcutaneous injection of ghrelin in the morning and afternoon of proestrus. The mice treated with ghrelin ate significantly more chow than saline-treated mice. However, 8 of 8 mice treated with saline and 7 of 8 mice treated with ghrelin showed signs of estrus on the day after treatment and all mice cycled regularly for at least ten days after treatment. Together these studies suggest that ghrelin signals directly to Kiss1 neurons in the arcuate nucleus to acutely diminish Kiss1 gene expression but acute administration of ghrelin on proestrus is not sufficient to disrupt estrous cycles in mice.

Disclosures: H.M. Dungan Lemko, None; **C.E. Lee**, None; **D.A. Lauzon**, None; **J.K. Elmquist**, None; **S.A. Rovinsky**, None; **S. Osborne-Lawrence**, None; **J.M. Zigman**, None; **C.F. Elias**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.9

Topic: E.01. Neuroendocrine

Support: NICHD-013254

NICHD-008610

Title: Identification of transcriptional regulators potentially involved in the arrest of pulsatile GnRH release during infancy in the male rhesus monkey (Macaca mulatta)

Authors: V. MATAGNE[1], S. RAMASWAMY[2], S. OJEDA[1], *T. M. PLANT[2];
[1]Oregon Natl. Primate Res. Ctr., Beaverton, OR; [2]Dept Cell Biol & Physiol, Univ. Pittsburgh, PITTSBURGH, PA

Abstract: In primates, two critical hypothalamic switches contribute to timing puberty. The infantile switch is operational during the first year of life and leads to decreased pulsatile GnRH release, guaranteeing the quiescence of the gonads during juvenile development. The second is activated at the end of juvenile development to trigger the resurgence in pulsatile GnRH release that results in the onset of puberty. While the pubertal switch has been studied extensively, much less is known about the infantile switch and its underlying mechanism(s). We have previously proposed that the genes responsible for the regulation of GnRH secretion that will lead to the onset of puberty are under the control of transcriptional regulators organized as central nodes of hierarchically arranged gene networks. To determine if such upper-echelon genes operate in the infantile-juvenile (INF-EJ) transition, independently of the gonads, we interrogated the hypothalamus of agonadal male rhesus monkeys using rhesus monkey-specific Affymetrix DNA arrays. The cerebral cortex was used as a control region. Changes in gene expression were monitored in male monkeys that were castrated during the first week of age. GnRH pulse generator activity was tracked indirectly by assessing circulating LH levels. Animals were killed either when pulse generator activity was maximal (INF group, N=5) or shortly after the GnRH "turn-off" was established (EJ group, N=5). Using the Partek Genomics suite for transcriptome analysis, we found a total of 212 probe sets that were significantly up- or down-regulated (1.8 fold change) in the hypothalamus during the INF-EJ transition and identified 15 of those genes as involved in transcriptional regulation and/or neuronal development. We selected five of them for quantitative PCR verification and found that expression of ZNF438, a transcriptional repressor, increases at the time of the infantile switch, when GnRH release decreases. In contrast, expression of the other four genes decreased during the INF-EJ transition. These genes are: GPR17, a negative regulator of glial cell differentiation, Prickle 1 and Prickle 2 required for the transcriptional regulation of neurite outgrowth, and BZW2, which promotes translational elongation, and is a target of the Myc oncogene. Further studies will determine the cellular sites of expression of these genes in the primate hypothalamus, verify the array results for the remaining genes of interest, and attempt to organize the verified genes into a regulatory network using both in silico and in vitro experiments.

Disclosures: V. Matagne, None; **T.M. Plant**, None; **S. Ramaswamy**, None; **S. Ojeda**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.10

Topic: E.01. Neuroendocrine

Support: NIH HD25123

U5418185

RR000163

Title: Epigenetic regulation of female puberty

Authors: *A. LOMNICZI, A. LOCHE, S. R. OJEDA;
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Abstract: The potential role of epigenetics in the neuroendocrine control of puberty has never been addressed. To gain insights into the contribution of DNA methylation to this process, we inhibited *de novo* DNA methylation by *in vivo* treatment with 5'-azacytidine (5-Aza), a DNMT inhibitor (starting on postnatal day 21; 2 mg/Kg BW, i.p). Plasma LH levels and estradiol production were reduced, puberty was strikingly delayed, and estrous cyclicity was disrupted. 5-Aza treated rats failed to respond to ovariectomy with increased LH output, indicating a central impairment; however, both the pituitary response to GnRH and the GnRH response to kisspeptin were normal, suggesting that loss of DNA methylation affects upstream cellular systems regulating GnRH neurons. The results also suggested that disruption of this epigenetic mechanism of gene silencing delays puberty by activating repressor genes whose expression would normally decrease at puberty. To identify such repressors, we searched the peripubertal female hypothalamus using Illumina and Affymetrix gene expression arrays, genome-wide DNA methylation analyses, and quantitative (q)PCR. This strategy yielded the Polycomb group (PcG) gene silencing system as a compelling candidate. The PcG complex regulates genomic programs by defining which sets of genes are active and which ones are quiescent at different stages of development. Of several PcG members analyzed, we found that Cbx7 and Eed expression decreases in the hypothalamus during puberty. Because these genes are core PcG components, we selected them for further study. We observed that the pubertal decrease in Cbx7 and Eed expression was accompanied by increased methylation of their promoter regions. We next selected the Kiss1 gene as a prototype of a potential PcG target gene, and found that premature puberty induced by PMSG (single dose on day 26) resulted 2 days later in promoter

demethylation (measured by TDMA-qPCR). Chromatin immunoprecipitation (ChIP) assays-qPCR revealed that these changes were accompanied by association of activating histone modifications (H3K9,14ac and H3K4me3) and loss of H3K27me3, a repressive histone form catalyzed by PcG proteins. Additional ChIP assays showed that PcG proteins do interact with the Kiss1 promoter. These results suggest that an epigenetic mechanism of transcriptional repression plays a significant role in timing the initiation of mammalian puberty, and that the PcG group of transcriptional silencers is a major contributor to this repressive mechanism. These repressors appear to target downstream genes involved in the stimulatory control of GnRH secretion at puberty.

Disclosures: A. Lomniczi, None; **A. Loche**, None; **S.R. Ojeda**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.11

Topic: E.01. Neuroendocrine

Support: NIH Grant R01 DK044838

NIH Grant U54 HD012303

NIH Grant F32 HD058427

NIH Grant T32 DK007494

NIH Grant R01 HD020377

Title: Specification of gonadotropin-releasing hormone gene expression to differentiated hypothalamic neurons is regulated by chromatin structure and histone modifications

Authors: *A. K. IYER, P. L. MELLON;
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Abstract: Targeting of hypothalamic neuropeptide hormone gene expression to individual populations of neurons is specified during development as cells adopt unique cell fates. The mature differentiated phenotype and function of these secretory cells is ultimately defined by the cell-specific expression of the neuropeptide hormone gene. Gonadotropin-Releasing Hormone

(GnRH), the central regulator of reproductive function, is produced by only ~800 highly specialized hypothalamic neurons. Accurate expression of GnRH and proper development of the GnRH neuron are critical for normal reproductive function as dysregulation leads to hypogonadotropic hypogonadism and infertility. The mechanisms through which GnRH gene expression is restricted to this functionally distinct subset of neurons are not fully understood. We have previously shown that neuron-specific GnRH expression is mediated through specific transcription factors acting on GnRH regulatory elements, which include the GnRH minimal promoter, and three upstream enhancer regions. Modulation of chromatin structure is thought to promote neuronal differentiation. Since GnRH gene expression gradually increases during development, reaching its highest levels in the mature, postmitotic state, neuronal specification of GnRH expression could be regulated at the level of chromatin structure. DNase Sensitivity and Chromatin Immunoprecipitation (ChIP) assays were performed using GT1-7 mature GnRH neuronal cells, GN11 immature GnRH neuronal cells, and NIH3T3 non-neuronal fibroblast cells. The GnRH promoter and enhancer regions were sensitive to DNase only in GT1-7 cells, and not in GN11 and NIH3T3 cells. Only in GT1-7 cells did GnRH regulatory elements display histone modification markers of active chromatin, including high enrichment of histone H3 lysine 4 tri-methylation (H3K4-Me3) at the promoter, and H3 acetylation at the promoter and enhancer regions. The GnRH promoter in GT1-7 cells displayed high levels of both total RNA Polymerase II (Pol II) as well as the Serine 5 phosphorylated form of Pol II (p-Pol II), associated with transcriptional initiation. Total Pol II and p-Pol II occupancy were low in both GN11 and NIH3T3 cells. Enrichment of H3K9-Me2, a marker of inactive chromatin, was high in NIH3T3 cells, low in GT1-7 cells, and at intermediate levels in GN11 cells. Taken together, these results indicate that the chromatin of the GnRH gene is open and active in mature GnRH neurons, and closed and inactive in non-neuronal cells, but not fully inactive in immature GnRH neurons. Chromatin structure and histone modifications therefore specify GnRH expression to differentiated hypothalamic neurons.

Disclosures: A.K. Iyer, None; **P.L. Mellon**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.12

Topic: E.01. Neuroendocrine

Support: AG-023477

AG-029612

HD-029186

RR-000163

Title: Aging-related changes in hypothalamic gene expression in the male rhesus macaque

Authors: *D. H. EGHLIDI[1], S. G. KOHAMA[1], H. F. URBANSKI[1,2];
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[2]Physiol. and Pharmacology, Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: The neuropeptides kisspeptin, neurokinin B, and dynorphin A (respectively encoded by *KiSS-1*, *NKB* and *PDYN*) are highly expressed in the primate hypothalamic arcuate nucleus (ARC), where they are thought to modulate the release of GnRH. We recently examined the expression of these genes in the ARC-median eminence (ARC-ME) of aging female rhesus macaques, and observed a perimenopausal increase in *KiSS-1* and *NKB.* In the present study we used real-time qPCR to determine if similar changes also occur in old males. In addition to *KiSS-1*, *NKB* and *PDYN*, we also examined changes in the expression of genes that encode associated receptors (*GPR54, NK3* and *KOR)*, as well as *GnRH-I* and *GnRH-II,* and *DIO2* and *DIO3* (two enzymes expressed in tanycyte cells within the ARC-ME). No obvious differential expression was observed in the expression of *KiSS-1*, *NKB* and *PDYN* or *GPR54, NK3* and *KOR* between the young adult (7.5 ± 3.2 years, n=5) and old adult (25.0 ± 4.0 years, n=14) animals. Similarly, no aging-related differences were observed in GnRH-I or GnRH-II gene expression. These data give further support to the view that the elevated *KiSS-1* and *NKB* expression previously observed in old females likely stems from the marked menopausal attenuation of ovarian sex-steroids levels, rather than from aging *per se*. In the present study, however, we did detect a significant ($P<0.01$) aging-related decrease in the expression of *DIO3*, but not *DIO2*. In photoperiodic mammals these genes regulate the availability of thyroid hormone (TH), which has reciprocal effects on GnRH-I release. This raises the interesting possibility that *DIO3* may play a role in reproductive senescence in aging primates.

Disclosures: D.H. Eghlidi, None; **H.F. Urbanski**, None; **S.G. Kohama**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.13

Topic: E.01. Neuroendocrine

Support: NIH AG25047

NIH RR015592

Title: Identification of the estrogen receptor-α positive neurons that innervate GnRH neurons

Authors: *L. H. JENNES, A. CENTERS, V. ADJAN;
Univ. Kentucky Col. Med., Lexington, KY

Abstract: The activity of gonadotropin releasing hormone (GnRH) neurons is regulated by feedback loops that include the pituitary gonadotropes and the ovarian steroid hormones, estradiol and progesterone. Estradiol inhibits the release of GnRH during all phases of the reproductive cycle except for a short period when rising estradiol levels cause a switch to a positive feedback which induces the release of GnRH which will cause ovulation. This effect of estradiol is not caused by a direct activation of estrogen receptor-α (ERα) in GnRH neurons but instead by actions of estradiol on other neurons in the brain which in turn convey the steroid signal to the GnRH neurons through synaptic interactions. In the present study we used a transgenic mouse model which expresses a fusion protein of DsRed2 and wheat germ agglutinin (WGA) under the control of the GnRH promoter in order to identify the cells that innervate GnRH neurons and we used immunohistochemistry for ERα to determine if the afferent neurons express the steroid receptor. The results show that about 20-30% of the neurons in the bed nucleus of the stria terminalis, anterior hypothalamic area, lateral hypothalamus and arcuate nucleus that innervate GnRH neurons, contain ERα. About 10-20% of the GnRH neurons-innervating cells in the septo-hippocampal nucleus, tenia tecta, medial septum and lateral septal nucleus, intermediate and ventral parts, horizontal limb of the diagonal band, medial preoptic nucleus and area and median preoptic nucleus express ERα. Few neuron containing both, DsRed2-WGA and ERα immunoreactivity are seen in the semilunar nucleus, lateral septal nucleus, dorsal part, vertical limb of the diagonal band, anterodorsal preoptic nucleus, lateral preoptic area, ventromedial nucleus, and paraventricular thalamic nucleus. The results show that GnRH neurons receive extensive input from estradiol-receptive neurons that are located in a variety of regions, some of which were known to be involved in the control of GnRH neuronal activity, such as the arcuate and ventromedial nuclei. These studies also reveal several additional brain regions that express ERα and project to GnRH neurons, such as the septo-hippocampal nucleus or tenia tecta which were previously not associated with the control of GnRH neurons.

Disclosures: L.H. Jennes, None; **A. Centers**, None; **V. Adjan**, None.

Nanosymposium

633. Kisspeptin and Co

Location: Room 1B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 633.14

Topic: E.01. Neuroendocrine

Support: R01HD049651

U54HD12629

Title: Generation and confirmation of a Kiss1 eGFP-Cre knock-in mouse

Authors: M. L. GOTTSCH[1], J. K. LAWHORN[2], S. M. POPA[3], A. E. OAKLEY[2], M. ALREJA[5], V. M. NAVARRO[2], M. MCCLEAN[1], *D. K. CLIFTON[6], R. D. PALMITER[4], R. A. STEINER[1];
[1]OB/GYN, [2]Physiol. and Biophysics, [3]Mol. and Cell. Biol., [4]Biochem. and the Howard Hughes Med. Inst., Univ. of Washington, SEATTLE, WA; [5]Psychiatry, Yale Univ. Sch. of Med. and the Ribicoff Res. Facilities, Connecticut Mental Hlth. Ctr., New Haven, CT; [6]Univ. Washington, SEATTLE, WA

Abstract: Kisspeptin (a product of the Kiss1 gene) and its receptor (Kiss1r) are critical players in the regulation of reproduction. In the rodent, the Kiss1 gene is co-expressed with the alpha form of the estrogen receptor by neurons in the arcuate (Arc) and anteroventral periventricular (AVPV) nuclei. However the two populations of Kiss1 neurons differ significantly. Estradiol (E) inhibits Kiss1 expression in the Arc, where Kiss1 neurons appear to mediate the negative feedback control of luteinizing hormone (LH) secretion by E. In contrast, E stimulates Kiss1 expression in the AVPV, an area responsible for driving the preovulatory release of LH. Furthermore, Kiss1 neurons in the Arc express neurokinin B (NKB) and dynorphin (Dyn), whereas those in the AVPV do not. We do not understand the molecular basis for the differential action of E on Kiss1 neurons in the Arc and AVPV or the functional significance of NKB and Dyn as cotransmitters with kisspeptin_primarily because we lack an appropriate model to genetically manipulate and analyze Kiss1 neurons. To this end, we have generated a Kiss1-CreGFP knock-in mouse, which can be used to produce cell-specific knockouts of genes expressed in Kiss1 neurons and to perform electrophysiological experiments to dissect the conductances involved in the short-term response of Kiss1 neurons to various neuromodulators, including E, NKB, and Dyn. Analysis of the brains of Kiss1-CreGFP mice show that males express eGFP in neurons within the Arc only, whereas females express eGFP in both the Arc and AVPV, thus establishing the fidelity of expression of the knock-in construct. The use of these Kiss1-CreGFP mice will allow us to examine the electrophysiological properties of Kiss1 neurons in slice preparations, identify the afferent inputs to Kiss1 neurons, and investigate the physiological significance of sex steroid receptors and cotransmitters in Kiss1 neurons.

Disclosures: M.L. Gottsch, None; **J.K. Lawhorn**, None; **S.M. Popa**, None; **A.E. Oakley**, None; **M. Alreja**, None; **V.M. Navarro**, None; **M. McClean**, None; **D.K. Clifton**, None; **R.D. Palmiter**, None; **R.A. Steiner**, None.

Nanosymposium

634. Learning the Value of Actions

Location: Room 30E

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 634.1

Topic: F.01. Human Cognition and Behavior

Support: James S. McDonnell Foundation (Collaborative Activity Award)

Title: Hierarchical reinforcement learning

Authors: *M. M. BOTVINICK[1], Y. NIV[1], C. DIUK[1], J. FERNANDES[1], A. G. BARTO[2];
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Abstract: Research on human and animal behavior has long emphasized its hierarchical structure, according to which tasks are comprised of subtask sequences, which are themselves built of simple actions. The hierarchical structure of behavior has also been of enduring interest within neuroscience, where it has been widely considered to reflect prefrontal cortical functions. In recent work, we have been reexamining behavioral hierarchy and its neural substrates from the point of view of recent developments in computational reinforcement learning. Specifically, we've been considering at a set of approaches known collectively as hierarchical reinforcement learning, which extend the reinforcement learning paradigm by allowing the learning agent to aggregate actions into reusable subroutines or skills. A close look at the components of hierarchical reinforcement learning suggests how they might map onto neural structures, in particular regions within the dorsolateral and orbital prefrontal cortex. It also suggests specific ways in which hierarchical reinforcement learning might provide a complement to existing psychological models of hierarchically structured behavior. A particularly important question that hierarchical reinforcement learning brings to the fore is that of how learning identifies new action routines that are likely to provide useful building blocks in solving a wide range of future problems. Here and at many other points, hierarchical reinforcement learning offers an appealing framework for investigating the computational and neural underpinnings of hierarchically structured behavior. In addition to introducing the theoretical framework, I will also describe a first set of fMRI, single-unit recording and behavioral studies, in which we have begun to test specific predictions.

Disclosures: M.M. Botvinick, None; **Y. Niv**, None; **C. Diuk**, None; **J. Fernandes**, None; **A.G. Barto**, None.

Nanosymposium

634. Learning the Value of Actions

Location: Room 30E

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 634.2

Topic: F.01. Human Cognition and Behavior

Support: NIH DA023462

NIH DA026457

AFOSR FA9550-07-1-0454

Title: A computational model of how medial prefrontal cortex learns to predict the value of actions

Authors: W. H. ALEXANDER[1], *J. W. BROWN[2];
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Abstract: Medial prefrontal cortex (mPFC) is widely recognized as a key locus of executive function and cognitive control. An open question, however, is what information is utilized by mPFC in determining when cognitive control is required. Human fMRI and EEG studies have suggested that activity in mPFC is associated with detection and processing of error, with detection of conflicting behavioral responses, or with the prediction of the likelihood of error commission. In contrast, single-unit recordings from monkey suggest that mPFC primarily predicts and detects rewarding events. To date, no single model of mPFC has been able to account for the diverse range of empirical results.
We present a new model of learning, the Prediction of Response-Outcome (PRO) model, which suggests a novel interpretation of mPFC activity as signaling surprising non-occurrences of predicted outcomes of actions. The PRO model extends previous models of reinforcement learning (Sutton, 1988) in four ways. First, rather than learning associations between stimuli and outcomes, the model predicts conjunctions of responses and outcomes (R-O learning) predicted by task stimuli. Second, the model learns independent timed predictions of all likely R-O conjunctions based on current stimuli. Third, the learning of R-O predictions is driven by a vector-valued reinforcement signal which signals the occurrence of an unpredicted event, regardless of affective valence (i.e., aversive events are treated in the same way as rewarding events). Finally, the model error is decomposed into its positive and negative parts. The positive error component indicates an unpredicted occurrence, while the negative error component signals the non-occurrence of a predicted event. Using only this derived measure of surprising non-occurrence, the PRO model simulates an unprecedented array of data from human imaging and

EEG studies, including effects of error, conflict, and error likelihood, as well as single-unit studies which show activity related to reward and reward prediction. Additional simulations of the model suggest novel hypotheses regarding the role of mPFC in decision-making and learning.

Disclosures: W.H. Alexander, None; **J.W. Brown**, None.

Nanosymposium

634. Learning the Value of Actions

Location: Room 30E

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 634.3

Topic: F.01. Human Cognition and Behavior

Support: Hersenstichting Nederland F2008(1)-01

Alkemade-Keuls foundation

Title: Striatal dopamine mediates the interface between motivational and cognitive control in humans: Evidence from Parkinson's disease

Authors: *E. AARTS[1,2], R. C. HELMICH[1,3], B. R. BLOEM[3], R. COOLS[1,2];
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Abstract: Striatal dopamine has been implicated in cognitive and motivational control. We have recently demonstrated, using genetic imaging in healthy volunteers, that striatal dopamine might also mediate the interaction between motivational and cognitive control (Aarts et al., Neuropsychopharmacology, in press). Anatomical evidence suggests a clear direction of information flow such that dopamine in the ventral striatum can influence processing in the dorsal striatum in motivationally relevant contexts. Here, we investigated this motivation-cognition interface with a rewarded task-switching paradigm in patients with mild to moderate Parkinson's disease (PD) after >12 hour withdrawal of their dopaminergic medication. In early PD, dopaminergic impairments are relatively restricted to the dorsal striatum, not yet extending to the ventral striatum. This dorsal-to-ventral striatal gradient suggests that intact function of the ventral striatum in early PD might compensate for impaired dorsal striatal function.
Consistent with prior work, PD patients (OFF medication; n = 32) demonstrated enhanced switch costs compared with matched healthy controls (n = 26). However, this switching deficit was

restricted to the low reward condition (1 cent) and was completely remediated when subjects anticipated high reward (10 cents). This reward benefit in terms of switching was greater than that in controls, a finding that was substantiated by the observation that PD patients (OFF medication) exhibited increased subjective reward responsiveness compared with controls as measured by the BIS/BAS questionnaire.
The motivation-cognition interaction in patients was highly correlated with the amount of dopamine depletion in dorsal striatum as measured with dopamine transporter Single Photon Emission Computed Tomography (DAT-SPECT), suggesting that dopamine depletion triggers a compensatory mechanism to restore cognitive flexibility. Intact dopaminergic ventral striatal functioning, as shown by the SPECT results, might provide the basis for this reward-based compensatory mechanism. The present results demonstrate that incentive motivation can compensate for cognitive inflexibility in mild PD, consistent with the idea that the motivation-cognition interface is mediated by ventral striatal dopamine. These findings elucidate a mechanism by which motivational goals can drive cognitive control processes.

Disclosures: E. Aarts, None; **R.C. Helmich**, None; **R. Cools**, None; **B.R. Bloem**, None.

Nanosymposium

634. Learning the Value of Actions

Location: Room 30E

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 634.4

Topic: F.01. Human Cognition and Behavior

Support: NWO Vidi 016.095.340 Innovational Research Incentives Scheme, Dutch Organisation for Scientific Research

Title: Dopaminergic modulation of punishment-based reversal learning is d2 receptor dependent and accompanied by modulation of the amygdala

Authors: *M. E. VAN DER SCHAAF[1], D. GEURTS[1], A. F. A. SCHELLEKENS[1], B. FRANKE[1], R.-J. VERKES[1], J. BUITELAAR[2], R. COOLS[1];
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Abstract: Our ability to adapt flexibly to environmental changes requires the flexible updating of reward and punishment predictions. The mesolimbic dopamine system plays an important role in such outcome-based learning. It has been suggested that dopamine D1 and D2 receptor mechanisms play different roles in learning from reward and punishment (Frank, 2005).

Consistent with this theorizing we have previously shown that bromocriptine, an agonist with high affinity for D2 receptors, altered punishment-based, but not reward-based reversal learning (Cools et al, 2009). In the present study we investigated the D2-dependency of this effect by pre-treating subjects with the selective D2 receptor antagonist sulpiride prior to administration of bromocriptine. Furthermore, we tested whether these effects were accompanied by modulation of the striatum and/or the amygdala. Twenty-seven healthy participants participated in four fMRI sessions, after intake of placebo, bromocriptine (1.25mg), bromocriptine and sulpiride (400mg) or sulpiride in a randomized, double-blind repeated measures design. Subjects performed a reversal learning task, in which reversals were signaled by either unexpected rewards or unexpected punishments. Compared with placebo, bromocriptine improved punishment-based reversal learning, while not affecting reward-based reversal learning. Consistent with our prediction, the punishment effect was partly abolished by pretreatment with sulpiride. fMRI data showed that this effect was accompanied by modulation of the amygdala response to unexpected punishments. These results support our hypothesis that effects of dopaminergic drugs on outcome-based reversal learning are outcome-specific and, additionally, suggest a role for D2 receptors in the amygdala in the dopaminergic modulation of punishment-based reversal learning.

Disclosures: M.E. Van Der Schaaf: None. **D. Geurts:** None. **A.F.A. Schellekens:** None. **B. Franke:** None. **R. Verkes:** None. **J. Buitelaar:** None. **R. Cools:** Research Grant; NWO Vidi 016.095.340 Innovational Research Incentives Scheme, Dutch Organisation for Scientific Research.

Nanosymposium

634. Learning the Value of Actions

Location: Room 30E

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 634.5

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01 MH080066-01

Title: Dopaminergic genes predict influence of verbal instruction on learning of action values

Authors: *B. B. DOLL, M. J. FRANK;
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Abstract: A large body of research suggests that the brain learns to maximize reward and minimize punishment through dopaminergic prediction error signals. Though such a learning

system is adaptive, it is slow and time consuming, requiring repeated experience with the outcomes of actions. A quicker way to learn about the environment is through rules, instructions, and advice from others. Such learning, however, comes at the cost of sensitivity to true reward contingencies. Here we explore and test the genetic underpinnings of how simple verbal information alters the neurocomputation of reinforcement values. Subjects were given inaccurate information about how to best perform in a probabilistic selection task. Instruction-consistent responding was persistent and specific to the instructed stimulus. Notably, performance did not indicate subjects were blindly following the inaccurate instructions, but rather combining outcome information with instruction information.
Neural network simulations suggest two possible neural circuits underlying the effect. In the first, verbal information representations in prefrontal cortex directly project to and bias striatal action-values learned through dopaminergic prediction errors, constituting a confirmation bias. In the second, the striatum learns the true reward contingencies through feedback, but does not express this learning in behavior because it is overridden at motor cortex by prefrontal instruction representations. Reinforcement learning models fit to behavior support the confirmation bias possibility, suggesting prediction errors for instruction-consistent outcomes are amplified, while those for instruction-inconsistent outcomes are diminished. Genetic results show that bias increases with striatal efficacy, impairing accuracy, as indexed by genes shown to enhance accuracy in uninstructed learning. Specifically, D2 receptor function was associated with learning from punishment on uninstructed trials, and learning to dismiss punishment produced by instruction-following. D1 pathway efficacy was associated with learning from uninstructed reward, and amplification of reward learning from instructed trials. A gene indexing prefrontal function showed that prefrontal efficacy increased the tendency to follow the instructions, despite the suboptimal outcomes they produced.

Disclosures: B.B. Doll, None; **M.J. Frank**, None.

Nanosymposium

634. Learning the Value of Actions

Location: Room 30E

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 634.6

Topic: F.01. Human Cognition and Behavior

Title: SEF encodes and monitors action value in a gambling task

Authors: *N. SO[1], V. STUPHORN[1,2];
[1]Neurosci., Johns Hopkins Univ., BALTIMORE, MD; [2]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: The value of an action depends on its anticipated outcome. Prior to executing an action, action value signals allow the selection of the action yielding the best possible outcome. The outcome of the selected action can be used to update the action value signal, if the actual outcome is different from the anticipated one.

The process of computing and learning action value signals is expected to recruit several brain regions. Those brain areas should be able to represent the motor aspect of an action and the value of that action's potential outcome. Supplementary eye field (SEF) is one of those candidates based on its anatomical connections to limbic and oculomotor areas.
To test this hypothesis, we recorded single cell activities from SEF while monkeys performed a gambling task. In this task, monkeys had to make saccades to targets that were associated with rewards of varying amount and uncertainty of delivery. Two types of trials in the task were designed to serve different purposes. Choice trials allowed us to use the monkeys' behavior, to directly measure the subjective value of an action. No-choice trials allowed us to investigate the neural correlates of action value. The different stages in the trial were associated with various value-related signals. In order to select a saccade, neural activity needed to reflect the anticipated value of each saccadic target. This value signal may represent only the anticipated reward (option value), or the association between a specific saccade and its expected outcome (action value). While the monkey awaited the final outcome to be revealed, another type of signal had to hold the anticipated outcome in working memory. Lastly, as the final reward amount was revealed on screen, different types of signals had to register the actual outcome and compare it to the expected outcome.
Our results show that SEF neurons encoded all of the aforementioned types of value signals throughout the different stages of the gambling task. We found neurons representing either option or action value before the monkey selected a saccade. While the monkey waited for the result, neurons reflected the anticipated reward of the saccade, the uncertainty of reward delivery (sure / gamble), or the combination of those signals. After the final reward amount was revealed, neurons reflected either absolute, or relative reward amount (win / loss). Single neurons could reflect different value signals at different trial stages. This implies that SEF neurons play an active role in using action value to select a saccade, and in monitoring its outcome. These feedback signals might be used to learn and update the action value signals.

Disclosures: N. So, None; **V. Stuphorn**, None.

Nanosymposium

634. Learning the Value of Actions

Location: Room 30E

Time: Tuesday, November 16, 2010, 1:00 pm - 2:45 pm

Program Number: 634.7

Topic: F.01. Human Cognition and Behavior

Support: Science Foundation of Ireland

Title: Contribution of the human dorsal striatum to model-free reinforcement learning

Authors: *R. K. JESSUP[1], D. CAHILL[1], J. P. O'DOHERTY[1,2];
[1]Trinity Col. Dublin, Dublin, Ireland; [2]Caltech, Pasadena, CA

Abstract: Model-free and model-based reinforcement learning are two distinct processes by which an individual may learn about an environment. A model-free system makes no assumptions about the underlying generating process within an environment whereas a model-based system assumes that there is a structure to the environment which can be used to scaffold novel events so as to learn more efficiently. The gambler's fallacy is an example of a particular model-based strategy that assumes a negative correlation between sequential outcomes. This contrasts with a simple model-free reinforcement learning strategy in which any relationships are learned exclusively from events occurring within the environment. We used a roulette wheel gambling task in order to compare the neural regions involved in model-free reinforcement learning and the model-based strategy known as the gambler's fallacy, using fMRI. On each trial, participants selected one of three non-equiprobable outcomes and then watched a spinner spin until stopping on the winning option. When the spinner stopped on the chosen option participants won €2. Participants were clearly instructed that the amount of area covered by an option indicated the probability that the spinner would land on that option and that the stopping probability of the spinner was completely independent from one trial to the next. Neuroimaging results indicated that when choosing according to a model-free reinforcement learning strategy - in contrast to a gambler's fallacy model-based strategy - greater dorsal caudate activation was observed. These findings provide evidence of a role for dorsal striatum in the implementation of model-free reinforcement learning.

Disclosures: R.K. Jessup, None; **D. Cahill**, None; **J.P. O'Doherty**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.1

Topic: F.02. Animal Cognition and Behavior

Support: Psychopharmacology Research Support Association (AFIP)

Research Innovation and Dissemination Centers – SLEEP (CEPID-Sleep)

The State of São Paulo Research (FAPESP)

the Psychobiology and Exercise Research Center (CEPE)

National Council for Scientific and Technological Development (CNPq)

Title: The impact of 8 wks of aerobic or resistance exercise on spatial memory and hippocampal BDNF of rodents

Authors: *R. C. CASSILHAS[1], J. FERNANDES[1], M. G. M. OLIVEIRA[1], L. B. FERREIRA[2], S. TUFIK[1], M. T. DE MELLO[1];
[1]Psychobiology, [2]Phisiology, Univ. Federal De São Paulo, São Paulo, Brazil

Abstract: Rodent studies demonstrated that exercise can improve learning and memory in various hippocampal-dependent tasks including Morris water maze (MWM), whereas these studies used aerobic exercise, running wheel or swimming to show these improvements. Although the resistance training can impact brain function in humans, we do not know if a rodent model of resistance exercise can affect learning and memory in the same way aerobic exercise does. In this sense, the goal of this study was to investigate the impact of 8 wks of aerobic or resistance exercise on spatial learning and memory and hippocampal BDNF of rodents. Wistar adult rats were distributed in four groups (n=10): Control Group (CTRL), Sham Group (SHA), Aerobic Group (AERO), and Resistance Group (RES). The AERO and RES groups were submitted to 8 wks of five weekly sessions of exercise in treadmill or vertical ladder apparatus (with a load secured to the tail), respectively. CTRL group was kept in the cage during all experiments with five minutes a day of manipulation. SHA group was kept in the same room and the apparatus (treadmill and vertical ladder) during the training of AERO and RES groups. After the last training session, all groups were submitted to MWM. In the training phase, rats were exposed 4 times to the MWM over 2 days. Each rat was given 60s to randomly explore water maze. After the training phase, in the spatial acquisition test (spatial learning) rats were submitted to 3 blocks of 4 swims separated by a 30 min interval, each rat was given 60s to reach the platform. The probe trial test which was conducted after a 30min break of spatial acquisition test, involved removing the platform and the rats undergoing a single trial of 60s. After 8 wks intervention, all four groups showed a reduction on escape latency, across blocks of trials, indicating spatial acquisition. Moreover, when compared to CTRL and SHAN groups, AERO and RES groups decreased the latency time in the three blocks of trials. On the probe trial, AERO and RES groups spent more time in the platform zone than the CTRL and SHA groups. For the hippocampal BDNF, both AERO and RES groups showed higher levels in comparison to CTRL and SHAN groups. AERO and RES groups showed better and faster spatial learning and memory also higher BDNF concentration when compared to CTRL and SHA groups. Furthermore, it was not founded any statistically difference between AERO and RES groups in the variables measured. In this sense, these results had been showed which aerobic and resistance exercise can improve spatial memory and can activate the BDNF pathway similarly.

Disclosures: R.C. Cassilhas, None; **J. Fernandes**, None; **M.G.M. Oliveira**, None; **L.B. Ferreira**, None; **S. Tufik**, None; **M.T. de Mello**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.2

Topic: F.02. Animal Cognition and Behavior

Support: 7R01DK077106-02

Title: Saturated fatty acids impair, while unsaturated fatty acids enhance, hippocampal memory and metabolic processes

Authors: *V. E. COTERO, C. JORGE, E. C. MCNAY;
Univ. at Albany (SUNY), Albany, NY

Abstract: Peripheral adiposity is associated with an increase in plasma fatty acid (FA) levels, which correlates with the development of insulin resistance (Boden et al., 2001, Wyne, K.L., 2003). This is supported by findings suggesting that a decrease in circulating FAs significantly improves insulin sensitivity (Oakes et al., 2001). Furthermore, a dietary increase in omega-3 and -6 FAs has been reported to improve memory (Page et al., 2009; Yehuda et al., 1994). In contrast, dietary saturated FAs have been associated with cognitive decline (Greenwood et al., 1996). We hypothesized that the effects of fatty acids on memory occur at least partly within the hippocampus and are dependent on the level of bond saturation. To test this hypothesis, we administered FAs with varying levels of saturation to the left hippocampus of adult male Sprague-Dawley rats prior to spatial memory testing. Animals receiving saturated FA showed a significant decrease in performance; conversely, animals receiving an unsaturated FA showed improved performance. However, no significant difference in enhancement was seen between mono- and polyunsaturated fatty acids (Figure 1). No effects on motor activity were observed, suggesting an effect on cognitive processes in the hippocampus rather than a nonspecific effect. Additionally, administration of a saturated FA was associated with a significant decrease in hippocampal metabolism during testing, assessed by concurrent in-vivo hippocampal microdialysis, and also with reduced hippocampal neurotransmitter (ACh, GABA) release, Our data suggest that in addition to the chronic effects of elevated FAs, adiposity and insulin resistance lead to cognitive impairment in part via direct, acute impairment of hippocampal function by saturated FAs; further, we provide a mechanism to explain the reported beneficial

effects of dietary unsaturated FAs on cognitive performance.$$graphic_{175D2D36-B2D8-4B4F-BD15-0DD3789B3BA1}$$

Disclosures: V.E. Cotero, None; **C. Jorge**, None; **E.C. McNay**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.3

Topic: F.02. Animal Cognition and Behavior

Support: Dutch Organization for Scientific Research (NWO) Grant ALW2PJ/08079

Title: Left hemispheric dominance of Zenk expression in response to tutor song in the NCM of juvenile zebra finch males

Authors: *S. MOORMAN[1], S. M. H. GOBES[2], M. KUIJPERS[1], M. A. ZANDBERGEN[1], J. J. BOLHUIS[1];
[1]Behavioural Biol., Utrecht Univ., Utrecht, Netherlands; [2]Organismic and Evolutionary Biol. and Ctr. for Brain Sci., Harvard Univ., Cambridge, MA

Abstract: Songbirds, such as zebra finches, learn their song from an adult conspecific (a 'tutor') early in life, much like human infants learn to speak. Zebra finches learn to sing in two partly overlapping phases: a memorization phase during which they form an internal representation (a 'template') of the song of their tutor, and a sensorimotor learning phase in which they start to produce their own song. We have shown previously that the caudal medial nidopallium (NCM) is a likely neural substrate for the representation of tutor song memory in male zebra finches. The NCM is thought to be analogous to the auditory association cortex in the human superior temporal lobe, which contains Wernicke's area, a region that is involved in speech processing. In human infants early speech processing occurs predominantly in the left temporal lobe. Here we investigated whether there is a similar hemispheric dominance in song processing in juvenile zebra finches. To this end, we exposed three groups of juvenile male zebra finches in the middle of their sensorimotor learning period (54-59 days post hatching) to either song of their tutor, an unfamiliar conspecific song or no song. Birds were reared with their nest mates, mother and father, after which they were kept in sibling groups from day 47 onwards, and thus did not hear their song tutor for at least 6 days prior to exposure. The juveniles had already copied parts of their tutor's song, as measured in the morning prior to stimulus exposure (similarity score: 57% ± 2.9 SEM). There were no significant differences in mean similarity scores between the three

groups of birds (F(2,15) = 0.027, n.s.). We measured the expression of Zenk, the protein product of the immediate early gene ZENK (a marker for neuronal activation) in both the left and right NCM. A repeated measures ANOVA showed a significant interaction between the factors Stimulus (tutor song, novel song or silence) and Hemisphere (F(2, 19) = 6.302, P = 0.008). Post-hoc tests (with Simes correction for multiple comparisons) revealed that in birds that were exposed to tutor song, there was a significantly greater number of Zenk-positive cells in the left NCM than in the right NCM (P = 0.019). In the other groups, there were no significant differences between the hemispheres. These results show that in young zebra finch males, there is a left NCM bias for processing the tutor song, but not for unfamiliar song. Interestingly, a similar left hemispheric dominance was found in the superior temporal lobe of human neonates that listened to words, compared to reversed words or silence. Thus, there is similar left hemispheric dominance in early auditory memory processing in humans and songbirds.

Disclosures: S. Moorman, None; **S.M.H. Gobes**, None; **M. Kuijpers**, None; **M.A. Zandbergen**, None; **J.J. Bolhuis**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.4

Topic: F.02. Animal Cognition and Behavior

Support: NIDA Grant DA025962

NIH Grant AG09464

NIDA Grant P0110044

Title: Control of cognition and adaptive behavior by the epigenetic regulators GLP and G9a

Authors: *A. SCHAEFER[1], S. SAMPATH[3], A. TARAKHOVSKY[2], P. GREENGARD[2]; [1]The Rockefeller Univ., NEW YORK, NY; [2]The Rockefeller Univ., New York, NY; [3]Stanford Univ., Stanford, CA

Abstract: The genetic basis of cognition and behavioral adaptation to the environment remains poorly understood. Here we demonstrate that the histone methyltransferases GLP and G9a that function as a GLP/G9a complex in vivo control cognition and adaptive responses to environmental signals in a region-specific fashion in the adult brain. Using conditional

mutagenesis in mice, we show that postnatal, neuron-specific deficiency of GLP/G9a leads to de-repression of numerous non-neuronal and neuron progenitor genes in adult neurons. This transcriptional alteration is associated with complex behavioral abnormalities, including defects in learning, memory, locomotion, motivation and environmental adaptation. The behavioral changes triggered by the postnatal neuron-specific GLP/G9a deficiency are similar to key symptoms of the human 9q34 mental retardation syndrome that is associated with structural alterations of the GLP/EHMT1 gene. The likely causal role of GLP/G9a in mental retardation in mice and humans suggests a key role for the GLP/G9a controlled histone H3K9 di-methylation in regulation of brain function through maintenance of the transcriptional homeostasis in adult neurons.

Disclosures: A. Schaefer, None; **S. Sampath**, None; **A. Tarakhovsky**, None; **P. Greengard**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.5

Topic: F.02. Animal Cognition and Behavior

Support: NSC 96-2314-B-320-003

Title: Behavioral phenotyping and hippocamal gene expression profiling for Cav3.2 knock out mice

Authors: *Y.-C. I. LIU[1], C.-C. CHEN[2], C.-W. SHEN[1], N.-C. CHUNG[1], M.-Y. MIN[3], S.-J. CHENG[3], R. F. THOMPSON[4];
[1]Mol. Biol. and Human Genet., Tzu Chi Univ., Hualien, Taiwan; [2]Academia Sinica, Taipei, Taiwan; [3]Zoology, Natl. Taiwan Univ., Taipei, Taiwan; [4]Neurosci., Univ. Southern California, Los Angeles, CA

Abstract: Voltage-dependent calcium channels trigger many intracellular biochemical events, including muscle contraction, gene expression and secretion of hormones and neurotransmitters. Among all the voltage-gated calcium channels, the α_1H T-type Ca^{2+} channel encoded by the $Ca_v3.2$ gene is highly expressed in the hippocampus, where it is correlated with contextual, temporal and spatial learning/memory. In order to investigate the functional role of the α_1H channel in learning and memory, we performed hippocampal-dependent behavioral tasks including trace fear conditioning, Morris water-maze and passive avoidance on $Ca_v3.2$

homozygous knock-out, $Ca_v3.2$ heterozygous knockout and wild-type mice. Results showed that $Ca_v3.2^{-/-}$ mice are normal in Morris water-maze and auditory trace fear conditioning but impaired in context-cued trace fear conditioning, step-down and step-through passive avoidance. In addition, long term potentiation (LTP) can be induced in hippocampal slices of $Ca_v3.2^{-/-}$ mice but last for 120 minutes only while LTP can last longer 180 minutes in WTs and $Ca_v3.2^{+/-}$ mice. Subsequently, the T-type Ca^{2+} channel blockers, mibefradil, was infused into the 3rd ventricle to locally knock down the function of α_1H T-type Ca^{2+} channel near the hippocampus. We also used expression microarray to establish a differentially expression gene profile for $Ca_v3.2$ knockout mice. Our results suggest that $Ca_v3.2$ encoded T-type calcium channel is required for expressing L-LTP, memory of context-cued trace fear conditioning and passive avoidance.

Disclosures: Y.I. Liu, None; **N. Chung**, None; **C. Shen**, None; **C. Chen**, None; **R.F. Thompson**, None; **M. Min**, None; **S. Cheng**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.6

Topic: F.02. Animal Cognition and Behavior

Title: Lead during postnatal developmental period: Water maze learning and memory, genotoxic, light and electron microscopic study on denate granule cells in rats

Authors: *M. S. RAO[1], S. SMITHA[2];
[1]Dept. of Anatomy, Fac. of Medicine, Kuwait University,Safat, Kuwait, [2]Anatomy, Fac. of Med., Kuwait Univ., Safat, Kuwait

Abstract: Lead, a heavy metal used in several products, particularly in paints, contaminates drinking water and food. Its toxic effects have been shown on liver, brain and spinal cord in developing and adult experimental animals as well as in humans. Though lead even at low doses, was reported to cause learning and memory deficits, it is not known which precise neural substrates are involved and mechanism of such deficit. The present study was aimed to investigate the effects of low doses of lead during postnatal developmental period on water maze learning and memory and correlate with genotoxic effects, cell proliferation, and electron microscopic structure of dentate granule cells. The neonatal rat pups were divided into (i) normal control group (n=18), which were drinking normal water, (ii) Lead group (n=18), which were intubated with lead nitrate solution (125mg/kg/day) for 50 days from birth. The control and treated rats were sacrificed on 57th day, after water maze test from (50th -56th days) for

micronuclei, cell density and electron microscopic study on dentate gyr4366560us. Blood smear was taken at the time of sacrifice for RBC stippling study. Results revealed (i) stippling of RBC suggesting lead toxicity, (ii) deficit in water maze learning and memory, (iii) increased number of micronuclei,(iv) decreased cell density, and (v) pathological features of granule cells at electron microscopic level in lead treated rats. Thus correlation of structural and functional results indicates that, lead induced pathological changes in dentate gyrus of the hippocampus may be one of the bases for lead induced functional deficit.

Disclosures: **M.S. Rao**, ..., Employment; **S. Smitha**, Department of Anatomy, Faculty of Medicine, Kuwait University, P.O. box 24923, Safat,13110 Kuwait, Employment.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.7

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01 NS054911-01A1 from NINDS.

Title: Transformation of inputs in a model of the rat hippocampal CA1 network

Authors: *A. V. OLYPHER[1,2], W. W. LYTTON[2], A. A. PRINZ[1];
[1]Dept. Biol., Emory Univ., ATLANTA, GA; [2]Dept. of Physiol. and Pharmacol., SUNY, Downstate Med. Ctr., Brooklyn, NY

Abstract: Information processing in the hippocampus involves synchronized spiking of subsets of neurons. The functional properties of synchronous spiking are unclear. According to one hypothesis, synchronously spiking neurons form relatively stable assemblies. We used computer simulations and theoretical analyses to study whether such assemblies could result from intrinsic neuronal properties and connectivity patterns alone without synaptic weight tuning. More generally, we assessed conditions under which CA1 principal cells that receive synchronous inputs could generalize similar input patterns and discriminate distinct patterns.
Network effects were simulated by partially overlapped inputs to 23,500 copies of a CA1 principal cell model corresponding to the approximate number of cells in one square millimeter in the CA1 rat hippocampus. Overlap of inputs was based on hippocampal neuroanatomy. Interactions between the CA1 principal cells were not considered. We assumed that all the cells received their excitatory and inhibitory inputs synchronously, as occurs due to strong modulation by ongoing theta and gamma rhythm. Each cell was modeled by biophysically realistic

multicompartment models of reconstructed CA1 principal cells using NEURON.
We characterized the network performance by its response to synchronous inputs within 40 milliseconds. If a modeled CA1 cell spiked within this interval, it contributed “1” to the output of the network, otherwise the cell contributed “0”. In our analyses, we compared the distances between pairs of input patterns and the distances between the corresponding pairs of CA1 activity (output). We performed this analysis for CA3, entorhinal, and mixed input patterns. These data suggests the importance of the input overlap to different CA1 cells on the variability of the CA1 network responses.
Our results provide a benchmark for comparing the CA1 network performance under normal and psychotic conditions, such as in schizophrenia. Revealed changes in the network ability to process similar and distinct inputs in pathological states could underlie cognitive deficits.

Disclosures: A.V. Olypher, None; **A.A. Prinz**, None; **W.W. Lytton**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.8

Topic: F.02. Animal Cognition and Behavior

Support: CELEST, an NSF Science of Learning Center (SBE-0354378)

the SyNAPSE program of DARPA (HR0011-09-3-0001)

Title: From path integration to place cells: Self-organized learning and oscillatory dynamics of hexagonal grid cell maps in the entorhinal cortex

Authors: *H. MHATRE, A. GORCHETCHNIKOV, S. GROSSBERG;
Cognitive and Neural Systems and Ctr. for Adaptive Systems, Boston Univ., BOSTON, MA

Abstract: Grid cells in the dorsal segment of the medial entorhinal cortex (dMEC) show remarkable hexagonal activity patterns during spatial navigation (Hafting et al., 2005). Furthermore, there exists a gradient of spatial scales along the dorsoventral axis of the dMEC, with neighboring cells sharing the same spatial scale but having offset spatial phases while maintaining the same orientation. Past studies have failed to explain why grid cells fire at only hexagonal locations when a rat explores an open field, and how such a structure may rapidly be learned as an animal explores such an environment. The GRIDSmap neural model shows how hexagonal firing fields may be learned by a self-organizing map whose inputs come from

multiple one-dimensional small-scale stripe cells that integrate linear velocity in layer III of dMEC, and whose learned categories are grid cells in layer II of dMEC. These stripe cells are predicted to exist in multiple orientations and phases. GRIDSmap explains how the grid cell firing field learns a hexagonal structure, with the observed phase, orientation and scale properties, based on simple trigonometric properties of spatial navigation, as an animal explores an open environment. A hexagonal grid is learned even when stripe cell orientations differ by 7, 10, 15, 20, 60, or random numbers of degrees that could generate quite different patterns in other models, such as the interference model (Burgess et al., 2007). The GRIDSmap habituative dynamics which control map learning also generate subthreshold membrane potential oscillations that may be used to clarify how experimentally observed oscillations in dMEC layer II stellate cells arise (Hasselmo et al., 2007). Gorchetchnikov and Grossberg (2007) earlier showed how multiple scales of grid cells, through a self-organizing map, can learn to form hippocampal place cell firing fields that are capable of spatial representation on a much larger scale; namely, the least common multiple of the grid cell scales. Taken together, these successive self-organizing maps clarify how vestibular path integration signals may give rise to place fields capable of representing the large spaces that are experienced during spatial navigation.

Disclosures: H. Mhatre, None; **A. Gorchetchnikov**, None; **S. Grossberg**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.9

Topic: F.02. Animal Cognition and Behavior

Support: HHMI

R01-MH78821

Title: Preplay and CA3 NMDAR-mediated plasticity facilitate rapid encoding in the hippocampus

Authors: *G. DRAGOI[1,2], S. TONEGAWA[1,2];
[1]Picower Inst. Learning & Memory, MIT, CAMBRIDGE, MA; [2]RIKEN-MIT Ctr. for Neural Circuit Genetics, MIT, Cambridge, MA

Abstract: We investigated the internal hippocampal network dynamics associated with the formation of novel spatial representations in naïve and experienced adult mice. In naïve animals,

the overall place cell sequence expressed during the first time exploration of a linear track matched the compressed temporal sequence of firing of the same cells during the sleep period preceding the exploration, a phenomenon called preplay. This spatial representation of the novel track, however, was not stable from the beginning to the end of the session and was associated with increased place field size and reduced coordination between overlapping place cells. Re-exploration of the same track after a period of sleep resulted in a stable spatial representation associated with increased spatial tuning and coordination of place cells. This new place cell sequence was replayed during the following sleep session, and the replay was stronger than the preplay recorded during the sleep session preceding the re-exploration. Subsequent exploration of a novel arm in contiguity with the now familiar track resulted in stable spatial representation and increased spatial tuning and coordination of place cells on the novel arm, and increased sleep replay over sleep preplay of the novel place cell sequence. Genetic blockade of CA3 NMDAR-mediated plasticity resulted in slower development of a spatial representation of the first track in naïve animals and elimination of the experience-dependent increase in sleep replay versus sleep preplay. Altogether, these results indicate that prior experience can accelerate encoding of related novel spatial information by facilitating the rapid conversion of preplay temporal sequences into stable spatial sequences and their further consolidation during sleep. Intact CA3 NMDAR-mediated plasticity can accelerate the encoding of unexpected novel spatial information by facilitating the rapid shift of the hippocampal network from an internally-driven into an externally-driven state and the stabilization of the newly-formed representation.

Disclosures: G. Dragoi, None; **S. Tonegawa**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.10

Topic: F.02. Animal Cognition and Behavior

Support: the National Institutes of Health (MH-090258)

James S. McDonnell Foundation

the Lookout Fund

the Kavli Institute for Brain and Mind

Title: Maturation-dependent function for adult-born dentate granule cells in context learning and

context discrimination

Authors: *W. DENG[1], F. H. GAGE[2];
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Abstract: The dentate gyrus of the hippocampus is one of the brain regions where new neurons are continuously born throughout the life of mammals. The function for the addition of new neurons remains elusive. Because the newborn neurons exclusively develop into dentate granule cells, these newborn neurons may have an impact on the function of the dentate gyrus. Here, we developed a context discrimination task in order to test whether newborn neurons are involved in pattern separation. We have previously shown that newborn neurons at different stage of maturation may contribute distinctively to learning and memory by using a Nestin-tk transgenic mouse model. We showed here that adult-born granule cells are involved in context learning and context discrimination depending on their maturation status. Furthermore, the newborn neurons, with enhanced plasticity, were involved in discriminating the closely related contexts but not the drastically different contexts.

Disclosures: W. Deng, None; **F.H. Gage**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.11

Topic: F.02. Animal Cognition and Behavior

Support: NSF grant IOS 0725001 to AF

Title: Investigations into the functional organization of grid cells in the entorhinal cortex and their role in navigation

Authors: *J. L. KUBIE[1], E. PARK[1], A. A. FENTON[3,2];
[1]Cell Biol., SUNY Downstate Med. Ctr., BROOKLYN, NY; [2]physiology and Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [3]Ctr. for Neural Sci., NYU, New York, NY

Abstract: The grid cells of entorhinal cortex (EC) have the remarkable property of exhibiting a regular triangular lattice pattern of spatial firing. Although numerous studies have focused on the role of hippocampal place cells in navigation, we feel there is compelling evidence that the set of place cells is unlikely to be the navigational map. The problem is are that the set of place cells

exhibits remapping in novel environments, which requires learning a new map for each environment. It is also clear that within single environments, place cells exhibit rapid map switching that resembles remapping. Our hypothesis is that grid cells are a universal map, capable of computing directions and distances between any pair of instantaneous grid cell firing vectors, vectors that are tied to specific environmental locations. For any environment the relationship between place cells and grid cells must be learned. After this the distance and direction between a pair of place cell loci can be computed from the grid cell universal map. Preliminary theoretical investigations indicate that potential algorithms for route computation depend on the functional organization of grid cells in EC. In the initial description of grid cells, Hafting et al (Nature, 2005, p801) described a small-to-large gradient of grid scale along the DV axis of medial entorhinal cortex. Although this pattern is accepted, there are two points of dispute about the organization of grid cells. The first question is whether the gradient continuous or organized in discrete steps. The second is whether there is a single angular orientation of the grid pattern for all grid cells in EC with the same spatial scale. Studies by Barry et al (Nat Neurosci, p682 2007) and Haftung et al suggest different answers.
We have begun recording grid cells in a large rectangular enclosure (1.8m x 1.4m) using a 3D tracking system to eliminate optical and parallax distortion. Preliminary findings from 8 cells recorded from one rat support the discrete-step organizational pattern of grid cell scale: 7 of 8 cells had virtually the same scale, while the 8th was larger by a factor of 1.45. Additionally, all 8 cells exhibited virtually identical grid orientations. These findings suggest that each set of grid cells with identical scale form a rigid network in that movement of any direction and distance from one firing vector of grid cells will predict a unique firing vector. Additionally, rigid grid cell sets with different scales permit the use of this system over a large range of distances. This, in turn, permits computation of direction and distance between the firing vectors associated with two familiar locations.

Disclosures: J.L. Kubie, None; **E. Park**, None; **A.A. Fenton**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.12

Topic: F.02. Animal Cognition and Behavior

Title: Computational model and prediction of three-dimensional properties of grid cells in entorhinal cortex

Authors: *T. ISLAM, Y. YAMAGUCHI;

Brain Sci. Institute, RIKEN, Wako-shi, Japan

Abstract: The discovery of grid cells in the entorhinal cortex (EC) of the rat (Hafting et. al, 2005) has provided many hints of the mechanism of spatial computation in brain during animal movement. Since the discovery of grid cells, several computational models have been proposed to explain the functional mechanism of the periodic tessellation of the firing patterns. Observation of the two dimensional hexagonal grid fields generated in rat entorhinal cortex cells raises an obvious question: Do grid fields have three-dimensional properties? What will be the firing field of grid cells during locomotion in three dimensions? Because the natural movement of rat is restricted to mainly two dimensions, there have not been many experimental studies to find out the three dimensional property of grid cells. Few earlier studies found that during movement of a rat on a tilted track, or in a virtually 3D environment in a NASA space shuttle, firing fields of many place cells remap even though the other environmental cue was same. Because place cells receive their major inputs from EC layer II and III grid cells, we hypothesize that grid fields are actually three dimensional, and the two dimensional grid fields that we observe are special cases of a more generalized property of grid cells. It should be noted that by "three-dimension" we mean space that is not a horizontal plane. Therefore, a tilted conic space (used in the related simulations) or a slope is three-dimensional in space in our definition because they are not horizontal planes. We assumed that the animal's sense of a 'tilt-angle', which can be relayed from sensory system to EC, is additionally required in our extended model of three-dimensional grid fields. With these assumptions of the existence of three-dimensional grid fields, we proposed a computational model of 3D grid fields. Based on our model, we have showed some simulation results of possible 3D grid fields of various shapes, considering various possibilities of their layered formation along the 3D space. It is also shown that in case of movement in horizontal plane, our three-dimensional model is reduced to the original two-dimensional model to generate hexagonal grid fields. Without much experimental data to support our hypothesis, and given that the existence of 3D grid fields are yet to be found, we cannot conclude any one of these polyhedral shapes as the most probable one, but we show some comparisons among these possible shapes in terms of change of field shape in accordance with change in slope of space. We believe our model can be helpful to make some predictions on the characteristics of EC grid cells.

Disclosures: T. Islam, None; **Y. Yamaguchi**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.13

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant

NIH Intramural Support

Title: Combined lesions of rostral superior temporal gyrus and rhinal cortex nearly abolish short-term memory in monkeys

Authors: J. B. FRITZ[1], *M. MISHKIN[2], R. C. SAUNDERS[2];
[1]Neural Systems Lab, Inst. for Systems Res., Univ. of Maryland, College Park, MD; [2]NIMH, BETHESDA, MD

Abstract: In order to better understand the neural basis of differences between auditory memory and memory in other sensory modalities observed in human subjects (Cohen et al., 2009), the current study explores auditory memory in a non-human primate model, where such differences have also been shown. Although both visual and tactile memory in the monkey are severely impaired after bilateral ablation of the rhinal cortex (RhC), a previous study showed that such lesions caused no impairment in auditory memory (Fritz et al., 2005). However, this same study did find impairment in auditory memory after bilateral ablation of the rostral superior temporal gyrus (rSTG), a lesion that reduced the monkeys' forgetting thresholds (i.e. the delay between sample and test that yields 75 percent accuracy) from ~35 sec to ~10 sec. We proposed that this loss was due to partial disconnection of prefrontal cortex, particularly its ventral medial region (Munoz et al., 2009), from acoustic input. In the present study we examined the effects on auditory WM of a more complete disconnection of auditory input to the ventral medial frontal cortex by combining the rSTG and lesions of RhC, which also projects to this prefrontal region. Three experimentally naïve rhesus monkeys (Macaca mulatta) were trained, as in the earlier study, to learn the rule for delayed matching-to-sample (DMS) with trial-unique sounds at delays of 2 sec, after which they were tested at longer, variable delays to allow determination of a forgetting threshold, which again reached a mean of ~35 sec. Postoperatively, the bilateral rSTG+RhC lesions resulted in a deficit far more severe than that produced by the rSTG lesion alone. None of the animals performed DMS reliably even after a year of postoperative retraining, and, when given performance tests at variable delays ranging from 0.125 to 8 sec, their mean forgetting threshold was found to have dropped to ~1 sec. The rSTG+Rh lesion appears to have produced this dramatic impairment by depriving the ventral medial frontal cortex of auditory input far more completely than did the rSTG lesion alone, thereby either preventing reliable reacquisition of the DMS rule with trial-unique sounds, or reducing the retention of acoustic stimuli to an echoic type of sensory memory, or both.

Disclosures: J.B. Fritz, None; **R.C. Saunders**, None; **M. Mishkin**, None.

Nanosymposium

635. Cognitive Learning and Memory Systems

Location: Room 7B

Time: Tuesday, November 16, 2010, 1:00 pm - 4:30 pm

Program Number: 635.14

Topic: F.02. Animal Cognition and Behavior

Title: Comparing active and passive visual short term memory in monkeys: Recency versus working memory

Authors: *J. H. WITTIG, Jr., B. J. RICHMOND;
NIMH, BETHESDA, MD

Abstract: We trained six rhesus monkeys to hold a touch bar while watching a sequence of images appear and disappear on a video monitor. They were rewarded at the end of each sequence if they correctly reported (via bar release timing) whether the final image occurred previously in the sequence. Three control monkeys learned the task within seven sessions, performing significantly above chance ($p < 0.01$) with variable sequences ranging from two to eleven images. After training, all monkeys reached steady state performance between 70% and 85% correct for sequences ranging from 2 to 8 images (16 seconds for an 8 image trial). On trials ending in a repeated image, the first presentation of the repeated image could have occurred early or late in the sequence. For all monkeys, the likelihood of correctly identifying a repeat was higher when the first presentation of that image was late in the sequence, i.e., all monkeys exhibited a recency effect.
The recency effect has been ascribed to the finite half-life and capacity of working memory, a form of short term memory attributed to the lateral prefrontal cortex. Three monkeys with symmetric bilateral lesions of the lateral prefrontal cortex readily learned the task, had comparable steady state performance to control monkeys, and exhibited a recency effect. Possibly this task does not depend on working memory, so we trained two monkeys to perform a task thought to require active rehearsal of just the first image in the sequence: delayed-match-to-sample with distractor repeats of non-first images (so called “ABBA” trials). During training, the monkeys had a strong bias towards solving the task by correctly rejecting recently occurring repeat distractors. This apparent strategy suggested they were not actively maintaining the first image, but instead were passively remembering recent images to achieve >80% correct with sequences less than six images long. Both monkeys eventually satisfied rigorous performance metrics indicative of active rehearsal of the first image with sequences ranging from 4 to 8 images. Our results suggest that recency and working memory are mediated by different neural mechanisms in rhesus monkeys.

Disclosures: J.H. Wittig, None; **B.J. Richmond**, None.

Nanosymposium

724. Molecular Regulation of Neural Stem Cells

Location: Room 24A

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 724.1

Topic: A.03. Stem Cells

Support: Howard Hughes Medical Institute

NIH 5DP1 OD00448-04

Title: A universal mechanism of stem cell induction

Authors: *R. A. ROSSELLO[1,2], C.-C. CHEN[3], J. T. HOWARD[3], R. DAI[3], J. TENOR[4], A. ABALLAY[4], U. HOCHGESCHWENDER[3], E. D. JARVIS[3,5];
[1]Jarvis Lab/Duke Univ., Durham, NC; [2]Howard Hughes Med. Institute , Durham, NC; [3]Neurobio., [4]Mol. and Cell Biol., Duke Univ., Durham, NC; [5]Howard Hughes Med. Inst., Durham, NC

Abstract: Stem cells have the potential to develop into any cell type; that is, they are pluripotent. Accordingly, stem cells provide a powerful platform to study development, tissue regeneration, disease mechanisms, and gene therapeutic approaches to the brain and other organs. Recently, induced pluripotent stem cells (iPSC) have been shown to be inducible from adult cells of a few mammalian species using just 4 transcription factors (Oct4, Klf4, Sox2, and c-myc). Creating stem cells in other model organisms besides mammals, such as birds, fish, flies, and worms, could be used for addressing many important issues in biology. Here we demonstrate that these same four transcription factors can induce iPSC in all of these non-mammalian species, and can do so with the mammalian genes (mouse homologs). Similar to induced mouse cells, the transfected avian (chicken, quail, zebra finch) and fish (zebrafish) iPSCs showed a suite of typical stem cell features, which included colonies with characteristic stem cell morphology, self-renewal, immortalized growth, alkaline phosphatase activity, telomerase activity, reprogramming of endogenous stem cell genes, and pluripotency. Pluripotency was demonstrated by the induced cell's ability to differentiate into the three primordial germ layers (ectoderm, mesoderm, and endoderm) in structured embryoid bodies. The fly (drosophila) cells produced activity subset of these features, including morphology, exogenous gene reprogramming and pluripotency. For the worm (C. elegans), we induced a stem-like phenotype within transgenic animals. These findings are the first that we are aware of to generate iPSCs in non-mammalian species, the first pluripotent stem cells isolated for songbirds and drosophila, and stem-like cells for C. elegans. The findings suggests a highly conserved, universal mechanism for reprogramming cells to a primordial stem-cell state across a phylogenetically diverse range of species spanning at least 600 million from their common ancestor. We anticipate that this universal principle can be used to generate a valuable stem cell resource for neuroscientists to

create in-vitro neural disease models and small tractable screens in systems that are either easier to study or that have similar traits to humans (such as vocal learning in songbirds).

Disclosures: R.A. Rossello, None; **C. Chen**, None; **J.T. Howard**, None; **R. Dai**, None; **J. Tenor**, None; **A. Aballay**, None; **U. Hochgeschwender**, None; **E.D. Jarvis**, None.

Nanosymposium

724. Molecular Regulation of Neural Stem Cells

Location: Room 24A

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 724.2

Topic: A.02. Neurogenesis and Gliogenesis

Title: The brahma related gene 1 (Brg1)-containing chromatin remodelling complex interacts with the transcription factor Pax6 to maintain the neurogenic potential of adult neural progenitors

Authors: *J. NINKOVIC[1,2], M. JAWERKA[2], L. BEILSCHMIDT[1], R. RUTH ASHERY-PADAN[3], M. GÖTZ[1,2];
[1]Helmholtz Zentrum München, Neuherberg, Germany; [2]Inst. of Physiology, Physiological Genomics, Ludwig-Maximilians Univ. Munich, Munich, Germany; [3]Human Genet. Fac. of Med., Tel Aviv Univ., Tel Aviv, Israel

Abstract: Neural stem cells (NSC) persist in the adult subependymal zone life-long and have the capacity to produce both olfactory bulb (OB) interneurons and oligodendrocytes of the corpus callosum. The intrinsic fate determinants Pax6 or Olig2 influence this fate decision and are regulated by extrinsic signals, such as BMP inhibiting Olig2 expression (Colak et al., 2008; Jablonska, et al, 2010). However, the molecular mechanisms of how Pax6 endows cells with a neurogenic fate are still elusive. Here we show that Brg1, an ATP-dependent chromatin remodelling factor, is required for the neurogenic fate maintenance. In the absence of Brg1 function, adult-generated neuroblasts convert to glial cells expressing the hallmarks of glial progenitors such are NG-2 and Olig2. We further showed that Brg1 directly interacts with Pax6 in the migrating neuroblasts at the late stage of their differentiation. Similar to Brg1 loss-of-function, the loss of Pax6 in neuroblasts, even when already migrating along the rostral migratory stream (RMS) towards the OB, results in the fate conversion of these neuroblasts towards an NG2+ glia fate. Interestingly, Pax6 expression is not altered in the Brg1 mutant and Pax6 can not mediate neurogenesis in the absence of Brg1. These data suggest that Brg1 and Pax6 form a functional complex necessary for the maintenance of neuroblast fate in the adult RMS. We shall further present data demonstrating a transient role of this complex in neuronal differentiation, as this complex is no longer needed in mature OB neurons, such as the

dopaminergic periglomerular neurons that do not alter their identify upon loss of either Pax6 or Brg1. In summary, our data reveal the functional complex of the transcription factor Pax6 with the Brg1-containin chromatin remodelling machinery as a key mechanism to maintain neuronal fate in migrating neuroblasts, thereby identifying for the first time a mechanism necessary for the active maintenance of commitment in adult neurogenesis.

Disclosures: J. Ninkovic, None; **M. Jawerka**, None; **L. Beilschmidt**, None; **R. Ruth Ashery-Padan**, None; **M. Götz**, None.

Nanosymposium

724. Molecular Regulation of Neural Stem Cells

Location: Room 24A

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 724.3

Topic: A.03. Stem Cells

Support: NIH Grant R01 NS059546

Title: MicroRNA let-7b regulates neural stem cell fate determination by targeting nuclear receptor TLX signaling

Authors: *C. ZHAO[1], G. SUN[2], S. LI[2], M.-F. LANG[2], S. YANG[2], W. LI[2], Y. SHI[2];
[1]California State Univ. Channel Islands, Thousand Oaks, CA; [2]Neurosciences, Beckman Res. Inst. of City of Hope, Duarte, CA

Abstract: MicroRNAs have emerged as central post-transcriptional negative regulators and have been implicated in a wide array of biological processes including cell cycle control, proliferation, and differentiation. Let-7b, a member of the let-7 microRNA family, is expressed in mammalian brains and exhibits increased expression during neural differentiation. Here we show that let-7b regulates neural stem cell proliferation and differentiation by targeting the stem cell regulator TLX and the cell cycle regulator cyclin D1. Overexpression of let-7b led to reduced neural stem cell proliferation and increased neural differentiation, whereas antisense knockdown of let-7b resulted in enhanced proliferation of neural stem cells. Moreover, in utero electroporation of let-7b to embryonic mouse brains led to reduced cell cycle progression in neural stem cells. Introducing an expression vector of Tlx or cyclin D1 that lacks the let-7b recognition site rescued let-7b-induced proliferation deficiency, suggesting that both TLX and cyclin D1 are important targets for let-7b-mediated regulation of neural stem cell proliferation. Let-7b, by targeting TLX and cyclin D1, establishes an efficient strategy to control neural stem cell proliferation and differentiation.

Disclosures: C. Zhao, None; **G. Sun**, None; **S. Li**, None; **M. Lang**, None; **W. Li**, None; **S. Yang**, None; **Y. Shi**, None.

Nanosymposium

724. Molecular Regulation of Neural Stem Cells

Location: Room 24A

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 724.4

Topic: A.03. Stem Cells

Support: R-181-000-130-720

Title: Analysis of epigenetic factors in mouse embryonic neural stem cells exposed to different glucose concentrations

Authors: *S. SHYAMA SUNDAR[1,2], B. BOON HUAT[3], S. TAY SAM WAH[3], D. RANGASAMY[4], T. DHEEN[3];
[1]SINGAPORE, Singapore; [2]Anat., Natl. Univ. of Singapore, Singapore, Singapore; [3]Natl. Univ. of Singapore, SINGAPORE, Singapore; [4]Australian Natl. Univ., Canberra, Australia

Abstract: Maternal diabetes has been shown to induce patterning defects in the developing brain during embryogenesis which subsequently induced impairment of memory performance in adults. We therefore hypothesized that maternal diabetes alters the epigenetic mechanisms (namely, histone modifications, DNA methylation and microRNA mediated) and expression of genes involved in various signalling pathways resulting in brain defects in embryos of diabetic mice. To verify our hypothesis, we have used mouse embryonic neural stem cells (NSCs) isolated from E13.5 embryos of normal and diabetes-induced Swiss Albino mice as an *in vitro* model and analyzed the epigenetic factors and expression of genes involved in neurogenesis in NSCs exposed to different glucose concentrations. Transmission electron microscopy revealed chromatin re-organization in NSCs exposed to high glucose concentrations *in vitro* and *in vivo*. Based on bioinformatics prediction using microarray data derived from the brains of embryos from normal and diabetic pregnancy, a number of microRNAs (miRNAs) that target on genes involved in neurogenesis, neuronal differentiation, and neuronal migration were identified. The quantitative real time RT-PCR analysis showed that high and low glucose concentrations altered the expression levels of some of the miRNAs and their predicted target genes in NSCs. The miRNA expression in NSCs was further studied by *in situ* hybridization. Histone acetylation and methylation patterns and DNA methylation of selected genes appeared to vary with the different glucose concentrations in NSCs as revealed by immunocytochemistry and chromatin

immunoprecipitation assay. The findings would provide novel insight into how maternal hyper- or hypo-glycaemia influence the fate of stem cells during development and also ascertain possible therapeutic strategies to prevent functional disturbances in the brain of infants exposed to maternal glucotoxicity or glucose deprivation.

Disclosures: S. Shyama sundar: Employment; National University of Singapore. Research Grant; R-181-000-130-720. **B. Boon Huat:** None. **S. Tay Sam Wah:** None. **D. Rangasamy:** None. **T. Dheen:** None.

Nanosymposium

724. Molecular Regulation of Neural Stem Cells

Location: Room 24A

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 724.5

Topic: A.03. Stem Cells

Support: Spina Bifida Association

Spastic Paralysis Research Foundation of Illinois-Eastern Iowa District of Kiwanis

Title: Role of C-terminal lysine residues of Pax3 in stem cell maintenance and differentiation

Authors: *C. K. MAYANIL[1], S. ICHI[1], V. BOSHNJAKU[1], B. MANIA-FARNEL[1,2], N. MANSUKHANI[1,3], D. G. MCLONE[1], T. TOMITA[1];
[1]Dev Biol, CMRC, CHICAGO, IL; [2]Dept. of Biol. Sci., Purdue Univ. Calumet, Hammond, IN; [3]Univ. of Illinois, Urbana-Champagne, IL

Abstract: Neuronal differentiation of dorsal root ganglion cells is preceded by down-regulation of Pax3 and vice versa. Although the mechanism of Pax3 down-regulation via ubiquitination and degradation is extensively studied, very little is known about how Pax3 activates a differentiation program prior to getting down-regulated. Here we report that lysine residues K437 and K475 are responsible for Pax3 regulation of Hes1 and Neurog2 activity. Removal of these lysine residues of Pax3 caused an increase in Hes1 but a decrease in Neurog2 promoter activity. Chromatin immunoprecipitation assays showed that SIRT1 is associated with Hes1 and Neurog2 promoter during early embryonic caudal neural tube development (E9.5), a period of active stem cell proliferation and not during later development (E12.5), a period which marks the beginning of neurogenesis. Over-expression of SIRT1 resulted in a decrease in acetylation of Pax3 and decreased Brn3a positive staining and neurogenesis. Conversely, siRNA-mediated silencing of SIRT1 caused an increase in acetylation of Pax3, increase in Brn3a positive staining and

increased neurogenesis. Taken together, these studies show that the C-terminal lysine residues of Pax3 upon acetylation decrease the stem cell proliferation and maintenance by down-regulating Hes1 activity and promote sensory neuron differentiation by increasing Neurog2 activity.

Disclosures: **C.K. Mayanil:** Research Grant; Spina Bifida Association, Spastic Paralysis Research Foundation of Illinois-Eastern Iowa District of Kiwanis. **S. Ichi:** None. **V. Boshnjaku:** None. **B. Mania-Farnel:** None. **N. Mansukhani:** None. **D.G. McLone:** None. **T. Tomita:** None.

Nanosymposium

724. Molecular Regulation of Neural Stem Cells

Location: Room 24A

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 724.6

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH grant RO1NS022518

HHMI-to Gail Mandel

Title: Conditional loss of REST in mammalian brain reveals its role as a differentiation timer and uncovers a p53-dependent check mechanism for precocious differentiation

Authors: *T. NECHIPORUK[1], T. FLOSS[2], J. MILLER[3], P. MICHA[3], G. MANDEL[3]; [1]Oregon Hlth. and Sci. University, Vollum Institute, HHMI, Portland, OR; [2]Inst. of Developmental Genet., Helmholtz Ctr. MunichInstitute of Developmental Genet., Neuherberg, Germany; [3]Vollum Inst., Portland, OR

Abstract: REST is the master repressor of a large network of neuronal genes in non-neuronal cells, including neural progenitors. As embryonic stem cell/neural progenitors differentiate into neurons, REST is down-regulated concomitant with the acquisition of a terminally differentiated neuronal phenotype and increased expression of neuronal genes. These observations predict that early loss of REST in progenitors would cause precocious neuronal differentiation. To test this idea, we generated mouse lines carrying REST Gene Trap allele, $Rest^{Gt(D047E11)Wrst/+}$ in non-conditional and conditional state. To generate a conditional knockout of REST in neural progenitors we crossed mice with conditional REST Gene trap allele to mice transgenic for Cre recombinase under Nestin promoter. Unlike non-conditional $Rest^{Gt(D047E11)Wrst/\ Gt(D047E11)Wrst}$ mice, which were embryonic lethal, more than 50% of Nestin CRE $Rest^{Gti(D047E11)Wrst\ /\ Gti(D047E11)Wrst}$ mice survived into adulthood. Conditional REST knockout mice exhibited fully penetrant

phenotypes of a smaller brain size, abnormal cortex histogenesis, and dysgenesis of corpus callosum. During embryogenesis, some of the cortical progenitors exited the cell cycle precociously and differentiated into early-born (layer V and VI) neurons, reducing the number of late-born (layers IV-II) neurons. The early cell cycle exit likely explained a depletion of stem cells and progenitors and led to a significant decrease in proliferating cells in both the developing brain and subventricular neurogenic region of the adult brain. Numbers of postnatal gliogenic GFAP-positive cells were also reduced. Loss of REST from embryonic neural progenitors activated widespread p53-dependent apoptosis. In double mutants, Nestin CRE $Rest^{Gt(D047E11)Wrst/Gt(D047E11)Wrst}$; $p53^{fl/fl}$ mice, loss of p53 completely rescued apoptosis in developing brain, but only partially rescued the small brain size, and did not prevent precocious up regulation of neuronal genes or premature cell cycle exit, suggesting that these are REST-specific functions. Taken together, our results point to REST as a crucial timer of neuronal differentiation and expose a p53-dependent surveillance mechanism that eliminates cells with premature expression of neuronal genes.

Disclosures: T. Nechiporuk, None; **T. Floss**, None; **J. Miller**, None; **P. Micha**, None; **G. Mandel**, None.

Nanosymposium

724. Molecular Regulation of Neural Stem Cells

Location: Room 24A

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 724.7

Topic: A.02. Neurogenesis and Gliogenesis

Support: Reproductive Biology Training Grant

NIH grant NS042205

Title: Sonic hedgehog signaling is required for the expansion of multipotent progenitors in the postnatal cerebellar white matter

Authors: *J. T. FLEMING, C. CHIANG;
Cell and Developmental Biol., Vanderbilt Univ., Nashville, TN

Abstract: Table of Contents

- The Sonic Hedgehog (Shh) signaling pathway is essential in vertebrate development. This is illustrated in the cerebellum, a hindbrain region required for coordinated

movement and cognition. Cerebellar neurons originate from two germinal neuroepithelia, the ventricular zone (VZ) and rhombic lip (RL). During development, granule precursors of glutamatergic neurons generated from the RL proliferate in response to Purkinje-derived Sonic hedgehog (Shh) during late embryogenesis and the early postnatal period. Aberrant Shh pathway activation in mice results in medulloblastoma (MB), a malignant cerebellar tumor, which is thought to stem from deregulated granule precursor proliferation. In contrast to glutamatergic neurons, GABAergic interneurons are generated from Sox2+ multipotent progenitors, situated in the embryonic VZ and in the postnatal white matter. However, the signaling mechanism that maintains the proliferative capacity of Sox2+ progenitors in the white matter remains elusive. Here, we show that mutants deficient in primary cilia, a cellular organelle required for Shh signaling, exhibit severely reduced proliferation of Sox2+ progenitors and a concomitant deficit in interneuron progenitor cell number in early postnatal stages. Through inducible Gli1 fate mapping studies, we showed that Sox2+ progenitors are Shh responsive and conditional deletion of Shh pathway activity in Sox2+ progenitors during postnatal stages lead to significant lost of interneuron progenitors. Collectively, the results are the first to highlight a novel function for Shh signaling in postnatal cerebellum development and provide insight into the poorly understood process of cerebellar interneuron neurogenesis. Ongoing experiments are directed at establishing the local source for the Shh signal targeting white matter progenitors.

Disclosures: J.T. Fleming, None; **C. Chiang**, None.

Nanosymposium

724. Molecular Regulation of Neural Stem Cells

Location: Room 24A

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 724.8

Topic: A.02. Neurogenesis and Gliogenesis

Support: JSPS postdoc fellowship (M.M.)

NIA Grant AG20603 (T.W.-C.)

NIA Grant AG27505 (T.W.-C.)

Title: Complement receptor 2 is expressed in neural progenitor cells and regulates adult hippocampal neurogenesis

Authors: *T. FUKUHARA[1,2], M. MORIYAMA[2], M. BRITSCHGI[2], R. NARASIMHAN[2], S. A. VILLEDA[2], H. D. MOLINA[3], M. HOLERS[4], T. WYSS-CORAY[2,5];
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Abstract: Injury and inflammation are potent regulators of adult neurogenesis. The complement system forms a key immune pathway activated in injury and inflammation and may thus have a role in neural development and neurodegeneration. Most recently, the human complement receptor 1 (CR1) locus was linked to Alzheimer`s disease in a large genome wide association study, although the biological role of CR1 in that disease, or in the brain in general, is unknown. To address whether complement receptor(s) have a role in adult neurogenesis in vivo, we asked if murine complement receptor 2 (Cr2), a homologue of human CR1, regulates neurogenesis. We discovered that Cr2, classically known as a co-receptor of the B lymphocyte antigen receptor, is expressed in adult neural progenitor/stem cells (NPCs) of the dentate gyrus. Lineage tracing studies, using Cr2-Cre and GFP reporter (Rosa26-lox-STOP-lox-EGFP) mice, revealed that GFP positive cells in the dentate gyrus express both GFAP and Sox2 characteristics of radial glial stem cells. Indicative of functional Cr2 expression, two of its ligands, C3d and interferon-α (IFN-α), inhibited proliferation of wildtype NPCs but not NPCs derived from mice lacking Cr2 (Cr2-/-) in vitro. Furthermore, the decrease in proliferation seen in wildtype NPCs was reversed by the addition of antibody 7G6, which blocks ligand binding to the Cr2 gene product. Interestingly, young and old Cr2-/- mice exhibited 2-3 fold increases in basal neurogenesis compared with wildtype littermates and also a roughly 40% increase in mature neurons in the young adult hippocampus, while intracerebral injection of C3d reduced neurogenesis in wildtype but not Cr2-/- mice. We also analyzed neurogenesis in mice deficient in Cr3, a major phagocyte receptor restricted to microglia and macrophages in the brain, or in crosses resulting from these mice and lacking both, Cr2 and Cr3. Mice deficient in Cr3 showed no changes in neurogenesis although mice lacking Cr2 and Cr3 showed equal increases in neurogenesis. Summarizing these results, we conclude that Cr2 regulates hippocampal neurogenesis and propose that increased C3d and IFN-α production associated with brain injury or viral infections may inhibit neurogenesis.

Disclosures: T. Fukuhara, None; **M. Moriyama**, None; **M. Britschgi**, None; **R. Narasimhan**, None; **S.A. Villeda**, None; **H.D. Molina**, None; **M. Holers**, None; **T. Wyss-Coray**, None.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.1

Topic: C.02. Alzheimer's disease and other dementias

Support: T.L.L. Temple Foundation Discovery Award from the Alzheimer's Association

Title: Convergence of insulin signaling pathway abnormalities on insulin receptor substrate-1 (IRS-1) may mediate effects of amyloid beta (Aβ) oligomers on episodic memory in mild cognitive impairment (MCI) and Alzheimer's disease (AD)

Authors: *K. TALBOT[1], H. KAZI[2], L.-Y. HAN[2], J. A. SCHNEIDER[3], R. S. WILSON[3], Z. ARVANITAKIS[3], D. A. BENNETT[3], J. Q. TROJANOWSKI[4], B. A. WOLF[5], S. E. ARNOLD[2];
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Abstract: An increasing number of studies find that type 2 diabetes is a risk factor for AD and that the two disorders share pathophysiogical features, including alterations in levels and activation states of insulin signaling molecules consistent with insulin resistance. It is unknown, however, what causes these alterations and if they actually impair neuronal insulin signaling in AD. To address these questions, we studied five levels of the insulin signaling pathway (i.e., insulin receptor, IRS-1, Akt, and mTOR) and its regulatory kinases (e.g., IKK, JNK, and PKC ζ/λ) in hippocampal tissue of non-cognitively impaired (NCI, n = 61), MCI (n = 29), and AD (n = 62) cases from the University of Pennsylvania and the Religious Orders Study at Rush University. Quantitative immunohistochemistry with phosphospecific antibodies was used to screen for neuron-specific abnormalities in insulin signaling and regulatory molecules. Ex vivo stimulation was then used to test the responsiveness of normal and AD hippocampal tissue to insulin (1 and 10 nM) as described by Hoau-Yan Wang et al. at this meeting. The most consistent and striking changes across diagnostic groups were step-wise increases from NCI to MCI to AD in density of hippocampal CA1 neurons with extra-nuclear levels of IRS-1 phosphorylated at serines (pS) 312, 636, and especially 616 ($p < 3 \times 10\text{-}12$). These NCI to AD changes were accompanied by upstream reductions in activated forms of neuronal insulin receptors ($p < 1.0 \times 10\text{-}5$) and downstream increases in density of neurons with detectable, activated forms of molecules promoting IRS-1 pS, namely Akt ($p < 4.5 \times 10\text{-}7$), IKK ($p < 0.0001$), JNK ($p < 0.0001$), mTOR ($p < 0.0001$), and PKC ζ/λ ($p < 0.0001$). Diverse abnormalities in AD thus appear to converge on IRS-1, serine phosphorylating it and thereby inhibiting insulin signaling. This may mediate effects of Aβ oligomers (Aβo) on cognition since (a) such oligomers induce IRS-1 pS, (b) the density of CA1 neurons with extra-nuclear IRS-1 pS is correlated positively with Aβo plaque load ($p < 0.001$) and negatively with episodic memory ($p < 0.001$), and (c) the

correlation of Aβo plaque load with such memory loses significance after controlling for density of neurons with extra-nuclear IRS-1 pS, which remains significantly correlated with episodic memory ($p < 0.0001$) after controlling for age, sex, educational level, Aβo plaque load, and density of neurobrillary tangles.
In conjunction with our ex vivo stimulation results presented separately at this meeting, these findings support the view that impaired insulin signaling is a common feature of AD closely related to its episodic memory deficits.

Disclosures: K. Talbot: None. **H. Kazi:** None. **L. Han:** None. **J.A. Schneider:** None. **R.S. Wilson:** None. **Z. Arvanitakis:** None. **D.A. Bennett:** None. **J.Q. Trojanowski:** None. **B.A. Wolf:** None. **S.E. Arnold:** None.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.2

Topic: C.02. Alzheimer's disease and other dementias

Title: Ex vivo demonstration of impaired insulin signaling in the hippocampal formation of Alzheimer’s disease (AD) cases

Authors: *H.-Y. WANG[1], K. TALBOT[2], A. STUCKY[1], J. Q. TROJANOWSKI[3], S. E. ARNOLD[2], J. A. SCHNEIDER[4], R. S. WILSON[4], Z. ARVANITAKIS[4], D. A. BENNETT[4], K. BAKSHI[1];
[1]Dept Physiol & Pharmacol, CUNY Med. Sch., NEW YORK, NY; [2]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA; [3]Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA; [4]Alzheimer's di. Ctr., Rush Univ., Chicago, IL

Abstract: Several discoveries over the last decade have led to the proposal that brain insulin resistance promotes development of AD (e.g., de la Monte, BMB reports 42: 475-481, 2009). It has been established, for example, that type 2 diabetes is a risk factor for AD and that abnormalities in the activation states of diverse insulin-signaling molecules, especially insulin receptor substrate-1, are common in the brains of those with the disorder as reported at this meeting by Talbot et al. Yet the hypothesis that brain insulin signaling is impaired in AD cases has not been tested directly. To do so, we used a validated ex vivo stimulation method (Wang et al., J. Neurosci. 29: 10961-10973, 2009) to test responses of hippocampal formation (HF) tissue from normal and AD cases to insulin doses low enough (1 and 10 nM) to activate insulin, not IGF-1, receptors. Tissue

was obtained from brain banks of Rush University and the University of Pennsylvania. Normal (n = 6) and AD (n = 6) cases were matched for sex and age (within 5 y) and had low postmortem intervals (PMI, mean ± SD = 6.0 ± 2.6 h).
The integrity of the insulin signaling pathway in the postmortem tissue was established in pilot tests on normal HF tissue, which showed that ex vivo stimulation with 0, 0.1, 1, 10, and 100 nM insulin caused increasing levels of activated IRβ, IRS-1 recruited to IRβ, Akt1(pS473), and ERK2(pY204). Reliably strong activation was obtained with 1-10 nM doses. Using those doses, ex vivo tests on the 6 pairs of normal and AD cases showed that while insulin stimulated phosphorylation of the catalytic (Y1162+1163) and IRS-1 binding (Y972) sites in IRβ in both diagnostic groups ($p<0.01$), the average level of such tyrosine phosphorylation per IRβ molecule was lower in AD ($p<0.01$). Reduced IRβ (Y972) was associated with attenuated insulin-induced IRS-1 recruitment in AD cases ($p < 0.01$). As expected given these findings, downstream responses to insulin stimulation at 1 and 10 nM were blunted, as shown by lesser increases in activated levels of Akt1 (S473), mTOR (S2448), and ERK2 (Y204). The activated forms of these downstream molecules were nevertheless higher in the absence of insulin stimulation (i.e., in their basal state), as were levels of IRS-1 pS312 and pS636, which may reflect other factors (e.g., NMDAR activation) driving downstream portions of the insulin signaling pathway feeding back upon IRS-1. These results provide direct evidence that insulin signaling is impaired in AD brains and that such signaling can be studied experimentally in human brain tissue from cases with low PMI, providing an assay system for testing causes and treatments of insulin abnormalities in this disorder.

Disclosures: H. Wang: None. **K. Talbot:** None. **A. Stucky:** None. **J.Q. Trojanowski:** None. **S.E. Arnold:** None. **J.A. Schneider:** None. **R.S. Wilson:** None. **Z. Arvanitakis:** None. **D.A. Bennett:** None. **K. Bakshi:** None.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.3

Topic: C.02. Alzheimer's disease and other dementias

Support: Human Frontier Science Program (HFSP)

FAPERJ

CNPq

Title: Alzheimer's toxic Aβ oligomers trigger insulin resistance by mechanisms common to type 2 Diabetes

Authors: *F. G. DE FELICE[1], T. R. BOMFIM[1], H. DECKER[1], M. SILVERMAN[2], K. TALBOT[3], W. L. KLEIN[4], S. T. FERREIRA[1];
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Abstract: Alzheimer's disease (AD) has been linked to impaired brain insulin signaling, a novel type of brain diabetes. Serine phosphorylation of IRS-1 (IRS-1pSer), a central feature in peripheral insulin resistance, blocks insulin signaling. Here we show that AD brains present elevated IRS-1pSer$^{636/639}$ levels, reminiscent of what is found in muscle and adipose tissue in type 2 diabetes. To determine the mechanism underlying pathological IRS-1pSer, we investigated the role of synaptotoxic Aβ oligomers, increasingly recognized as AD pathogenic agents and recently implicated in neuronal insulin resistance. Oligomers induced IRS-1pSer^{636} and inhibited physiological IRS-1pTyr^{465} in mature hippocampal neurons in culture. IRS-1pSer was blocked in neurons expressing a dominant negative form of c-Jun N-Terminal Kinase (JNK) and by the JNK inhibitor, SP600125, as well as by infliximab, a tumor necrosis factor-α (TNF-α) blocking antibody. Involvement of JNK and TNF-α in oligomer-induced neuronal IRS-1pSer parallels the pathway underlying peripheral insulin resistance. Consistent with aberrant activation of JNK, SP600125 blocked oligomer-induced disruption of axonal transport, a defect linked to JNK dysregulation in neurodegenerative diseases. Insulin and exendin-4, drugs used to treat diabetes, blocked oligomer-induced pathologies. By establishing a molecular link between AD-dysregulated insulin signaling and diabetes, results open new avenues for rapid implementation of therapeutics in AD.

Disclosures: F.G. De Felice: None. **T.R. Bomfim:** None. **H. Decker:** None. **M. Silverman:** None. **K. Talbot:** None. **W.L. Klein:** None. **S.T. Ferreira:** None.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.4

Topic: C.02. Alzheimer's disease and other dementias

Support: Alzheimer Society UK

Alzheimer Research Trust UK

Title: Novel GLP-1 analogues developed to treat type 2 diabetes are neuroprotective in an APP/PS1 model of Alzheimer's disease

Authors: *C. HOLSCHER;
Sch. Biomed Sci., Univ. Ulster, Coleraine, United Kingdom

Abstract: Recent research has shown that type 2 diabetes (T2DM) is a risk factor for Alzheimer's disease (AD). The underlying link may be that insulin receptors in the brains of AD patients desensitize. Insulin acts as a growth factor in the brain and has neuroprotective properties. The incretin hormone Glucagon-like peptide-1 (GLP-1) normalizes insulin signaling in T2DM by facilitating insulin release and re-sensitizing insulin receptors. It has also been shown that GLP-1 has neuroprotective properties, increases neuroneogenesis, reduces apoptosis and increases dendritic growth. Novel protease-resistant GLP-1 analogues have been developed as treatments for T2DM. We have tested several GLP-1 agonists in animal models of AD which express the Swedish mutation of amyloid precursor protein and a mutation of human presenilin-1. We found that GLP-1 analogues such as Liraglutide or Val(8)GLP-1 cross the blood brain barrier and protect cognitive abilities in APP/PS1 mice, prevent the amyloid plaque- dependent impairment in synaptic plasticity, reduce plaque numbers and ß-amyloid levels, and reduce the inflammation response in the brain. Since Liraglutide (Victoza) is currently on the market as a treatment for type 2 diabetes and has shown few side effects in chronic use, the use of such GLP-1 analogues as a treatment for Alzheimer's disease is most promising. We also showed that GLP-1 receptors are expressed in pyramidal neurons in the cortex and hippocampus, and that GLP-1 receptor KO mice are impaired in learning tasks and in synaptic plasticity in the hippocampus. Furthermore, GLP-1 analogues increase the proliferation of neuroprogenitor cells in the dentate gyrus region of the brain and also increase the numbers of new neurons together. These results show that GLP-1 is a neuropeptide that acts as a neuroprotective growth factor and as a neurotransmitter, and that activation of GLP-1 receptors in the brain have important protective effects in the brain. Further development of GLP-1 agonists as treatments for AD or other neurodegenerative diseases appears to be a promising strategy.

Disclosures: C. Holscher: Research Grant; Alzheimer Research Trust UK project grant.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.5

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH grant AG030942

NIH grant AG19740

Alz Assn grant IIRG-07-59802

Alz Assn grant NIRG-09-133302

NIH grant AG18478

Title: Anti-Aβ immunotherapy dramatically alters the inflammatory state of the brain and activates matrix metalloproteinases; implications for vascular adverse events

Authors: *D. M. WILCOCK[1], D. MORGAN[2], M. N. GORDON[2], M. P. VITEK[1], C. A. COLTON[1];
[1]Med. / Neurol., Duke Univ. Med. Ctr., DURHAM, NC; [2]Univ. of South Florida, Tampa, FL

Abstract: Anti-Aβ immunotherapy is a promising approach to the prevention and treatment of Alzheimer's disease (AD). There is extensive evidence, both in mice and humans that a significant adverse event is the occurrence of microhemorrhages. In order to overcome these vascular adverse effects it is critical that we understand the mechanism(s) by which they occur in order to circumvent such events. We have now characterized the inflammatory response to immunotherapy in both passively immunized APPSw mice and actively vaccinated APPSw/NOS2-/- mice. APPSw transgenic mice have only amyloid pathology while APPSw/NOS2-/- transgenic mice show amyloid pathology plus disease progression to tau pathology and neuron loss.
APPSw transgenic mice have primarily an alternative inflammatory state in the brain. This state involves markers such as arginase 1, YM1 and IL-4. Importantly, levels of classical inflammatory genes such as TNFα, IL-1β and IL-6 are just in the detectable range, with only a slight increase compared to wildtype mice. APPSw/NOS2-/- mice show a mixed inflammatory response with high alternative and classical inflammatory genes. Following treatment with ant-Aβ immunotherapy, the inflammatory state shifts. Alternative activation genes, normally high, are reduced significantly, even lower than wildtype mice in some cases. Concomitantly, classical activation genes appear to increase transiently during the same time periods that we observe significant amyloid reductions. We have previously shown that microglial activation significantly, albeit transiently, increases in response to both active vaccination and passive immunization. We are now

more closely characterizing this inflammatory response in order to understand the mechanisms of both amyloid reductions and increased vascular events. The shift in the inflammatory state is likely key to the removal of amyloid from the brain.
Along with this shift in inflammatory state is also an activation of the matrix metalloproteinase (MMP) degradation system. This includes increased expression of MMP9, MMP2 and MMP14, along with a concomitant reduction in the TIMP1 metalloproteinase inhibitor. Zymogram data to indicate that the activity of these proteinases is also increased by immunotherapy. The MMP system is heavily implicated in the pathophysiology of intracerbral hemorrhage, and may therefore provide a potential mechanism of microhemorrhage due to immunotherapy. These data show that immunotherapy, regardless of active or passive means of administration, results in a dramatic shift in inflammatory profiles and increased activity of the MMP system.

Disclosures: D.M. Wilcock, None; **D. Morgan**, None; **M.N. Gordon**, None; **M.P. Vitek**, None; **C.A. Colton**, None.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.6

Topic: C.02. Alzheimer's disease and other dementias

Support: Biostar/Discovery

Cure Alzheimer’s Fund

Title: Non human amyloid oligomer epitope reduces Alzheimer’s-like neuropathology in 3xTg-AD transgenic mice

Authors: *S. RASOOL[1], H. M. CORIA[2], L. BREYDO[1], J. WU[1], S. MILTON[1], A. TRAN[1], R. ALBAY[1], C. G. GLABE[1];
[1]Mol. Biol. & Biochem., Univ. California, Irvine, CA; [2]Neurol., Memory impairment and Neurolog. disorders, Irvine, CA

Abstract: Accumulation of beta-amyloid (Aß) is an important molecular event in Alzheimer’s disease (AD). It is now well known that vaccination against fibrillar Aß prevents amyloid accumulation and preserves cognitive function in transgenic mouse models. To study the effect of vaccination against generic oligomer epitopes, Aß

oligomers, islet amyloid polypeptide (IAPP) oligomers, random peptide oligomer (3A) & Aß fibrils were used to vaccinate 3xTg-AD, which develop a progressive accumulation of plaques and cognitive impairment. It was found that all vaccinated mice have a significant improvement in cognitive function compared to controls. Subcutaneous administration of these antigens markedly reduced total plaque load (Aβ burden) in the 3xTg-AD mouse brains. We demonstrated that vaccination with this non human amyloid oligomer generated high titers of oligomer specific antibodies recognizing Aβ oligomers, which in turn inhibited accumulation of Aß pathology in mice. In addition to amyloid plaques, another hallmark of AD is tau pathology. It was found that there was a significant decline in the levels of total tau and hyperphosphorylated tau**.** We conclude that amyloid Aß sequence is not necessary to produce a protective immune response as the random peptide (3A) gives rise to an oligomer-specific immune response. The critical epitope is a pathology-specific conformation of the peptide backbone that is independent of the specific amino acid sequence. It is therefore suggested that vaccination against a non-human amyloid oligomer epitope may be useful for clearing both hallmark lesions of AD. It may be an effective strategy for developing a vaccine that circumvent auto-inflammatory immune complications.

Disclosures: S. Rasool: None. **H.M. Coria:** None. **L. Breydo:** None. **J. Wu:** None. **S. Milton:** None. **A. Tran:** None. **R. Albay:** None. **C.G. Glabe:** Consultant/Advisory Board; Kinexis Inc..

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.7

Topic: C.02. Alzheimer's disease and other dementias

Title: Progranulin-targeting by ND-602 reduces plaque burden in a mouse model of Alzheimer’s disease

Authors: *J. M. VAN KAMPEN, D. KAY;
Neurodyn Inc., Charlottetown, PE, Canada

Abstract: Progranulin (PGRN) is a multifunction protein expressed by neurons and microglia and has recently been implicated in neuronal survival and CNS inflammatory processes. In preliminary studies, we have discovered decreased neuronal PGRN expression to be an early event in the development of an animal model of Amyotrophic

lateral sclerosis-Parkinsonism dementia complex of the western pacific (ALS-PDC), suggesting that PGRN may have some involvement in the neurodegenerative process. Indeed, following a series of pre-clinical assessments, we have found PGRN to have a disease-modifying effect in models of various neurodegenerative disorders. Here, we present data describing the effects of ND-602, a proprietary construct designed to target PGRN synthesis. Using a transgenic mouse model of Alzheimer's disease (AD), Tg 2576 mice, ND-602 was found to elevate neprilysin, a key enzyme involved in the degradation of Aβ. High levels of Aβ are a prime risk factor for AD, making strategies designed to target this protein, of great therapeutic value. Indeed, we also discovered a significant reduction in Aβ and plaque burden ipsilateral to ND-602 treatment, while no change was observed in equivalent regions of the contralateral, untreated, hemisphere. Together, the data reported here indicate that ND-602 treatment regulates Aβ, a key causal factor in AD, by reducing tissue levels in Tg2576 mice as well as plaque deposition. Thus, ND-602, by targeting PGRN, may represent a potential therapy designed to slow or halt disease progression in AD.

Disclosures: **J.M. Van Kampen**, Neurodyn Inc., Employment; **D. Kay**, Neurodyn Inc., Employment.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.8

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH Grant AG027818

Title: Rational design peptide-based inhibitors for Aβ42-induced neurotoxicity

Authors: ***H. LI**, E. FRADINGER, R. ZEMEL, G. BITAN;
Neurol., Univ. of Calfornia at Los Angeles, Los Angeles, CA

Abstract: Neurotoxic oligomers of amyloid β-protein (Aβ) are believed to be the main cause of Alzheimer's disease (AD). Previously, we have prepared a series of C-terminal fragments (CTFs) of Aβ42, evaluated their bioactivities, and identified Aβ(39-42) and Aβ(31-42) as lead inhibitors of Aβ42-induced neurotoxicity that also rescued Aβ42-induced inhibition of synaptic activity at micromolar concentration (Fradinger et al., *Proc. Natl. Acad. Sci. USA (2008) 105: 14175-14180*). We now present studies in which

second-generation candidates were developed based on each lead. In each case, we performed structure-activity relationship studies and identified key structural features. In the case of Aβ(39-42), we modified structural characteristics including chirality, side chain identity, and N-/C-terminus charge. The side chain of Ile41 and a free N-terminus were found to be crucial for the inhibitory activity. To characterize the binding site(s) of Aβ(39-42) on Aβ42 we used Tyr as a probe along the Aβ42 sequence and monitored changes in intrinsic fluorescence as a function of Aβ(39-42) binding. Surprisingly, the N-terminus of Aβ42 was found to be the main binding site of Aβ(39-42). Aβ(31-42) was modified using N-methyl amino acids. These modifications were found to increase the aqueous solubility of Aβ(31-42) substantially. Ile31 was found crucial for the inhibitory activity and N-methylation at positions 33, 38, 39, or 41 increased the inhibitory activity significantly. The data suggest that inhibition of Aβ42-induced neurotoxicity by CTFs is highly specific providing basis for design and testing of new peptidomimetics derivatives with improved activity and metabolic stability.

Disclosures: H. Li, None; **E. Fradinger**, None; **R. Zemel**, None; **G. Bitan**, None.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.9

Topic: C.02. Alzheimer's disease and other dementias

Support: Volkswagen-Stiftung Grant I/82 649

EU-FP6 Grant (cNeupro)

Title: Combining independent drug classes into hybrid molecules targeting Abeta oligomers

Authors: *A. MUELLER-SCHIFFMANN[1], J. MAERZ-BERBERICH[2], A. H. C. HORN[3], A. ANDREYEVA[4], R. ROENICKE[5], K. REYMANN[5], K. GOTTMANN[4], H. STICHT[3], T. SCHRADER[2], C. KORTH[1];
[1]Neuropathology, Heinrich Heine Univ. of Duesseldorf, Duesseldorf, Germany; [2]Inst. for Organic Chem., Univ. Essen-Duisburg, Essen, Germany; [3]Inst. for Biochem., Friedrich Alexander Univ. Erlangen-Nuernberg, Erlangen, Germany; [4]Inst. of Neuro- and Sensory Physiol., Heinrich Heine Univ. Duesseldorf, Duesseldorf, Germany; [5]German Ctr. for Neurodegenerative Dis., Magdeburg, Germany

Abstract: Cross beta-sheeted Abeta oligomers or amyloid are a hallmark of Alzheimer´s disease. Misprocessing of the amyloidogenic fragment Abeta, derived from proteolytic processing of amyloid precursor protein (APP) is critical in a cascade of events starting with the oligomerization of Abeta ultimately leading to neuronal death in the CNS. Rationally designed small molecule beta-sheet breakers proofed to be highly efficient in preventing or disassembling beta-sheet structures in cell-free in vitro systems but failed to show convincing effects in vivo, including cell based assays due to their unspecific binding to Abeta. The aim of our study was to overcome this weakness by the addition of a molecular Abeta recognition unit directing the beta-sheet moiety to its target molecule. As beta-sheet breaker we chose aminopyrazoles (AP) that possess a specific hydrogen bond donor-acceptor-donor sequence complementary to that of a beta-sheet. Interaction of AP with the diaromatic motif F19 F20 in Abeta had been determined by NMR and near UV CD spectroscopy. The AP was covalently linked to a D-enantiomeric dodecapeptide (D3), which had been selected by mirror phage display as a potent Abeta binder. Simulations revealed multiple interactions between D3 and the Glu22 residues present in oligomeric Abeta. As linker we used triethylene glycol exactly matching the distance of the candidate interaction sites.
Utilizing an in vitro cell culture model we demonstrated that only the hybrid compound (JM169) but not its single components or combinations of both prevented the assembly of naturally secreted oligomeric Abeta. Synaptic pathology, as a key biological effect of oligomeric Abeta, was efficiently reversed by JM169 in two independent models. JM169 blocked Abeta induced decrease in mEPSC frequency mediated by AMPA receptors in cultured cortical neurons and impairment of long-term potentiation (LTP) in hippocampal slices.
As predicted by comparative modelling and molecular dynamics simulations, shorter linkers favoring a tight interaction of AP and D3 to Abeta due to a smaller entropic loss upon binding, had stronger effects on inhibiting Abeta oligomer formation.
We generated a compound which selectively reduces naturally secreted oligomeric Abeta and counteracts synaptotoxicity caused by this Abeta form. Moreover we demonstrate that covalent linkage of two entirely different substance classes acting on the same target can yield dramatic synergistic effects and leads to novel pharmacological properties.

Disclosures: A. Mueller-Schiffmann, None; **J. Maerz-Berberich**, None; **A.H.C. Horn**, None; **A. Andreyeva**, None; **R. Roenicke**, None; **K. Reymann**, None; **K. Gottmann**, None; **H. Sticht**, None; **T. Schrader**, None; **C. Korth**, None.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.10

Topic: C.02. Alzheimer's disease and other dementias

Support: American Health Assistance Foundation A2008-350

Jim Easton Consortium for Alzheimer's Drug Discovery and Biomarker Development at UCLA

Title: Molecular tweezers reduce amyloid β-protein burden in transgenic mice by inhibiting Aβ assembly

Authors: *A. ATTAR[1], S. SINHA[2], P. MAITI[2], M. TAN[3], R. BAKSHI[2], P.-Y. KUO[2], F. YANG[2,6], M. R. JONES[2,6], C.-W. XIE[3,4], F.-G. KLÄRNER[7], T. SCHRADER[7], S. A. FRAUTSCHY[2,6], G. BITAN[2,4,5];
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Abstract: In Alzheimer's disease (AD), as in other diseases of aberrant protein assembly, traditional compound library screens have not yet resulted in safe and efficacious pharmacotherapy. Amyloid β-protein (Aβ) has been a poor target for these compound screens due to the metastable nature of Aβ oligomers, which exist in dynamically changing mixtures. As an alternative to library screening, we used a rational approach to target the hydrophobic and electrostatic interactions that mediate the earliest steps of Aβ monomers' assembly into oligomers and the disruption of cell membranes by the oligomers. We identified small molecules, termed "molecular tweezers," that inhibit Aβ assembly and promote Aβ fibril disaggregation *in vitro*. Our lead compound, CLR01 was found to rescue cell viability, dendritic spine density, and electrophysiologic activity in primary neurons. Administration of low doses of CLR01 subcutaneously in the triple-transgenic mouse model of AD caused robust reduction in Aβ burden and improved spatial working memory. Importantly, at doses 30-times higher than the efficacious dose, no signs of toxicity were observed. Taken together, the data suggest that molecular tweezers are highly promising compounds for development of efficacious therapy for AD in the near future.

Disclosures: A. Attar, None; **S. Sinha**, Patent co-inventor, Ownership Interest; **P. Maiti**, None; **M. Tan**, None; **R. Bakshi**, None; **P. Kuo**, None; **F. Yang**, None; **M.R. Jones**, None; **C. Xie**, None; **F. Klärner**, Patent co-inventor, Ownership Interest; **T. Schrader**, Patent co-inventor, Ownership Interest; **S.A. Frautschy**, Patent co-inventor, Ownership Interest; **G. Bitan**, Patent co-inventor, Ownership Interest; Clear Therapeutics, Ownership Interest.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.11

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH-NS#49442

Title: Novel CNS druglike small molecules specifically block soluble Abeta oligomer-induced memory deficits

Authors: *S. M. CATALANO[1], J. RAVENSCROFT[1], C. REHAK[1], R. YURKO[1], N. IZZO[1], H. SAFFERSTEIN[1], G. RISHTON[1], A. STANISZEWSKI[2], O. ARANCIO[2]; [1]Cognition Therapeutics, Inc., PITTSBURGH, PA; [2]Columbia Univ., New York, NY

Abstract: Small molecules that rapidly block the negative effects of Abeta oligomers on the molecular mechanisms of synaptic plasticity underlying memory have the potential to be disease-modifying Alzheimer's therapeutics. Membrane trafficking is central to the process of synaptic plasticity, and Abeta oligomers affect the rate of membrane trafficking as measured by the number of dye-stained cycling vesicles. We have discovered several structurally distinct compound series that block the effects of Abeta oligomers on membrane trafficking in primary neurons with low micromolar potency, but have no effect on primary neurons in the absence of Abeta oligomers. These molecules appear to act via partial antagonism of Abeta oligomer binding to the surface of the neuron and/or disruption of oligomer structure, are plasma stable (t1/2 = 3hrs) and are capable of reaching high concentrations in the brain (171 ng/g at 3 hours, 57 times the behaviorally efficacious concentration). Representative members of these compound series were tested in the fear conditioning behavioral task for their ability to preserve normal associative memory. Compounds were injected bilaterally via intrahippocampal cannula (2 pmol) one hour prior to the injection of Abeta 1-42 oligomers (200nM total Abeta) in wild-type C57Bl/6mice. After an additional 20 minutes, animals received a mild electric foot shock. Animals were tested for context-dependent learning 24 hours later. Animals receiving Abeta oligomer injections exhibited significant memory deficits as measured by decreased freezing behavior vs. vehicle (13 +/- 2% vs. 27 +/- 1% respectively). Compounds administered prior to Abeta oligomer completely blocked the effects of Abeta oligomers on memory (CT0109 =30 +/- 2%, CT0093 = 25 +/- 1%), and had no effect on memory when administered without Abeta oligomers (CT0109 =28 +/- 1%, CT0093 = 31 +/- 1%). Systemic administration of CT0109 does not induce motor deficits or abnormal behavior. These compounds represent promising Alzheimer's

therapeutics.
Supported by Cognition Therapeutics Inc. and NIH-NS#49442 to O.A.

Disclosures: **S.M. Catalano**, Cognition Therapeutics Inc., Employment; **J. Ravenscroft**, Cognition Therapeutics Inc., Employment; **C. Rehak**, Cognition Therapeutics Inc., Employment; **R. Yurko**, Cognition Therapeutics Inc., Employment; **N. Izzo**, Cognition Therapeutics Inc., Employment; **H. Safferstein**, Cognition Therapeutics Inc., Employment; **G. Rishton**, Cognition Therapeutics Inc., Employment; **A. Staniszewski**, Columbia University, Employment; **O. Arancio**, Columbia University, Employment.

Nanosymposium

725. Alzheimer's Disease: Therapies Targeting Abeta and Abeta Oligomers

Location: Room 32B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 725.12

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH P01 HD29587

Blueprint Core Grant P30 NS057096

Title: Takusan alleviates Aβ-induced synaptic dysfunction

Authors: ***S. TU**, F.-F. LIAO, H. XU, S. A. LIPTON, N. NAKANISHI; Sanford|Burnham Med. Res. Inst., LA JOLLA, CA

Abstract: Soluble oligomers of Aβ peptide are thought to play an important role in the pathophysiology of Alzheimer’s disease (AD). Specifically, soluble Aβ oligomers prepared from various sources downregulate dendritic-spine density and the levels of synaptic proteins, including the presynaptic protein synaptophysin and postsynaptic proteins such as PSD-95 and glutamate receptor subunits. We recently identified and characterized a large gene family, which we named takusan (meaning many in Japanese), which are particularly enriched in neurons. Upon forced expression in cultured hippocampal neurons, α1-takusan increased dendritic-spine density, PSD-95 clustering, GluR1 surface expression, and AMPA-induced whole-cell current. Because of these synaptic effects, we hypothesized that forced expression of α1-takusan, the prototypical takusan variant, might interfere with toxic effects of Aβ oligomers on synapses in cultured neurons. We chose conditioned medium from 7PA2 cells (Aβ CM), Chinese

hamster ovarian (CHO) cells expressing mutant (V717F) human APP, as the source of Aβ oligomers, since it contains naturally-secreted soluble Aβ oligomers at physiologically relevant concentrations (estimated ~5 nM in our preparations). Cultured hippocampal neurons transduced with EGFP (control) or EGFP-α1-takusan were treated with Aβ CM or control CM from regular CHO cells. We then quantified dendritic spine densities, PSD-95 and PSD-95/synapsin clusters, representing the number of synapses. Compared to exposure to control CM, Aβ CM led to significant reductions of dendritic spine density and PSD-95 (or PSD-95/synapsin) clusters in cells expressing the control EGFP vector. In contrast, forced expression of EGFP-α1-takusan ameliorated reduction in dendritic spines and PSD-95 (or PSD-95/synapsin) clusters following Aβ CM exposure. These data suggest that takusan can alleviate Aβ-induced synaptic dysfunction not only because takusan increases dendritic spine density and synapses, but also by increasing resistance of dendritic spines and synapses to Aβ toxicity. RNAi suppression of takusan expression showed that endogenous takusan protects cells against Aβ-induced synaptic loss. Interestingly, forced expression of takusan increased PSD-95 clustering without altering the total amount of PSD-95 protein. Additionally, Aβ exposure led to a reduction in PSD-95 protein associated with takusan. These results suggest that direct interaction between takusan and PSD-95 may stabilize the integrity of synapses after exposure to Aβ oligomers. In summary, takusan proteins may be exploited as therapeutic agents against Aβ-induced synaptic dysfunction.

Disclosures: S. Tu: None. **F. Liao:** None. **H. Xu:** None. **S.A. Lipton:** None. **N. Nakanishi:** None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.1

Topic: C.06. Developmental Disorders

Support: National Alliance for Autism Research-AutismSpeaks (BRS)

NIH PO1 HD035471 (KAL)

NIH RO1 MH 072263 (DAP)

Title: Paired finger stimulation MEG studies of the autistic brain: A window into inhibition

Authors: *M. A. COSKUN[1], S. L. REDDOCH[2], D. A. PEARSON[2], K. A. LOVELAND[2], E. M. CASTILLO[3], A. C. PAPANICOLAOU[3], B. R. SHETH[1]; [1]Univ. Houston, Houston, TX; [2]Dept. of Psychiatry and Behavioral Sci., [3]Dept. of Pediatrics, Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Autism is a developmental disorder, thus abnormalities in the circuits of autistic brains are likely to be pervasive. A leading proposal in this regard is reduced inhibition. Physiological studies of paired versus single finger stimulation provide a window into synaptic inhibition. Difference in the amplitude of the early (30-50 ms) component of the somatosensory evoked potential (SSEP) in response to single versus paired finger stimulation reflects inhibition at sub-cortical relay nuclei whereas difference in the mid-latency (80-100 ms) SSEP component reflects cortical inhibition. Reduced inhibition means a larger response in the autistic brain to paired versus single finger stimulation than in the typical brain. We tested this prediction here.

We used magnetoencephalography (MEG) to record neural responses to the passive tactile stimulation of thumb (D1), index finger (D2), and both fingers combined (D1,D2) of the dominant (right) hand of young subjects (Ss) (13 high-functioning persons with autism spectrum disorder or ASDs and 17 typically developing persons or TDs) while they remained awake in an eyes-closed supine posture.

The data were analyzed in two complementary ways: (i) the three sensors that respectively recorded the largest evoked response to D1, D2 and D1,D2 were selected. (ii) For each S and finger combination, we factorized the 248 sensor time series data using singular value decomposition (SVD), extracted the component or "virtual sensor" that accounted for the largest variance in the data. For both i and ii, we measured the amplitudes of the short-latency M50 and mid-latency M100 SSEPs in response to the stimulation of D1/D2/D1,D2. For each SSEP component, the combined response (D1,D2) was linearly regressed to the sum of the responses to individual stimulation (D1+D2). Reduced inhibition implies a steeper slope for the ASD group.

M50: For both i and ii, the slope of D1,D2 vs. D1+D2 responses was significantly smaller than one in ASDs but comparable to one in TDs; group slopes significantly differed as well. The M50 response likely reflects afferent activity. Therefore, our result suggests enhanced inhibition in sub-cortical structures of the autistic brain.

M100: For both i and ii, D1,D2 response was comparable to D1+D2 response in both groups; the group slopes did not differ either. The M100 response reflects cortical activity. Therefore, our result argues against between-group differences in cortical inhibition.

Enhanced 'gating' of peripheral stimulation in the autistic brain lends physiological support to the idea of stimulus over-selectivity in autism. Our results do not support the idea of reduced inhibition in the autistic brain.

Disclosures: M.A. Coskun, None; **S.L. Reddoch**, None; **D.A. Pearson**, None; **K.A. Loveland**, None; **E.M. Castillo**, None; **A.C. Papanicolaou**, None; **B.R. Sheth**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.2

Topic: C.06. Developmental Disorders

Support: NIH Grant T32NS007413

NIH Grant R01DC008871

NLM Family Foundation

Autism Speaks

Pennsylvania Department of Health

Title: Resting-state power and functional connectivity abnormalities in autism spectrum disorders

Authors: *L. CORNEW, T. P. L. ROBERTS, T. LEI, J. C. EDGAR;
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Abstract: Neural oscillatory rhythms are thought to reflect a balance of inhibition and excitation in cortical and thalamo-cortical circuits. Oscillatory abnormalities, at rest and during task performance, have been observed in ASD, suggesting inhibitory/excitatory dysfunction in these circuits. The precise nature and clinical significance of oscillatory abnormalities, however, are unclear. The present study employed whole-cortex MEG to probe resting-state brain activity in 6- to 14-year-old children (22 with ASD, 20 neurotypical controls). Data were obtained while participants underwent a two-minute eyes-closed resting-state exam. Offline, a standard regional source model was used to transform each individual's raw MEG surface activity to brain space. To assess spectral power at each regional source, a Fast Fourier Transform (FFT) was applied to artifact-free two-second epochs. The two-second spectra were averaged, and oscillatory activity was examined from 0-120 Hz. At regional sources where group resting power differences were observed, functional connectivity was assessed using magnitude squared coherence (MSC). Finally, the Social Responsiveness Scale (SRS) provided a measure of ASD symptom severity. Results indicated that children with ASD exhibited greater alpha (8-12 Hz) power than controls. This effect was maximal at parietal regions, and in ASD, increased parietal alpha power was associated with increased symptom severity. Functional connectivity analyses focused on parietal sources: Short-range connectivity

was assessed between parietal midline and parietal left and right sources, and long-range connectivity between parietal midline and frontal left and right sources. Compared to controls, children with ASD exhibited reduced high gamma (70-90 Hz) connectivity, especially for long-range source pairs. In addition, in the 90-120 Hz very fast oscillation (VFO) range, children with ASD exhibited elevated short-range connectivity coupled with reduced long-range connectivity. Functional connectivity abnormalities were associated with increased symptom severity. Furthermore, in children with ASD, parietal alpha power correlated with both short- and long-range parietal connectivity in the high gamma and VFO bands, suggesting that a common mechanism may underlie both low- and high-frequency oscillatory abnormalities. In sum, these findings demonstrate abnormal resting-state oscillatory activity in children with ASD, show that these abnormalities are of clinical significance, and support an imbalance of neural inhibition/excitation as a putative neurophysiologic ASD biomarker.

Disclosures: L. Cornew, None; **T.P.L. Roberts**, None; **T. Lei**, None; **J.C. Edgar**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.3

Topic: C.06. Developmental Disorders

Support: NICHD P50 HD055784

NIMH 1R01 HD065280-01

NIH T32 GM008042

Title: Reduced coherence of intrinsic connectivity networks in children with autism spectrum disorders

Authors: *J. D. RUDIE[1,2], L. H. HERNANDEZ[2], Z. SHEHZAD[3], N. L. COLICH[2], S. Y. BOOKHEIMER[2], M. DAPRETTO[2];
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Abstract: Functional brain imaging studies have demonstrated that, even in the absence of an overt cognitive task, there exist synchronized low frequency spontaneous fluctuations in neuronal activity across different brain regions, suggesting that the brain is

intrinsically organized into several large-scale functional networks. Two such networks are the task negative “default mode” network, thought to reflect self referential/introspective processing, and the anticorrelated task positive network, thought to reflect externally-driven task-related processing (Fox & Raichle 2007). Converging evidence from neurobiological studies of autism spectrum disorders (ASDs) suggests that core deficits observed in ASD may be related to aberrant patterns of synchronized neural activity across long-range networks required for complex reciprocal social behavior (Just 2007, Geschwind & Levitt 2007). Previous studies examining these networks in adults with ASD have reported both reduced connectivity (Kennedy & Courchesne 2008; Monk 2009) and increased connectivity (Monk 2009) in the task negative network, as well as no differences in the task positive network (Kennedy & Courchesne 2008). Here we sought to further investigate both task negative and task positive networks in children and adolescents with ASD and typically-developing controls (matched for age, gender, IQ and head motion) who underwent resting state functional connectivity MRI (rs-fcMRI; 6 minutes eyes open). Whole brain positive and negative functional connectivity maps were generated from a 10 mm diameter sphere centered in the posterior cingulate/precuneus, a hub region of the task negative network, (Fox 2005, MNI: -5, -53, 41). We found significant reductions in the coherence of both task-negative and task positive networks in children with ASD, as well as local over connectivity in ASD. In light of evidence showing increased intrinsic connectivity and long-range connections during typical development (Kelly 2008, Fair 2009), the overall reduced connectivity observed in these large-scale networks in ASD may in part reflect developmental delay in this population. Our findings highlight the need to examine the development of these neural systems over time to disentangle effects, which may reflect a history of altered engagement with the environment from those reflecting an abnormal neurobiological substrate. Moreover, given the task-free nature of rs-fcMRI, studies using this technique could also be conducted in younger and more affected children with ASD which may help identify intermediate phenotypes as well as inform the development of new diagnostic tools and biologically-based treatments.

Disclosures: J.D. Rudie, None; **L.H. Hernandez**, None; **Z. Shehzad**, None; **N.L. Colich**, None; **S.Y. Bookheimer**, None; **M. Dapretto**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.4

Topic: C.06. Developmental Disorders

Support: NICHD Grant R01HD065280-Bookheimer

NICHD Grant P50HD055784-Bookheimer

NIMH Grant F32MH088142-Shirinyan

Title: Ventral striatum hyperactivates to highly salient, self-selected, non-social rewards in autism

Authors: *D. SHIRINYAN[1], M. DAPRETTO[2], J. HOPKINS[3], S. Y. BOOKHEIMER[2]; [1]UCLA, LOS ANGELES, CA; [2]Psychiatry and Biobehavioral Sci., [3]UCLA, Los angeles, CA

Abstract: The social motivation hypothesis (Dawson et al., 1998b; Dawson, et al., 2005; Schultz, 2005) of autism spectrum disorders (ASD) holds that social stimuli are not experienced as rewarding for individuals with ASD, leading to multiple sequelae including reduced social and affinitive behaviors and interests. Recent neuroimaging work from our group found reduced reward responsivity in the ventral striatum (VS), which encompasses nucleus accumbens and ventral putamen, in response to monetary and social rewards in ASD (Scott-VanZealand et al 2010). Still, it is unclear whether VS hypoactivation is ubiquitous in ASD or whether there are stimuli that ASD children may find equally or more rewarding, such as those associated with their own restricted interest, resulting in a VS response. To further characterize the reward system in ASD and in typically developing children (TD), we conducted an fMRI study using individualized, highly salient but non-social rewards in the context of a modified Weather Prediction Task (Knowlton et al., 1994). High-functioning, verbal ASD subjects and age, IQ, performance, and head motion-matched controls made a simple button-press response which, if correct, was followed by a picture of items associated with each child's particular interest and which indicated that the child was earning points towards the agreed upon reward. The rewards were identified by asking the child and the accompanying parent "what is his/her favorite thing to do/play with?" or "is there something you have been very much looking forward to that you would like to work for?" Our results show that the ASD group exhibited greater VS signal (nucleus accumbens, and ventral putamen) to the highly salient stimulus than the TD group. This finding suggests that rather than a tonically depressed reward system, children with ASD may have a dysregulated VS that may be hypoactive to social rewards but is shown here to be hyperactivated when the rewards are self-selected and highly preferred. This finding complements prior research and extends the social motivation hypothesis of autism, suggesting that a heightened response to non-social stimuli may compensate for and/or displace reward from social stimuli. We hypothesize that a potentiated experience of reward may contribute to the rigid and anxious adherence to a small number of interests that is a core feature of the disorder.

Disclosures: D. Shirinyan, None; **M. Dapretto**, None; **J. Hopkins**, None; **S.Y. Bookheimer**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.5

Topic: C.06. Developmental Disorders

Support: NIH R01-DC006155

NIH R01-MH081023

NIDCD 1T32-DC007361-03

Title: Atypical maturation and functional segregation within the posterior superior temporal sulcus in autism spectrum disorder

Authors: *P. SHIH[1], B. KEEHN[3], J. ORAM[1], K. M. LEYDEN[1], C. L. KEOWN[2], R.-A. MUELLER[1,4];
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Abstract: Socio-communicative impairments are among the most salient features of autism spectrum disorder (ASD). It has been suggested that atypical connectivity plays an important role in ASD. One brain region that may be affected, the posterior superior temporal sulcus (pSTS), has been implicated in the processing of language, biological motion, and social context. Abnormalities in the development of pSTS may account for some of the socio-communicative deficits in ASD. In the present study, we investigated functional and anatomical maturation of pSTS with functional connectivity MRI (fcMRI) and anatomical measures, obtained with Freesurfer's automated cortical parcellation algorithm. FcMRI was used to examine intrinsically-occurring, low frequency BOLD fluctuations of pSTS subregions in 47 children and adolescents 8-19 years old. Twenty-one participants with ASD and 26 typically developing (TD) individuals were matched on age, gender, and IQ. Three functional subregions of pSTS were delineated in each hemisphere, and whole-brain, connectivity maps from each of the six pSTS fcMRI seeds were created. Two subregions of pSTS participated in overlapping networks, which were both topographically and temporally distinct from a third region located between the two.

In direct-group comparisons, the networks subserved by pSTS were significantly less differentiated in ASD. This was reflected in regions of increased connectivity relative to the TD group. There was a significant positive association between the differentiation of networks with age in the TD group that correlated with cortical thinning in pSTS and whole-brain white matter volume. However, in the ASD group, the differentiation of pSTS connectivity into distinct networks with age was not significant and did not correlate reliably with measures of anatomical maturation. Atypical maturation of pSTS may suggest altered trajectories for functional segregation and integration of networks, which may have ramifications in the development of higher-order processing. In addition, the present study provides a potential explanation for atypically increased connectivity in ASD, as observed in some fcMRI studies. Our findings highlight the importance of interpreting group differences in autism fcMRI studies from a developmental perspective.

Disclosures: P. Shih, None; **B. Keehn**, None; **J. Oram**, None; **K.M. Leyden**, None; **C.L. Keown**, None; **R. Mueller**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.6

Topic: C.06. Developmental Disorders

Support: NIH P50-NS22343

NIH R21-NS070296

NIH R21-MH089645

Title: A comparison of overt visuospatial orienting abilities in children with autism, Williams syndrome, specific language impairment, perinatal brain lesions, and typical development

Authors: *B. KEEHN[1,2], M. WESTERFIELD[2], C. LOCK[2], K. VO[2], M. ZINNI[2], D. TRAUNER[2], J. TOWNSEND[2];
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Abstract: Eye-movement studies can be an important tool for the study of brain function, particularly with young or cognitively impaired individuals. Designs such as the gap-

overlap task permit researchers to examine of the influence of attention on oculomotor function. We have conducted eye-tracking studies as part of an on-going multi-disciplinary program project to study cognitive function in children with developmental disorders, perinatal brain lesions, and typical development. The objective of the current study was to examine the similarities and differences in the accuracy and efficiency of overt visuospatial orienting in children with high-functioning autism (HFA), Williams syndrome (WS), specific language impairment (LI), pre- or perinatal stroke (FL), and typical development (TD). Participants were HFA, WS, FL, LI and TD children aged 7 - 11 years. We used a gap-overlap paradigm in which participants were instructed to fixate on a central crosshair and then move their eyes to a peripheral target once it appeared. Each trial began with a crosshair presented alone in the center of the display for a duration 1000ms. Following fixation on the crosshair, a peripheral target could appear: with the crosshair remaining on the screen (overlap condition), 400ms after the crosshair disappeared (gap condition), or with the simultaneous offset of the crosshair (baseline condition). There were 16 possible target locations arranged on two invisible concentric circles. The circles surrounded the central fixation cross at eccentricities of 4.9° and 9.8°, with eight possible locations on each circle. Latency and accuracy of participants' saccades were monitored using an EyeLink 1000 Remote eye-tracking system. Preliminary analyses suggest that children with WS have reduced saccadic accuracy, while children with HFA show abnormal disengagement of attention. Furthermore, differences in saccadic RT to near and far targets in the FL group (as compared to the TD group) suggest that children with FL may be less efficient at orienting to information outside the current locus of attention. Finally, while children with LI showed a saccadic RT pattern that was similar to TD children, they demonstrated slowing across all conditions. Preliminary results suggest that children with HFA, WS, FL, and LI show evidence of syndrome-specific deficits in the adaptive allocation of attention and oculomotor function. Elucidating both shared and unique characteristics of low-level attentional and motor function within these groups may help reveal pathways that contribute to their phenotypic end-state.

Disclosures: B. Keehn, None; **M. Westerfield**, None; **C. Lock**, None; **K. Vo**, None; **M. Zinni**, None; **D. Trauner**, None; **J. Townsend**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.7

Topic: C.06. Developmental Disorders

Support: NIH Grant R01-DC006155, R01-MH081023

National Institute on Deafness and Other Communicative Disorders 1T32-DC007361-03 (author BK)

Title: Atypical lateralization of networks in autism spectrum disorder as detected by independent component analysis

Authors: *R. C. CARDINALE[1], P. SHIH[1], C. L. KEOWN[2,1], B. KEEHN[3], R.-A. MUELLER[4,1];
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Abstract: Autism spectrum disorder (ASD) is characterized by socio-communicative impairments, repetitive behaviors, and restricted interests. Lateralization of brain networks is an important aspect of functional development as it relates to the emergence of intrahemispheric specialization for language and other domains. Atypical lateralization in ASD may relate to socio-communicative impairments (e.g. typically left-dominant language and right-dominant face processing). Many studies have noted aberrant functional organization in ASD, but none have specifically examined atypical lateralization of functional networks. Our study aimed to provide insight into atypical connectivity patterns in ASD and their relation to abnormal cognitive profiles. Eleven ASD and 19 typically developing (TD) participants, matched on age, handedness, IQ, and gender, performed a visual search task during fMRI scanning (224 time points at 2.5 seconds each for a total of 560 seconds). Temporal concatenation independent component analysis (ICA), implemented within FMRIB Software Library's (FSL) MELODIC, was used to decompose fMRI data into separate spatial networks. One set of IC networks was obtained for each group and their Z-score connectivity maps, excluding regions of anti-correlation, were spatially correlated between groups to identify consistent network components. The lateralization of each network extracted with ICA was assessed with a lateralization index computed as (L-R)/(L+R). Model order, estimated by MELODIC, was similar for both groups. Thirty-five were estimated for the TD group, and 34 were estimated for the ASD group. Of those, eight spatial components were matched between the TD and ASD groups. Lateralization indices computed for the remaining IC networks indicated differences in the lateralization of three network maps. Both reduced and enhanced lateralization was observed in the ASD group. The first network, which included the left superior medial gyrus, precuneus, calcarine gyrus, and middle temporal gyrus and the right superior temporal gyrus, had greater right lateralization in the TD group. The second network, including the left inferior frontal gyrus (IFG), middle occipital gyrus, and inferior parietal lobe and the right angular gyrus and IFG, showed greater left lateralization in the ASD group. The third network, comprised of the left postcentral gyrus and middle cingulate cortex and the right precentral gyrus, displayed greater left lateralization in the TD group. Our exploratory study suggests that ICA is a

promising data-driven approach to the study of hemispheric asymmetries of functional networks in ASD.

Disclosures: R.C. Cardinale, None; **P. Shih**, None; **C.L. Keown**, None; **B. Keehn**, None; **R. Mueller**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.8

Topic: C.06. Developmental Disorders

Support: UK MRC (U.1055.02.001.0001.01)

Title: Reduced resting state activity is predicted by individual differences in autism spectrum traits across both the typical and ASD population

Authors: *E. VON DEM HAGEN, R. STOYANOVA, A. J. CALDER;
Med. Res. Council, Cambridge, United Kingdom

Abstract: Autism Spectrum Disorders (ASD) comprise a group of neurodevelopmental disorders characterised by marked abnormalities in the social domain, in addition to restricted interests and repetitive behaviour. Recent evidence has suggested that traits associated with ASD extend into the typical population and that these affect behavioural performance in typical participants on tasks that are impaired in ASD. Neuroimaging studies have further underscored the existence of an extended autism spectrum by correlating measures of autistic traits in healthy controls with brain structure and function. However these studies have all examined the effect of these traits in healthy participants only. Here, we use a validated questionnaire measure, the Autism Spectrum Quotient (AQ), to determine the extent of autistic traits across a group of typical controls and a group of individuals with ASD, and use this measure as a predictor of activity in the default mode network during the resting state. The default mode network comprises a number of well-defined regions, including mPFC, known to show abnormal function across various tasks and at rest in ASD. 25 healthy adults and 17 adults with ASD (2 High-functioning Autism and 15 Asperger's Syndrome) underwent a 10 min resting state fMRI scan on a Siemens 3T Tim Trio. Data were preprocessed in FSL for motion correction, spatial normalization, spatial smoothing and temporal filtering. 5 subjects with ASD had to be removed from the analysis due to excessive head movement. ICA

was performed on the remaining subjects (25 controls, 12 ASD) using group ICA for fMRI toolbox (GIFT). Individual subject IC patterns representing resting state network components were entered into one- and two-sample random-effects analyses in SPM5. We found a significant reduction in activity in dmPFC in the resting state which was predicted by individual differences in AQ scores. While this reduced activity was present at a less stringent statistical threshold when the groups were compared in a two-sample t-test, the effect was much more robust when AQ was used as a predictor in place of group membership. These results suggest that there is a spectrum of autistic characteristics which extends from the typical to the ASD population and affect neural response in a continuous fashion. Furthermore, the results suggest that a behavioural measure of autistic traits such as the AQ may afford additional sensitivity when studying differences between the typical and ASD populations.

Disclosures: E. von dem Hagen, None; **R. Stoyanova**, None; **A.J. Calder**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.9

Topic: C.06. Developmental Disorders

Support: NIH Grant R01-DC006155

NIH Grant R01-MH081023

National Institute on Deafness and Other Communicative Disorders 1T32-DC007361-03

Title: Investigation of functional networks with independent component analysis in autism spectrum disorder

Authors: *C. L. KEOWN[1], P. SHIH[2], B. KEEHN[3], R.-A. MUELLER[2,4];
[1]Computat. Sci. Program, San Diego, CA; [2]Dept. of Psychology, San Diego State Univ., San Diego, CA; [3]Joint Doctoral Program in Language and Communicative Disorders, San Diego State Univ. and Univ. of California, San Diego, San Diego, CA; [4]Dept. of Cognitive Sci., UCSD, San Diego, CA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder

characterized by sociocommunicative deficits and restricted behaviors. Research in ASD using functional connectivity MRI has consistently observed abnormal patterns of synchronization between disparate brain regions when compared to typically developing (TD) individuals. The diffuse variations in connectivity patterns may suggest aberrant development of functional networks in ASD. Temporal concatenation group independent component analysis (TC-GICA) is an exploratory, data-driven technique shown to reliably extract neurally relevant, spatially independent networks from the BOLD fMRI signal. A parameter of the TC-GICA algorithm is the dimensionality, the number of source signals or components. Previous work has shown that increasing the model order in ICA may dissociate networks into potentially cognitively relevant subnetworks. However, there is presently no empirical analysis that shows how individual networks differ between control and clinical groups. In the present study, we performed TC-GICA using FMRIB Software Library (FSL) MELODIC on one resting-state and two task-related fMRI data sets collected on TD participants and individuals with ASD. We iteratively varied the number of components in a group and reconstructed subnetworks by grouping components based on spatial map correlations and time series correlations to the parent network. Results from our analysis can be categorized by network behavior as follows: We found that some putative major networks broke down similarly between groups (e.g. the default mode network into separate medial prefrontal cortex and posterior cingulate cortex subnetworks). However, we also observed group differences in decomposition into subnetworks, in addition to networks that dissociated in one group but not in the other. To examine the distribution of relationships among individual networks extracted from each TC-GICA, temporal and spatial correlations between network components were investigated for each group in order to measure internetwork integration. Average magnitudes for positive and negative correlations were greater in the TD group than in the ASD group, indicating a stronger level on integration and segregation between networks in TD. Our results suggest that impaired internetwork cooperation in ASD may reduce integration between functional systems. This may not be limited to specific networks but is rather distributed across many networks.

Disclosures: C.L. Keown, None; **P. Shih**, None; **B. Keehn**, None; **R. Mueller**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.10

Topic: C.06. Developmental Disorders

Support: HIH Grant R01-DC006155

Title: Microstructural abnormalities of short-distance white matter tracts in autism spectrum disorder

Authors: *D. K. SHUKLA, D. M. SMYLIE, B. KEEHN, R.-A. MÜLLER;
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Abstract: Previous studies have shown white matter compromise in autism spectrum disorder (ASD), which may relate to impaired function in distributed networks. However, tract-specific evidence for short-distance white matter fibers remains limited in ASD. In the present study, short-distance white matter fibers tracts were estimated for the fractional anisotropy (FA), mean diffusivity (MD) and axial and radial diffusivity. Diffusion tensor imaging (DTI) data of 26 children with ASD and 24 typically developing (TD) children were acquired using a single-shot diffusion-weighted EPI pulse sequence (b=0 and 2000 s/mm^2, 15 non-linear directions, four repetitions). FA, MD and axial and radial diffusivity images were aligned into a common space using nonlinear registration method. Mean FA image was created and thinned to represent centers of all common tracts. Short-distance fibers close to the cortical boundary were indentified for frontal, temporal and parietal lobes in both hemispheres
Significant differences for all DTI indices were observed between ASD and TD groups for all short-distance fibers combined in both hemispheres. Furthermore, FA was significantly reduced in frontal lobe in ASD compared to TD group. MD and radial diffusivity were significantly increased in frontal, temporal and parietal lobes in ASD group compared to TD group. No significant group differences for axial diffusivity were found (Figure 1). Significant positive correlation between age and FA and negative correlation between age and MD and radial diffusivity were also found for short-distance fibers in each lobe in TD group but not in ASD group.
These results suggest the involvement of short-distance white matter compromise in ASD. Short-distance U-fibers have shown to be an important component of neural nets and thought to play a crucial role in cognitive function. Absence of age-related correlation with DTI indices may reflect altered maturation of short-distance fibers in ASD. Our observations open new doors to study the contribution of short-distance U-fibers in neuropsychological impairments in ASD.$$graphic_{7EF8D474-ACBC-48ED-8EC5-2B37B5845ECB}$$

Disclosures: D.K. Shukla, None; **D.M. Smylie**, None; **B. Keehn**, None; **R. Müller**, None.

Nanosymposium

726. Autism: Physiology and Systems II

Location: Room 10

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 726.11

Topic: A.07. Development of Motor, Sensory and Limbic Systems

Support: NARSAD

Autism Speaks

Title: DTI study of cingulum bundle in autism

Authors: *E. ANAGNOSTOU[1], S. AMEIS[2], C. ROCKEL[3], L. SOORYA[4], J. FAN[4]; [1]Univ. of Toronto, Pediatrics, toronto, ON, Canada; [2]Psychiatry, Univ. of Toronto, toronto, ON, Canada; [3]Psychology, SickKids Hosp., toronto, ON, Canada; [4]psychiatry, Mount Sinai Sch. of Med., New York, NY

Abstract: Objective: In the autism spectrum disorders (ASD), brain overgrowth in early life may drive white matter dysconnectivity. Impaired white matter connectivity within limbic circuitry may contribute to socio-emotional dysfunction, in ASD. Preliminary evidence has pointed to structural disturbance within the cingulum bundle in adults with ASD. This study aims to examine structural connectivity of the cingulum bundle in developing children and adolescents with ASD, compared to controls, using diffusion tensor tractography.
Methods: Diffusion tensor images (DTI) were acquired for 18 children and adolescents with ASD (age range 7-18 years; mean 12.2 ± 3.1) and 17 age and sex matched healthy controls (age range 8-17; mean 12.6 ± 3.3) on a 3T Siemens Allegra head-dedicated MRI system. Deterministic tractography was performed using a single region of interest approach to reconstruct the cingulum bundle. Average fractional anisotropy, axial diffusivity, radial diffusivity, and mean diffusivity values were quantified for the right and left cingulum bundle. Independent samples t-tests were performed to determine between-group differences in DTI indices: (1) in the overall sample of participants, (2) in a sub-group of developing children within the overall sample, and (3) in a sub-group of developing adolescents within the overall sample. We define children as those participants that were under the age of 12 at the time of scanning, and adolescents as participants 12 years of age and older at the time of DTI scanning.
Results: The results of our study show that mean diffusivity is significantly increased in ASD in the left cingulum bundle ($p = .044$), when considering the overall sample of participants. No other differences in the overall sample were found. However, when developing children with ASD were compared to matched controls, mean diffusivity was significantly increased in the ASD group within the left ($p = .004$) and right ($p = .042$) cingulum bundle. Similarly, radial diffusivity was significantly increased within the left ($p = .035$) and right ($p = .049$) cingulum bundles, in children with ASD. No other differences were found among the sub-group of developing children. Interestingly, when developing adolescents with ASD were compared to matched controls, no differences in

DTI measures were found between groups.
Conclusions: Our results suggest potential disruption of the cingulum bundle, in ASD, with mean diffusivity and radial diffusivity in the cingulum bundle in developing children with ASD but not in developing adolescents, possibly indicating immaturity of the cingulum bundle that is specific to children with ASD.

Disclosures: E. Anagnostou, None; **S. Ameis**, None; **C. rockel**, None; **L. Soorya**, None; **J. Fan**, None.

Nanosymposium

727. Toxic Pharmacology

Location: Room 23A

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 727.1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIDA Grant DA023205

NIDA Grant DA013978

Title: Attenuation of methamphetamine-induced neurotoxicity by SN79: Involvement of sigma-2 receptors

Authors: *N. KAUSHAL[1], C. R. MCCURDY[3], R. R. MATSUMOTO[2];
[2]Basic Pharmaceut. Sci., [1]West Virginia Univ., Morgantown, WV; [3]Dept. of Medicinal Chem., Univ. of Mississippi, Oxford, MS

Abstract: Methamphetamine (METH) causes hyperthermia as well as neurotoxicity to dopaminergic and serotonergic nerve terminals in the striatum at high or repeated doses. The damage caused by METH has been shown to be associated with various neurological disorders. However, the mechanisms of these neurotoxic effects are not very clearly understood and there is still no effective pharmacotherapy for treating the negative health consequences of METH. This project evaluates the effectiveness of antagonizing sigma-2 receptors to attenuate the neurotoxic effects of METH. It is based on three previous observations: 1) METH binds to sigma-2 receptors at physiologically relevant concentrations, 2) sigma-2 receptors are present on dopaminergic and serotonergic neurons and can modulate their actions, and 3) sigma-2 receptor activation causes cell death in tumor cells. Therefore, a sigma-2 receptor selective antagonist, 6-acetyl-3-(4-(4-(4-florophenyl)piperazin-1-yl)butyl)benzo[d]oxazol-2(3H)-one (SN79), was developed

and tested. Using radioligand binding studies, SN79 was shown to have high nanomolar affinity and selectivity for sigma-2 receptors, with >10,000 nM affinity for sigma-1 receptors and 59 other binding sites. In the in vivo part of the study, SN79 pretreatment dose-dependently attenuated METH-induced hyperthermia in Swiss Webster mice. In addition, SN79 pretreatment prevented METH-induced depletions in striatal dopamine and serotonin levels. In the in vitro part of the study, SN79 attenuated the cytotoxic effects of METH in NG108-15 cells maintained at a constant temperature of 37°C. The results demonstrated the ability of a highly selective sigma-2 receptor antagonist to prevent the neurotoxicity of METH. Together, the data suggest an important role of sigma-2 receptors in the neurotoxic effects of METH and identify a potential novel therapeutic target for medication development.

Disclosures: N. Kaushal, None; **C.R. McCurdy**, None; **R.R. Matsumoto**, None.

Nanosymposium

727. Toxic Pharmacology

Location: Room 23A

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 727.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-ES016554

NIH-ES010356

Title: Early developmental methylphenidate exposure causes persistent cognitive impairment in zebrafish

Authors: S. ROACH[1], D. SLEDGE[2], S. DONERLY[1], E. LINNEY[1], *R. D. SCHWARTZ-BLOOM[1], E. D. LEVIN[1];
[1]Duke Univ. Med. Ctr., DURHAM, NC; [2]North Carolina Central Univ., Durham, NC

Abstract: Methylphenidate (Ritalin) is a widely used treatment for attention deficit hyperactivity disorder (ADHD) in juveniles and adolescents. Methylphenidate is being taken by increasing numbers of adults to treat ADHD residual type. This raises the question of the risk, which may arise with regard to the potential risks of early developmental exposure if people taking the medication becomes pregnant. In the current study we assessed the neurobehavioral effects of methylphenidate in zebrafish. Zebrafish provide an outstanding model for the study of neurodevelopment and the adverse effects

of developmental neurotoxicity. They offer cellular reporter systems, continuous visual access and molecular interventions such as morpholinos as well as validated and sensitive behavioral test methods to help determine critical mechanisms underlying neurobehavioral teratogenicity. Previously, we had seen that persisting neurobehavioral impairment in zebrafish with developmental chlorpyrifos exposure was associated with disturbed dopamine systems and that learning proficiency is significantly correlated with levels of the dopamine metabolite DOPAC. Because methylphenidate is an indirect dopamine agonist, it was thought that it could disrupt development of dopamine systems and cause persistent cognitive impairment after developmental exposure. In a preliminary study, zebrafish embryos were exposed to methylphenidate 0-5 days post fertilization (6.25-100 mg/l). They were tested for persistent cognitive effects as adults. In the three-chamber spatial learning task developmental methylphenidate exposure caused a significant impairment in initial choice accuracy during the learning sequence in the fish exposed to 25, 50 and 100 mg/l of methylphenidate. These data show that early developmental exposure of zebrafish to methylphenidate causes a long-term impairment in neurobehavioral plasticity. The identification of these functional deficits in zebrafish enables further studies with this model to determine how molecular and cellular mechanisms are disturbed by developmental methylphenidate exposure to arrive at this compromised state.

Disclosures: S. Roach, None; **D. Sledge**, None; **S. Donerly**, None; **E. Linney**, None; **R.D. Schwartz-Bloom**, None; **E.D. Levin**, None.

Nanosymposium

727. Toxic Pharmacology

Location: Room 23A

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 727.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant DA013978

Title: AC927, a selective sigma receptor ligand, blocks the generation of reactive oxygen species induced by methamphetamine in NG108-15 cells

Authors: *R. R. MATSUMOTO[1], M. ELLIOTT[1], N. KAUSHAL[1], A. K. V. IYER[1], Y. ROJANASAKUL[1], A. COOP[2];
[1]Basic Pharmaceut. Sci., West Virginia Univ., Morgantown, WV; [2]Univ. of Maryland, Baltimore, MD

Abstract: Earlier studies have demonstrated the ability of the selective sigma receptor ligand AC927 (N-phenethylpiperidine oxalate) to attenuate the stimulant and neurotoxic effects of methamphetamine in vivo. However, the precise mechanisms through which AC927 conveys its protective effects remain to be determined. Using differentiated NG108-15 cells as a model system, the effects of methamphetamine on the generation of reactive oxygen and nitrogen species (ROS/RNS) and its implications for the ensuing development of neurotoxicity was examined in the absence and presence of AC927. Methamphetamine induced ROS/RNS which were detected by the fluorigenic substrate chloromethyl dihydrodichlorofluorescin diacetate (CMH_2DCFDA) in a concentration and time dependent manner. N-acetylcysteine, catalase, and L-N^G-monomethyl arginine (L-NMMA) inhibited the CMH_2DCFDA induced by methamphetamine, suggesting the formation of hydrogen peroxide and nitric oxide. Exposure to methamphetamine also stimulated the release of dopamine from NG108-15 cells into the culture medium. AC927 attenuated the methamphetamine-induced production of CMH_2DCFDA and also inhibited the secretion of dopamine by NG108-15 cells in response to methamphetamine, while producing no significant effects on its own on ROS/RNS and dopamine release. In contrast to the physiologically relevant concentrations of methamphetamine that induced ROS/RNS in vitro, only very high concentrations of methamphetamine were able to induce cell death in NG108-15 cells, suggesting that the radicals may contribute to neurotoxicity, but by themselves are not directly responsible for cell death. Together, the data suggest that the ability of AC927 to attenuate the generation of ROS/RNS may contribute to its alleviation of the hyperthermic and neurotoxic effects of methamphetamine in vivo.

Disclosures: R.R. Matsumoto, None; **M. Elliott**, None; **N. Kaushal**, None; **A.K.V. Iyer**, None; **Y. Rojanasakul**, None; **A. Coop**, None.

Nanosymposium

727. Toxic Pharmacology

Location: Room 23A

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 727.4

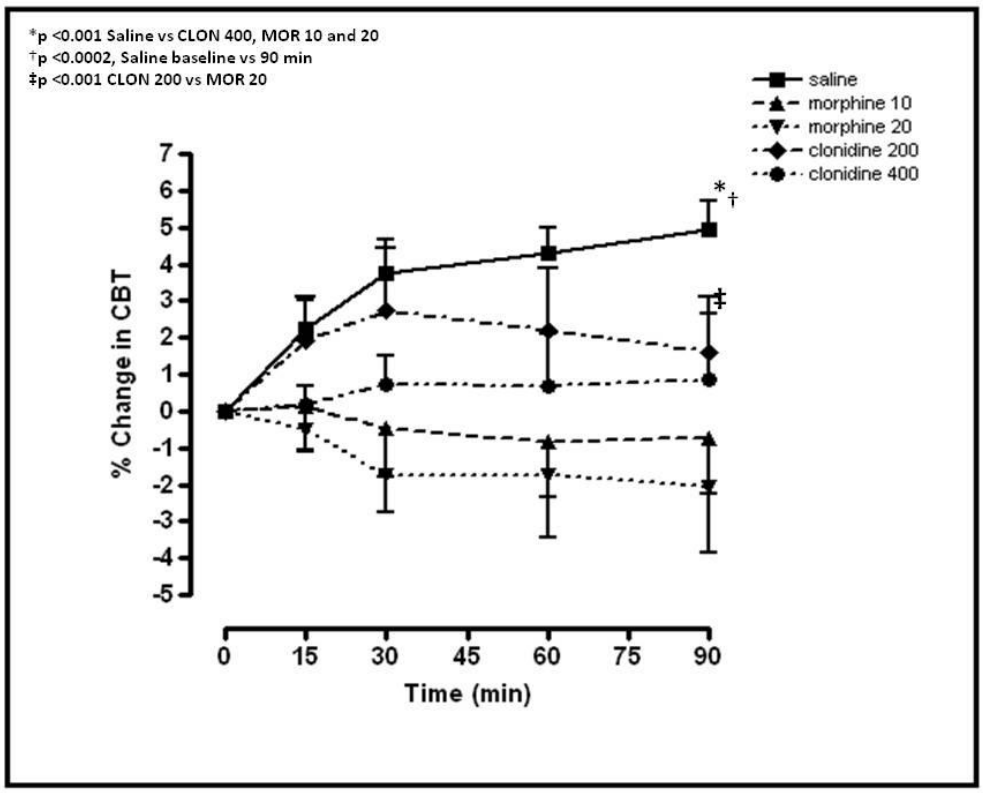
Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIDA Grant R25 DA021630-04

Title: The effect of clonidine and morphine on core body temperature in newborn rat pups

Authors: *K. KESAVAN[1], E. B. GAUDA[2], F. J. NORTHINGTON[2];
[2]Neonatology, [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Sedatives are used in critically ill newborns who are at risk for brain injury, and core body temperature (CBT) modifies outcomes in infants with brain injury. Specifically, avoiding hyperthermia is critical. The purpose of this study is to evaluate the role of two classes of sedatives; α2-adrenergic receptor agonist, clonidine (CLON); and μ-opioid receptor agonist, morphine (MOR) on core body temperature (CBT).
Hypothesis: MOR and CLON will differentially modify CBT in neonatal rodents.
Rectal probe thermometers (IT 18 Rectal Probe, AD instruments) were used to continuously measure CBT in freely moving Sprague- Dawley rat pups at postnatal day 7 (n=20/treatment group). The animals were placed in a thermoneutral environment (31-33^{o}C) and temperature was measured prior to and for 90 mins after intraperitoneal administration of MOR (10 or 20mg/kg), CLON (200 or 400 μg/kg), or saline, all in a volume of 200 μl.
Results: CBT was significantly affected by treatment (2-Factor ANOVA, $p<0.0001$), Figure. In animals receiving MOR or CLON, CBT did not significantly change from baseline; while animals receiving saline, CBT at 90 mins (35.9±0.2^{o}C) was increased by 5% from baseline (34.2± 0.2^{o}C) ($p < 0.0002$, baseline vs 90 mins). CBT was greater in the saline treated animals compared to animals treated with CLON 400 and MOR 10 and 20 (all $p<0.001$ vs. saline). The effect of MOR and CLON on CBT also differed ($p<0.005$, 2-Factor, ANOVA); MOR at 20mg/kg reduced CBT when compared to CLON at 200ug/kg; $p=0.002$ (Bonferroni correction).
Conclusion: Both MOR and CLON stabilized CBT in comparison to saline which significantly increased CBT. These data suggest that adequate sedation may be beneficial in stabilizing core body temperature (CBT) in infants at risk for brain injury and specifically reducing risk of developing hyperthermia. .



Disclosures: K. Kesavan, None; **E.B. Gauda**, None; **F.J. Northington**, None.

Nanosymposium

727. Toxic Pharmacology

Location: Room 23A

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 727.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Portland Alcohol Research Center Pilot Grant

NS054684

NS049210

NS20020

Title: Moderate perinatal ethanol exposure does not alter adult male or female experimental stroke infarct volume outcomes

Authors: *S. J. MURPHY, L. ZHANG, N. LIBAL, P. D. HURN;
Dept. of Anesthesiol. & Perioperative Med., Oregon Hlth. & Sci. Univ., PORTLAND, OR

Abstract: Alcohol is a widely abused drug in pregnant women that can affect the developing brain. Research on perinatal ethanol exposure in developing brain has mostly focused on chronic high doses in pregnant women, which can cause substantial CNS changes and behavioral deficits in offspring. However, low to moderate maternal alcohol consumption is more common than binge drinking or alcoholism during pregnancy. Relative to developing brain, little research has been done linking perinatal ethanol exposure to ischemic stroke susceptibility and outcomes in men versus women. We evaluated whether moderate perinatal ethanol exposure increases adult ischemic stroke sensitivity in a sex-specific manner. C57BL/6 female mice were given daily intraperitoneal ethanol (0.2 g/kg) to mimic moderate alcohol consumption in women or saline (0.015 ml/g) 1 week before timed-mating and during gestation. After delivery, pups were treated daily with intraperitoneal injections of ethanol (0.25 g/kg) or saline (3 µl/g) from P0 to P8, as brain development during this period would reflect the effects of ethanol during the third trimester of human pregnancy. At 8 to 10 weeks of age, mice underwent 1 h of middle cerebral artery occlusion via intraluminal filament. Brains were collected at 72 h of reperfusion. Cortical and striatal infarct volumes (% contralateral structure) were determined by digital image analysis of 2 mm thick coronal brain slices stained with 2,3,5-triphenyltetrazolium chloride. Overall, cortical and striatal infarct volumes were comparable among the groups regardless of sex (male vs. female) or perinatal treatment (ethanol vs. saline). No differences were seen in cortical (60 $\pm$ 2%) and striatal (106 $\pm$ 3%) infarct volumes between males with moderate perinatal ethanol exposure (n=17) and male mice perinatally exposed to saline (Cortex: 58 $\pm$ 4%, Striatum: 105 $\pm$ 4%, n=15). In female mice with moderate perinatal ethanol exposure (n=15), cortical (60 $\pm$ 4%) and striatal (111 $\pm$ 4%) infarct volumes were similar to female mice perinatally exposed to saline (Cortex: 61 $\pm$ 4%, Striatum: 108 $\pm$ 7%, n=11). Our findings suggest that moderate perinatal ethanol exposure may not alter ischemic stroke susceptibility in adults regardless of sex. Future studies will assess the effects of higher perinatal ethanol exposure levels (dose response) on adult stroke sensitivity. We will also examine how ethanol exposure alters ischemic brain outcomes when mice are exposed both perinatally and in adulthood.

Disclosures: S.J. Murphy, None; **L. Zhang**, None; **N. Libal**, None; **P.D. Hurn**, None.

Nanosymposium

727. Toxic Pharmacology

Location: Room 23A

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 727.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 NS05062 (to M.K.)

P50 DA026306 (TMARC, P5) (to M.K.)

1 P30 NS057096 (to A.J.R.)

Title: Methamphetamine and HIV-1 envelope gp120 mutually influence their effects on behavior and neuronal viability

Authors: *M. KAUL[1], R. MAUNG[1], N. E. SEJBUK[1], C. AKE[2], A. J. ROBERTS[3]; [1]Infect & Infl Dis Ctr., Sanford-Burnham Med. Res. Inst., LA JOLLA, CA; [2]Dep. of Psychiatry, UCSD, San Diego, CA; [3]Mol. and Integrative Neurosciences, The Scripps Res. Inst., La Jolla, CA

Abstract: Infection with Human Immunodeficiency Virus (HIV)-1 and AIDS are frequently associated with use of recreational drugs, such as Methamphetamine (METH). While there is evidence that both HIV-1 and METH can cause behavioral, neurocognitive and histopathological changes, the combined effect and potential interaction of both the virus and the drug is poorly understood. Recently, we observed that HIV/gp120-transgenic (tg) mice, which express the viral envelope protein in the brain, show an altered behavioral response to METH in comparison to non-transgenic, wild type littermate controls. The viral envelope protein seemed to primarily alter the reaction to METH exposure of the nigro-striatal dopamine pathway (stereotypy) while exerting a less pronounced effect on the mesolimbic dopaminergic system (locomotion). In a first approach to assess the effect of METH on gp120 neurotoxicity, we exposed rat cerebrocortical cell cultures for 24 h to increasing concentrations of METH in the presence and absence of HIV envelope protein (200 pM). Neuronal injury and death was assessed in fixed and permeabilized cell cultures using a combination of immunostaining for neuronal cell markers and fluorescence labeling of DNA which allowed scoring of cellular morphology, apoptosis and cell numbers. The results showed that 100 microM METH aggravated the toxic effect of gp120. Surprisingly however, 10 and 50 microM METH showed a trend toward the opposite effect, suggesting that the psychostimulant drug can exert, at least for a short term, at low concentrations and in combination with HIV env protein, an unanticipated protection. However, METH alone showed comparable but limited neurotoxicity at all three applied concentrations suggesting that 10 microM already achieved the maximum toxic effect under the given conditions. In summary, our results suggest a concentration-dependent interaction of the viral envelope

protein and the psychostimulatory drug that modifies behavior as well as neuronal viability.

Disclosures: M. Kaul, None; **R. Maung**, None; **N.E. Sejbuk**, None; **C. Ake**, None; **A.J. Roberts**, None.

Nanosymposium

727. Toxic Pharmacology

Location: Room 23A

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 727.7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Dexamethasone but not the equivalent doses of hydrocortisone induces apoptosis and neurodegeneration in neonatal rat brain

Authors: *A. J. BHATT, Y. FENG, P. G. RHODES;
Uni of Mississippi Med. Ctr., JACKSON, MS

Abstract: Studies to evaluate glucocorticoids' neurotoxicity in newborn are very important because the use of dexamethasone (Dex) in premature infants to prevent and/or treat bronchopulmonary dysplasia (BPD) adversely affects neuro-cognitive and behavioral development. Our previous studies using a neonatal rat model involving a regimen of tapering doses of Dex to investigate long-term effects of neonatal Dex exposure on developing CNS showed that Dex caused apoptotic neurodegeneration. Recent data suggest that hydrocortisone (HC) use may be safer than Dex. However, limited information is available about the effect of neonatal HC treatment on brain in an animal model. Objectives of this current project were to evaluate whether different regimens of HC cause apoptotic neurodegeneration in newborn rats and to compare them to neurotoxic regimen of Dex. The rat pups in each litter were randomly divided to the HC, Dex or vehicle groups. Rat pups in HC and Dex groups received tapering doses of i.p. HC or Dex, respectively, on postnatal day 3 to day 6 (HC: lower dose: 5, 2.5, 1.25, and 0.6 mg/kg; middle dose (equivalent to Dex): 15, 7.5, 3.75 and 1.9 mg/kg and high dose: 30, 15, 7.5 and 3.8 mg/kg, respectively and Dex: 0.5, 0.25, 0.125 and 0.06 mg/kg, respectively), or single dose of 15, 30 and 60 mg/kg for HC, or 0.125, 0.25 and 0.5 mg/kg for Dex on postnatal day 6. Pups in the corresponding vehicle groups received equivalent volumes of saline. The brain apoptosis was evaluated by caspase-3 activity, TUNEL stain and cleaved caspase-3 immunofluorescence and immunohistochemical analysis at 24 h after treatment. We found that all regimens of Dex, but not HC, significantly decreased

the gain of body and brain weight. A single dose did not induce brain apoptosis in either Dex or HC treatment groups. The tapering doses of Dex significantly increased caspase-3 activity, cleaved caspase-3 and TUNEL positive cells in the brain cortex and hippocampus. However, treatment with tapering doses of HC increased TUNEL positive cells in the brain cortex and hippocampus and caspase-3 positive cells in the hippocampus only at a high dose but not with doses in the middle or lower range. Our results suggest that Dex and high doses of HC cause mild but significant increases in apoptotic cell death in the developing rat brain, but equivalent and lower doses of HC do not affect the developing rat brain. Our findings support the assertion that HC is a safer alternative to Dex for the treatment of BPD in premature infants.

Disclosures: A.J. Bhatt, None; **Y. Feng**, None; **P.G. Rhodes**, None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS43991-07

NIH Grant NS43991-07S1

Title: Accumulation of mtDNA mutations and increased oxidative stress in a transgenic mouse model of HIV-associated sensory neuropathy

Authors: *A. HOKE, N. REED, C. ZHOU, W. CHEN;
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Abstract: Human immunodeficiency virus-associated sensory neuropathy (HIV-SN) is a common neurological complication of HIV infection. Underlying mechanisms are not well understood. Expression of HIV envelope protein, gp120, under the GFAP (glial fibrillary acidic protein) promoter leads to development of a sensory neuropathy affecting primarily the unmyelinated sensory fibers in mice aged 12-15 months. This process can be accelerated by administration of an antiretroviral drug, didanosine, for 4 weeks to young animals at 2-3 months of age (Keswani et.al. J. Neuroscience 2006). We examined the potential contribution of mitochondrial DNA (mtDNA) mutations and oxidative damage in this mouse model of HIV-SN. GFAP-gp120 transgenic mice treated with

didanosine develop increased mtDNA mutations with impaired function in freshly isolated mitochondria from distal sciatic nerves. In addition these animals accumulate markers of oxidative damage in their peripheral nerves. These data suggests that accumulation of mtDNA mutations leads to impaired mitochondrial dysfunction and oxidative damage that could contribute to distal axonal degeneration seen in this model of HIV-SN. These findings are similar what is seen in HIV patients with sensory neuropathy.

Disclosures: A. Hoke: Research Grant; NIH NS43991, NIH NS43991-07S1. **N. Reed:** None. **C. Zhou:** None. **W. Chen:** NS43991.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Animal model of neurologic autoimmunity induced by occupational exposure to aerosolized brain tissue

Authors: J. W. MEEUSEN[1], K. E. HASELKORN[1], T. J. KRYZER[1], S. T. EISENMAN[1], *V. A. LENNON[1,2,3];
[1]Neuroimmunology Laboratory, Dept. of Lab. Med. & Pathology, [2]Dept. of Immunol., [3]Dept. of Neurol., Mayo Clin., Rochester, MN

Abstract: Between November, 2006 and May, 2008, 21 of 500 workers (4.2%) who were exposed occupationally to aerosolized porcine brain tissue developed an inflammatory, painful sensory-predominant polyradiculoneuropathy that was associated with moderate motor disability and mild dysautonomia. The central nervous system (CNS) was involved in three patients (aseptic meningitis, meningoencephalitis and transverse myelitis). Individuals working closer to the point of brain extraction (executed by a blast of compressed air) had more severe disease. All patients' sera contained neural-reactive IgG that yielded a novel and complex immunostaining pattern. This "signature" IgG was also found in 34% of 85 at risk workers who were asymptomatic, but in none of 178 community controls. (Lachance et al, Lancet Neurol 9:55, 2010) Individual autoantigens identified by the patients' IgGs included voltage-gated potassium channel (VGKC; 79%) and myelin basic protein (76%). (Lennon et al, Ann Neurol 66:S63, 2009) After exposure stopped, serum antibody titers fell and patients improved.

Upon returning to work, relapse occurred and serum titers rose. These observations suggested an autoimmune basis for the neurological syndrome. To test the hypothesis that neurological autoimmunity is inducible by naso-pharyngeal exposure to neural tissue, we exposed mice to liquified porcine brain intranasally, twice daily. At 4 weeks, we detected serum IgG yielding the staining pattern characteristic of the patients' in 20% of mice. At 10 weeks, 100% of mice were seropositive for the "signature" IgG staining pattern and for myelin basic protein IgG, and 20% were positive for VGKC autoantibody. Induction of the same serum IgG markers of neurological autoimmunity in mice exposed to liquified porcine brain intranasally confirms the postulated autoimmune basis of the neurological disease encountered in swine abattoir workers. This unprecedented outbreak of occupational autoimmunity gives pause to consideration of therapeutic autoantigen tolerization by oro-nasal pharyngeal routes for human subjects with autoimmune neurological diseases, or multiple sclerosis.

Disclosures: J.W. Meeusen, None; **V.A. Lennon**, None; **K.E. Haselkorn**, None; **T.J. Kryzer**, None; **S.T. Eisenman**, None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: MS Society of Canada

Title: Granzyme-B as an effector molecule in T cell-mediated cytotoxicity on human Neurons: A novel pathogenetic mechanism for neurodegenerative processes of multiple sclerosis

Authors: Y. HAILE[1], K. SIMMEN[2], N. TOURET[2], T. SIMMEN[3], C. R. BLEACKLEY[2], *F. GIULIANI[4,1];
[1]Med., [2]Biochem., [3]Cell Biol., Univ. of Alberta, Edmonton, AB, Canada; [4]Edmonton, AB, Canada

Abstract: Introduction: Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by demyelination and neurodegeneration. Infiltrating T cells and macrophages are constituents of MS lesions. Activated cytotoxic T cells (CTLs) release a serine-protease granzyme B (GrB) which has been shown to

induce neurotoxicity. CTLs-mediated neuronal microtubule disruption was reported in animal model of MS, experimental autoimmune encephalomyelitis. Recent studies also showed alpha-tubulin, one of the main constituents of microtubules, as a new target for GrB. In this study, we investigated the mechanisms of GrB-mediated neuronal injury.
Methods: Human cortical foetal neurons (HFNs) were cultured alone, treated with GrB, co-cultured with unactivated or anti CD3-activated T cells on poly-ornithine coated culture plates. Viability assay was performed using immunocytochemistry for microtubule associated protein-2 or ß-tubulin to assess the survival of neurons. Expression of GrB by T cells was measured by RT-PCR. Confocal microscopy was used to follow the interaction of T cells and neurons, internalization of GrB into neurons and induction of apoptosis. Cleavage of alpha-tubulin, GrB substrate, was evaluated using Western blotting.
Results: The viability of HFNs was significantly low in cultures treated with activated T cells (32 ± 7%) or GrB (36 ± 9%). In mouse system, activated T cells from GrB knock out mice failed to kill mouse neurons. GrB entered into neurons via mannose-6-phosphate receptor (M6PR) and induced perforin-independent neuronal apoptosis. Western blotting showed caspase-dependent cleavage of alpha-tubulin in presence of GrB or activated T cells.
Conclusion: For the first time this study shows that GrB internalization into neuron is independent of perforin and occurs through M6PR mediation. When internalized, GrB destabilizes the cytoskeletal proteins, migrates into the neuronal soma and induces caspase-dependent neuronal apoptosis. These data support GrB as new potential target for neuroprotective strategies in MS treatments.

Disclosures: Y. Haile, None; **K. Simmen**, None; **N. Touret**, None; **T. Simmen**, None; **C.R. Bleackley**, None; **F. Giuliani**, None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NCL Foundation, Hamburg

DFG (SFB 581, to RM)

local funds of the University of Würzburg (to RM)

Batten Disease Family Association (BDFA)

Batten Disease Support and Research Association (BDSRA)

Title: Immune-related cells are pathogenic mediators in neuronal ceroid lipofuscinosis (NCL or Batten disease)

Authors: *J. GROH[1], T. KÜHL[2], A. KRONER-MILSCH[1], P. CROCKER[3], J. D. COOPER[2], R. MARTINI[1];
[1]Dept. of Neurol., Developmental Neurobiology, Univ. of Würzburg, Würzburg, Germany; [2]Dept. of Neurosci., Inst. of Psychiatry, King's Col. London, London, United Kingdom; [3]Wellcome Trust Biocentre, Sch. of Life Sciences, Univ. of Dundee, Dundee, United Kingdom

Abstract: The neuronal ceroid lipofuscinoses (NCLs or Batten Disease) are a group of fatal inherited lysosomal storage disorders. All display pronounced neurodegeneration that is preceded by a low grade of inflammation, but it is unclear whether this is pathogenic. We are investigating the role of inflammatory cellular components (lymphocytes and microglia) in two mouse models of NCL: early onset Infantile NCL (*Ppt1*$^{-/-}$ mice) and the slower progressing Juvenile NCL (*Cln3*$^{-/-}$ mice).
First, we characterised the types of immune cells present within the CNS and found evidence for progressive infiltration by different classes of immune cells in both forms of NCL. In *Ppt1*$^{-/-}$ mice there is a significant infiltration of CD8 cytotoxic T-cells (and to a lower extent CD4 helper T-cells) that occurs early in disease progression when neuron loss first starts, but these events happen much later in *Cln3*$^{-/-}$ mice. Both models also display microglial activation, with upregulation of the macrophage cell recognition molecule Sialoadhesin (*Sn*), which interacts with T-lymphocytes.
To analyse the pathogenic impact of lymphocytes, we crossbred *Ppt1*$^{-/-}$ mice with mice deficient in *Rag-1*, (which lack T- and B-lymphocytes) and scored neural damage in the optic nerve, an early target in the NCLs. In *Ppt1*$^{-/-}$ mice axonopathic changes (axonal swelling) were abundant in 3 month-old mutants, but were dramatically reduced in *Ppt1*$^{-/-}$/*Rag-1*$^{-/-}$ mice. Similarly, SMI32 staining as a marker of non-phosphorylated neurofilament was diminished in these double mutants. Next, we analysed the impact of microglial cells in INCL by crossbreeding *Ppt1*$^{-/-}$ and Sn$^{-/-}$ mice and found that axonopathic changes were robustly diminished in the absence of this microglial recognition molecule.
These observations suggest that both lymphocytes and microglia contribute to pathogenesis in *Ppt1*$^{-/-}$ mice and we are performing similar crosses with *Cln3*$^{-/-}$ mice. These studies are providing significant insights into the pathogenesis of individual forms of NCL and may provide information crucial for developing treatment strategies to block lymphocyte/microglial activation and encourage neuronal survival.

Disclosures: J. Groh: None. **T. Kühl:** None. **A. Kroner-Milsch:** None. **P. Crocker:** None. **J.D. Cooper:** None. **R. Martini:** None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: APDA Advanced Center for Parkinson Research at UAB

Parkinson Association of Alabama

Title: Fcγ receptors are required for NF-κB signaling, microglial activation and dopaminergic neurodegeneration in an AAV-synuclein mouse model of Parkinson's disease

Authors: *S. CAO, S. THEODORE, D. G. STANDAERT;
Dept. of Neurol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Overexpression of alpha-synuclein (α-SYN), a protein which plays an important role in the pathogenesis of Parkinson's disease (PD), triggers microglial activation and adaptive immune responses, and leads to neurodegeneration of dopaminergic (DA) neurons. We hypothesized a link between the humoral adaptive immune response and microglial activation in α-SYN induced neurodegeneration. To test this hypothesis, we employed adeno-associated virus type 2 (AAV2) to selectively over-express human α-SYN in the substantia nigra (SN) of wild-type mice and FcγR-/- mice, which lack high-affinity receptors for IgG. Using immunohistochemistry and quantitative PCR, we found that in wild type mice, α-SYN induced the expression of NF-κB p65 and pro-inflammatory molecules. In FcγR-/- mice, NF-κB activation was blocked and pro-inflammatory signaling was reduced. Microglial activation was examined using immunohistochemistry for gp91PHOX. At four weeks, microglia were strongly activated in wild-type mice, while microglial activation was attenuated in FcγR-/- mice. Dopaminergic neurodegeneration was examined using immunohistochemistry for tyrosine hydroxylase (TH) and unbiased stereology. α-SYN overexpression led to the appearance of dysmorphic neurites, and a loss of DA neurons in the SN in wild-type animals, while FcγR-/- mice did not exhibit neuritic change and were protected from α-SYN-induced neurodegeneration 24 weeks after injection. Our results suggest that the humoral adaptive immune response triggered by excess α-SYN plays a causative role in microglial activation through IgG-FcγR interaction. This involves NF-κB signaling, and leads to DA neurodegeneration. Therefore, blocking either FcγR signaling or specific

intracellular signal transduction events downstream of FcγR-IgG interaction, such as NF-κB activation, are viable therapeutic strategies in PD.

Disclosures: S. Cao, None; **S. Theodore**, None; **D.G. Standaert**, None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Investigations on neuroinflammation mediated by distinct Glutaminyl Cyclases (QCs)

Authors: *H. CYNIS[1], R. EICHENTOPF[1], S. GRAUBNER[2], S. SCHILLING[1], H.-U. DEMUTH[1];
[1]Probiodrug AG, Halle/Saale, Germany; [2]Ingenium Pharmaceuricals, Munich, Germany

Abstract: Neuroinflammation caused, e.g., by deposition of toxic Abeta-peptides in Alzheimer's disease leads to a profound activation of astrocytes and microglia cells in affected tissues. The proinflammatory chemokine CCL2 (MCP-1) plays a major role in mediating inflammatory activation and microglia migration. In this regard, CCL2 has been found to deteriorate plaque pathology in transgenic Alzheimer's disease mouse models. In addition, CCL2 is upregulated in early stages of Alzheimer's disease. We have shown, that posttranslational modification of CCL2 is mediated by Glutaminyl Cyclase (QC) activity leading to a proteolytically stable phenotype of CCL2 possessing a pyroglutamyl residue at the N-terminus (pGlu-CCL2).
During target validation studies of QC-activity inhibition in Alzheimer's disease (to prevent pGlu-Abeta formation) and inflammation/neuroinflammation (blocking pGlu-CCL2 maturation), we have discovered and isolated an isoenzyme of the secreted mammalian QC, which is sharing a similar substrate specificity but possessing a different subcellular localization as a resident enzyme of the Golgi complex. To gain further insights into the substrate conversion and regulation of QC and isoQC in vivo, we have challenged QC and isoQC knock out animals with inflammatory stimuli. First results point to a differential role of QC/isoQC in distinct inflammatory conditions. Furthermore, although both enzymes are located within the secretory compartment, evidence will be presented, that both enzymes convert different subsets of substrates in vivo. In addition, treatment of primary neuronal, astrocytic and microglial cell cultures with different Abeta

species reveals an upregulation of CCL2 upon stimulus.
The results point to a possible connection of inflammation and Abeta deposition mediated by the upregulation of QC-enzyme activity in Alzheimer's disease.

Disclosures: **H. Cynis**, Probiodrug AG, Employment; **R. Eichentopf**, Probiodrug AG, Employment; **S. Graubner**, Ingenium, Employment; **S. Schilling**, Probiodrug AG, Employment; **H. Demuth**, CSO Probiodrug AG, Ownership Interest; CEO Ingenium, Ownership Interest.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Contract N01-AI-30063

NIH Grant U54 AI-065357

NIH Grant RR020146

Title: Are West Nile virus (WNV) neurological symptoms caused by disruption of central autonomic pathways or focal lesions in the central nervous system?

Authors: *J. D. MORREY[1], H. WANG[1], V. SIDDHARTHAN[1], N. E. MOTTER[1], R. D. SKINNER[2];
[1]Utah State Univ., LOGAN, UT; [2]Ctr. for Translational Neurosci. and Dept. of Neurobio. and Developmental Sci., Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: To test the hypothesis that some WNV-induced neurological symptoms are caused by disruption of central autonomic pathways (dysautonomia) and some are caused by specific focal lesions, we employed a hamster model manifesting neurological disease signs similar to those occurring in human West Nile neurological disease. The following data suggests that suppression of electromyography (EMG) of the diaphragm is caused by dysautonomia. WNV-induced suppression of EMG begins as early as 3 days after subcutaneous (s.c.) viral challenge, which is before WNV-immunoreactive foci are readily found and long before H&E histopathology are noted in the brain stem or spinal cord. Moreover, we conclude that physiological events leading to EMG suppression have

already occurred before day 4, because a therapeutic antibody typically efficacious for other disease signs is not efficacious for EMG suppression when administered as early as day 4. Heart rate variability (HRV) was also employed as a marker for dysautonemia. Conversely, the following data suggests that suppression of motor neuron units (motor unit number estimation, MUNE) in hind limbs, gastrointestinal stasis, and reduced heart rate (bradycardia) is caused by specific focal lesions in the spinal cord, enteric intestinal plexus, or brain stem. All of these neurological diseases signs occur after day 7 when focal lesions are readily identified in the spinal cord and brain stem. MUNE suppression directly correlates to reduced choline acetyltransferase and numbers of motor neurons in the lumbosacral spinal cord, and to hind limb paralysis. Gastrointestinal stasis and bradycardia might also be caused by lesions in the thoracic and cervical cord, intestinal plexus or in the brain stem. In conclusion, WNV neurological symptoms are caused by focal lesions in the CNS, but disruption of central autonomic pathways typically caused by cell mediated factors such as cytokines may cause some WNV-induced neurological diseases signs.

Disclosures: J.D. Morrey, None; **H. Wang**, None; **V. Siddharthan**, None; **N.E. Motter**, None; **R.D. Skinner**, None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CNPq

FAPERJ

FUJB/UFRJ

Title: Identity of the cells recruited to a lesion in the protocerebral tract of a decapod crustacean

Authors: P. G. CHAVES DA SILVA[1], C. MONTEIRO DE BARROS[1], F. R. S. LIMA[1], A. BIANCALANA[1], A. M. B. MARTINEZ[1], *S. ALLODI[2];
[1]Programa de Biologia Celular e Desenvolvimento, Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [2]Programa de Biologia Celular e do Desenvolvimento, Inst. C

Biomedicas-CCS-UFRJ, Rio de Janeiro, Brazil

Abstract: In a previous paper of our laboratory (Corrêa et al., 2005), the protocerebral tract (PCT) of the crab *Ucides cordatus* was analised by light and electron microscopy, after extirpation of the eyestalk. The results of this paper showed that among axons with morphologic aspects of axoplasmic degeneration, granular cells resembling hemocytes were seen. This finding was very important because it motivated us to study the morpho-functional aspects of these cells, which are the blood cells of crustaceans present in the hemolymph. Since hemocytes were not described in this animal model so far, in this work we first used histochemistry, imunohistochemistry and (scanning and transmission) electron microscopy to characterize and classify them. Based on the literature and in the morphologic aspects observed, we classified the hemocytes in: hialinocytes, semigranular cells and granular cells. Then, we investigated the participation of these cells in the acute degenerative process of the PCT (24h after lesion) and evaluated some of their possible functions. We found that the majority of the cells recruited to the lesion zone were constituted by semigranular and/or granular hemocytes, which were labeled by a lectin that is used to identify macrophages, and with a morphology resembling cells in the hemolymph. The exception was a specific type of cell that was only seen by scanning electron microscopy in the injured PCT and was not found in the animals in normal conditions. Glial fibrillary acidic protein (GFAP)-positive cells were also increased in the injuried local. Our results suggest that hemocytes are attracted to the lesion site in the acute stage of degeneration. In addition, we believe that besides phagocitizing neural debris, a function that is shared with the local glial cells, hemocytes may have been attracted to the lesion in order to produce important factors necessary for glia proliferation and activation.

Disclosures: P.G. Chaves da Silva, None; **C. Monteiro de Barros**, None; **F.R.S. Lima**, None; **A. Biancalana**, None; **A.M.B. Martinez**, None; **S. Allodi**, None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: MSSC

NSERC

CIHR

Title: Recruitment of crawling inflammatory monocytes in the cerebral vasculature: Regulation by pertussis toxin

Authors: *L. VALLIERES, M. ROY, J.-F. RICHARD;
Endocrinol. and Genomics, Laval Univ. Hosp. Res. Ctr., Quebec, QC, Canada

Abstract: We have recently identified a new population of monocytic precursors that patrol the cerebral vasculature by crawling on the endothelial surface and that can penetrate the brain to give rise to mononuclear phagocytes, which are known to play essential roles in the development of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). We have shown that these cells are recruited in greater number in response to bacterial lipopolysaccharide by a mechanism involving TNF, IL-1ß, and angiopoietin-2. Here we report that pertussis toxin (PTX), which is commonly used to induce EAE, produces a similar recruitment of phagocytes without inducing these three mediators. Instead, it acted in part through IL-6, as demonstrated in IL-6 knockout mice. PTX also acts on the vasculature to induce ICAM1 and VCAM1 expression, likely via a mediator that is not IL-6 and that remains to be identified. Furthermore, we found evidence that the adhesion of phagocytes to the cerebral vasculature is independent of the receptors CCR1, CCR2, CX3CR1, ITGα4, ITGβ1, and ITGβ7. In conclusion, this study supports the concept that PTX induces EAE by promoting vascular changes necessary for leukocyte infiltration via a non-conventional mechanism. This study also helps to understand how environmental toxins could influence the development of MS.

Disclosures: L. Vallieres, None; **M. Roy**, None; **J. Richard**, None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS K08NS52550

Title: ABCD1 deficiency impairs mononuclear phagocytic cells: implications for

neurodegeneration

Authors: *F. EICHLER, K. GAROFALO, B. SCHMIDT, N. ELPEK, T. MEMPEL, J. EL KHOURY;
Massachusetts Genl Hosp., BOSTON, MA

Abstract: ABCD1 is an ATP-binding cassette transporter protein involved in fatty acid metabolism. Mutations in ABCD1 cause X-linked adrenoleukodystrophy (ALD), a genetic disorder of the peroxisome associated with devastating inflammatory demyelination in childhood. The role of ABCD1 in other neurological disorders is unknown. We previously reported that microglial apoptosis in perilesional white matter represents an early stage in lesion evolution in cerebral ALD. We investigated the peripheral mononuclear and brain microglial cell response in a mouse model with targeted inactivation of the ABCD1 gene. Using a sterile peritonitis model we assessed monocyte recruitment in ABCD1 -/- mice. We also investigated the role of ABCD1 in acute experimental autoimmune encephalitis (EAE). In order to study ABCD1-/- brain microglia in vivo, we crossed the ABCD1 -/- mouse with a mouse in which the chemokine receptor CX3CR1 has been replaced by the GFP reporter gene. Using in vivo two-photon microscopy we then studied the response of brain microglia to laser injury. In all three scenarios mononuclear phagocytic cells demonstrated aberrant function. Following thioglycolate stimulation only 50% of cells were found within the peritoneum of the ABCD1-/- mouse (30% macrophages compared to 60% in wild type). The ABCD1-/- mouse also showed an attenuated response to EAE induction. This manifested in both a delayed onset by 1-2 days and decreased severity of disability (mean maximum score of 1.9+/-0.33 versus 4.6+/-0.4). Lastly, following laser injury ABCD1 -/- microglial processes showed defective convergence of processes upon the site of injury as measured by difference in fluorescence intensity. These observations confirm that there is an aberrant innate immune response in ALD. These data also suggest that ABCD1 could be involved in regulating the mononuclear phagocytic response in other neurodegenerative disorders. We are in the process of investigating the molecular pathways disrupted by deficiency of the ABCD1 protein.

Disclosures: F. Eichler: Research Grant; NINDS-K08NS52550. **K. Garofalo:** None. **B. Schmidt:** None. **N. Elpek:** None. **T. Mempel:** None. **J. El Khoury:** None.

Nanosymposium

728. Neuroinflammation and Degeneration

Location: Room 31C

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 728.11

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR Frederick Banting and Charles Best Doctoral Award

CIHR Operational Grant

Title: Peripheral immune cell-cerebral endothelial cell interactions drive microglial activation within the brain during peripheral inflammatory disease

Authors: *C. D'MELLO, H. ZHOU, T. LE, M. SWAIN;
Immunol., Univ. of Calgary, Calgary, AB, Canada

Abstract: Pathways whereby the immune system communicates with the brain during peripheral inflammatory diseases are unclear. Peripheral inflammation is associated with activation of circulating immune cells which in turn can interact with endothelium to activate cells within tissues; however, this is not well defined for the brain. Therefore, we speculated that during peripheral inflammation intimate peripheral immune cell:cerebral endothelial cell (CEC) interactions may drive cerebral microglial activation. To investigate this, we used a well characterised model of hepatic inflammation due to bile duct resection (BDR) vs. Sham controls.
Results: Using intravital microscopy, we observed a striking increase in the number of peripheral leukocytes that rolled and adhered along CECs in BDR mice (# of rolling leukocytes- BDR: 6.1 ± 0.7 vs. Sham: 0.18 ± 0.07; n=5/group, $p<0.05$). In addition, we documented increased numbers of activated microglia in BDR (5940 ± 919 cells/brain) vs. Sham (3066 ± 564 cells/brain; n=7/group, $p<0.05$) mice, as reflected by increased monocyte chemoattractant protein (MCP)-1 expression. Intravital microscopy in neutrophil depleted (via anti-Ly6G treatment) Lysozyme-GFP BDR mice (in which myeloid cells express GFP), demonstrated monocytes to be the cells that rolled and adhered along CECs. In BDR mice treated with anti-P selectin antibody (prevented rolling and adhesion of monocytes to CECs) there was a marked reduction in the number of activated microglia. Tumor necrosis factor (TNF)-α is a well documented mediator of communication between the periphery and the brain during systemic inflammation. The number of circulating monocytes that expressed TNFα was ≈2-fold higher in BDR vs. Sham mice. Furthermore, in BDR mice treated with anti-TNFα serum intraperitoneally, rolling and adhesion of peripheral monocytes along CECs was inhibited (# of rolling monocytes- WT BDR: 6.3 ± 1.0 vs. anti-TNFα treated BDR: 1.4 ± 0.1; n=3/group, $p<0.05$) and this in turn prevented activation of cerebral microglia (# of MCP-1 expressing microglia/brain- WT BDR: 4957 ± 545 vs. anti-TNFα treated BDR: 2799 ± 338, n=4/group, $p<0.05$). TNFR1 expression was higher on CECs in BDR mice and TNFα signalling via TNFR1, but not TNFR2, was required for our observed effects.
Conclusions: Our results demonstrate that in mice with peripheral organ centered inflammation, the intimate interaction of peripheral immune cells with CECs can activate microglia within the brain, via a TNFα/TNFR1 dependent pathway. These findings may have implications for cerebral changes commonly encountered in inflammatory diseases occurring outside the CNS.

Disclosures: C. D'Mello, None; **H. Zhou**, None; **T. Le**, None; **M. Swain**, None.

Nanosymposium

729. Gene Therapy For Diseases of the Brain

Location: Room 5B

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 729.1

Topic: C.14. Gene Therapy

Support: International Rett Syndrome Foundation

Hannah's Hope Fund

Amyotrophic Lateral Sclerosis Association

Oregon National Primate Research Center P51 RR 000163

Title: Gene delivery to the nonhuman primate brain via systemic administration of adeno-associated virus (AAV) serotype 9

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Abstract: Adeno-associated viruses (AAV) are considered ideal vectors for gene therapy because of their low immunogenicity and long-term expression. Due to the inability of most viral vectors to cross the blood brain barrier (BBB), past efforts to regulate gene expression in the brain using AAV vectors have relied mostly on intracerebral, stereotaxically guided methods of administration. Very recently, AAV serotype 9 (AAV9) was shown to successfully deliver transgenes to the rodent brain via a transvascular approach (Foust *et al.* 2009, Nat. Biotech. 27:59). The objective of our study was to determine if a similar approach would be successful when targeting the nonhuman primate brain. To begin answering this question, we investigated the bio-distribution of self-complementary (sc) AAV9 after systemic injection to young (3-4 year-old) male rhesus monkeys. scAAV9-GFP was administered to 2 animals intravenously (i.v., saphenous vein; 8.7X10E12 vector genome (vg)/kgBW and 9.1X10E12 vg/kgBW) or via intra-carotid infusion (i.c., 9X10E12 vg/kgBW and

9.5X10E12 vg/kgBW); green fluorescent protein (GFP) expression was used as a reporter marker. All animals tolerated the infusion procedure well and exhibited no side effects. Twenty-eight days after scAAV9-GFP administration, animals were euthanized and the brain, the spinal cord and multiple peripheral organs (liver, heart, spleen, GI tract, testes, etc.) were collected to assess scAAV9-GFP bio-distribution by GFP staining and quantitative PCR. Results show that scAAV9-GFP crosses the BBB of rhesus monkeys and transduces both neurons and astrocytes of the brain and spinal cord, with a preference for astrocytes, and with higher efficiency after i.c. administration of the virus. As expected, peripheral transduction (e.g. heart) by scAAV9-GFP was observed after both i.v. and i.c injections. Ongoing studies are aimed at (i) quantifying the number of GFP+ cells in the brain by unbiased stereology, (ii) identifying the cells types transduced by scAAV9-GFP in the brain, and (iii) determining if the systemic injection of scAAV9-GFP results in any measurable response of the innate and/or adaptive immune systems. Results from these studies aimed at quantifying the extent of CNS transgene delivery will establish the feasibility of introducing a foreign gene to the primate CNS via a systemic route and provide usable translational data for clinical research and therapeutic purposes.

Disclosures: V. matagne, None; **S. Gray**, None; **J. McBride**, None; **S.R. Ojeda**, None; **J. Samulski**, None.

Nanosymposium

729. Gene Therapy For Diseases of the Brain

Location: Room 5B

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 729.2

Topic: C.14. Gene Therapy

Support: R/115161

Title: Systemic delivery of scAAV9 expressing SMN prolongs survival in a model of spinal muscular atrophy

Authors: C. F. VALORI, K. NING, M. D. WYLES, *M. AZZOUZ;
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Abstract: SMA represents a recessive autosomal disorder and is one of the most common genetic causes of death in childhood. It is caused by mutations of the survival motor neuron (*SMN*) gene, leading to depletion in SMN protein levels. To date there are no effective drug treatments for this disease. We previously reported that Lentiviral

vector expressing *SMN* was successfully used to increase the life expectancy by 5 days compared to control animals [1]. Additional studies are therefore needed to optimise delivery vector systems for optimal SMN protein distribution. The aim of the current project is to assess the efficiency of self-complementary AAV serotype 9 (scAAV9) mediated SMN gene replacement in SMA mouse model. scAAV9 expressing codon optimised version of SMN or GFP has been administered systemically into postnatal day 1 (P1) delta7SMA mice [2]. Each mouse was injected with 10^{11} viral particles. Animals were assessed by body weight and behavioral tests. scAAV9 mediates efficient and sustained transgene expression in cells of the nervous system following systemic administration. Here, we report that a single injection of SMN-expressing scAAV9 vector into the facial vein reversed the phenotype of delta7SMA mice [2]. Most notably, SMN replacement led to robust increase in the life expectancy compared to controls, thereby achieving one of the highest therapeutic effects reported in the field to date. Taken together, this study reports a preclinical proof-of-concept that scAAV expressing SMN has the greatest therapeutic efficiency in stimulating motor neuron survival in SMA. Based on these findings, we are planning to initiate clinical trials using the currently used SMN gene transfer methods in the very near future.

References

1. Azzouz, M., *et al. J. Clin. Inv.* **114**, 1726-1731 (2004).
2. Le, T.T., *et al*. *Hum. Mol. Gent.* **14**, 845-857 (2005).
This work was supported by JTSMA and SMA Trust.

Disclosures: C.F. Valori, None; **M. Azzouz**, None; **K. Ning**, None; **M.D. Wyles**, None.

Nanosymposium

729. Gene Therapy For Diseases of the Brain

Location: Room 5B

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 729.3

Topic: C.14. Gene Therapy

Support: NIH Grant RC2- NS069476

NIH Grant R01-NS038650

Title: scAAV9-mediated gene therapy in spinal muscular atrophy: Implications for autonomic involvement

Authors: *A. K. BEVAN[1,2], K. D. FOUST[1], L. BRAUN[1], L. SCHMELZER[1], A. H. M. BURGHES[3], B. K. KASPAR[1,3,2];
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Abstract: Proximal spinal muscular atrophy (SMA) is a fatal neurological disorder that causes dysfunction and death of spinal motor neurons which leads to subsequent atrophy of skeletal muscle. SMA affects about 1 in 8000 live births across the globe and is the leading genetic cause of infant death in the US. SMA is the result of a homozygous deletion or mutation of the *SMN1* gene, but an almost identical gene, *SMN2,* is a modifier of disease severity by producing low levels of SMN. While SMN is ubiquitously expressed in cells throughout the body, the primary pathology is classically thought to only include lower motor neurons of the spinal cord.
Recently, we have reported on a successful gene therapy approach using scAAV9 to introduce SMN into the CNS of an animal model of SMA. We have also recently shown that the success of our gene therapy approach is due not only to its ability to rescue motor neurons, but also to treat bradycardia, likely through the transduction of key autonomic loci.
In this study, we have further characterized the ability of scAAV9 to transduce key neural loci in both mice and non-human primates at different ages to further define the potential translatability of our gene therapy approach. In mice, highly efficient transduction of motor neurons is limited to the first 10 days postnatally. In non-human primates, we have found that this window of opportunity is open significantly longer, thereby increasing the likelihood of success in future human clinical trials.

Disclosures: A.K. Bevan, None; **K.D. Foust**, None; **L. Braun**, None; **L. Schmelzer**, None; **A.H.M. Burghes**, None; **B.K. Kaspar**, None.

Nanosymposium

729. Gene Therapy For Diseases of the Brain

Location: Room 5B

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 729.4

Topic: C.14. Gene Therapy

Support: International Rett Syndrome Foundation

Hannah's Hope Fund

Title: Promoter optimization for global CNS transgene delivery and expression by self-complementary AAV vectors

Authors: *S. J. GRAY[1], J. W. SCHWARTZ[2], S. FOTI[1], M. D. EHLERS[2], T. J. MCCOWN[1], R. J. SAMULSKI[1];
[1]Gene Therapy Ctr., Univ. of North Carolina, Chapel Hill, NC; [2]Neurobio., Duke Univ., Durham, NC

Abstract: Recombinant adeno-associated virus (AAV) is regularly used as a gene therapy vector for diseases affecting the Central Nervous System (CNS). Traditional AAV vectors utilize a single-stranded (ss) genome and package approximately 4.5 kb of foreign DNA. Self-complementary (sc) AAV vectors are up to 100 times more efficient, but their packaging capacity is only 2.2 kb of foreign DNA. Reports of global CNS gene delivery by intra-CSF or intravenous (i.v.) injection of AAV have relied on sc AAV vectors, as ss AAV vectors so far have not shown adequate transduction efficiency for these applications. While global CNS gene delivery offers immense promise for treating a wide range of CNS disorders, the packaging constraints of sc AAV vectors present a challenge for engineering compact gene expression cassettes, especially for transgenes over 1.2 kb.

For ubiquitous expression, sc AAV expression cassettes commonly utilize either the CMV promoter or a truncated chicken beta actin (CBA) promoter, each of which is 800 bp. Upon i.v. injection of AAV serotype 9 vectors in adult mice, we report differences in the cell-specific expression pattern of GFP driven by these so-called "ubiquitous" promoters in the CNS. The CMV promoter provides high expression in neurons and glia, but silences over time in some neural populations. For the CBA promoter, a striking difference was observed comparing identical constructs that differed only in an intron in the 5' untranslated region of the transgene cassette. A CBA promoter with the SV40 late 16S intron displayed high glial and neuronal expression, but highly reduced expression in motor neurons (MNs). The same construct utilizing a hybrid CBA/MVM intron (dubbed "CBh") gave robust long-term expression in all cells observed with CMV or CBA, including MNs. These results demonstrate the profound difference that an intron can play in regulating cell-specific gene expression. The 800 bp CBh promoter we developed offers truly ubiquitous and long-term gene expression *in vivo*. However, when combined with a 200 bp polyA signal, the CBh promoter restricts the use of only genes 1.2 kb or less to fit into a sc AAV vector.

In the interest of packaging a larger transgene into sc AAV vectors, we investigated the use of a 229 bp fragment of the murine MeCP2 promoter (MeP). MeP drives preferentially neuronal gene expression, albeit at lower levels compared to the CBh promoter. When combined with a 60 bp synthetic polyA, the MeP allows a gene up to 1.9 kb to be packaged into a sc AAV vector. Using this promoter, we have designed therapeutic vectors to package the 1.8 kb gigaxonin gene and the 1.5 kb MeCP2 gene for Giant Axonal Neuropathy and Rett Syndrome, respectively.

Disclosures: S.J. Gray, None; **J.W. Schwartz**, None; **S. Foti**, None; **M.D. Ehlers**, None; **T.J. McCown**, None; **R.J. Samulski**, None.

Nanosymposium

729. Gene Therapy For Diseases of the Brain

Location: Room 5B

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 729.5

Topic: C.14. Gene Therapy

Support: American Heart Association

NIH EYO16119

Title: AAV2 mediated hypoxia responsive astrocyte-specific gene therapy

Authors: *J. C. BLANKS[1], M. BISWAL[2], K. A. WEBSTER[4], H. PRENTICE[3];
[1]Ctr. Complex Systems, [2]Integrative Biol., Florida Atlantic Univ., BOCA RATON, FL;
[3]Col. of Biomed. Sci., Florida Atlantic Univ., Boca Raton, FL; [4]Vascular Biol. Inst., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Brain ischemia following vascular occlusion is characterized by tissue hypoxia as well as oxidative stress which under severe conditions results in neuronal cell death. Astrocytes play an important role in the brain under ischemic conditions including acting as a source of trophic factors and controlling reuptake of the excitatory neurotransmitter glutamate. While cell specific gene therapy vectors are valuable for ensuring sustained targeted protein expression, the potential for regulating expression by hypoxia has the added advantage of ensuring appropriate timing of expression as well as avoiding side effects of unregulated expression. We have designed astrocyte-specific promoters containing hypoxia responsive elements (HRE) as well as silencer domains that provide both cell-specific, hypoxia-inducible expression as well as normoxic silencing. To make the hypoxia responsive tissue specific promoter, several HREs and a human glial fibrillary acidic protein (GFAP) promoter were incorporated in a pGL3 based luciferase reporter vector. To restrict promoter activation in normoxia, aerobically silenced elements (HRSE) were also incorporated at the 5' end of HRE elements. The plasmids were transfected in rat primary astrocytes, as well as other cell lines: human embryonic kidney (HEK), C6 glioma, mouse neuronal (HT22), and human retinal pigment epithelial (ARPE-19) cells. After 40 hours of hypoxic and normoxic exposure, the dual luciferase assay was performed to measure the activity of promoters. Our preliminary results show that GFAP promoter was modestly activated (< 3 fold), whereas the regulated GFAP promoter was induced by more than 15-fold in hypoxia. The regulated GFAP promoter was silenced in astrocytes in normoxia, whereas this promoter was inactive in both

normoxia and hypoxia in all other cell lines (HEK, C6, HT22, and ARPE-19). Promoter constructs were also incorporated into a self-complementary Adeno Associated virus (scAAV) plasmid to produce scAAV2 vectors expressing green fluorescent protein (GFP) in rat primary astrocytes. The tranduced cells were exposed to hypoxia and normoxia for 40 hours and analyzed for GFP expression. Our results demonstrated that the regulated promoter construct was completely silenced in aerobic conditions in primary astrocyte cultures. Exposure to hypoxia induced a high level of GFP expression in transduced astrocytes. In conclusion, our hypoxia-regulated, astrocyte-specific promoter provides an effective platform for AAV-based gene therapy which is restricted to astrocytes in the brain under conditions of hypoxia or ischemia.

Disclosures: J.C. Blanks, None; **M. Biswal**, None; **H. Prentice**, None; **K.A. Webster**, None.

Nanosymposium

729. Gene Therapy For Diseases of the Brain

Location: Room 5B

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 729.6

Topic: C.14. Gene Therapy

Support: GPSA, ASU

Title: A novel biochip for the controlled delivery of siRNA molecules to a targeted population of primary neurons in a culture

Authors: *C. PATEL, A. SRIDHARAN, J. MUTHUSWAMY;
Harrington Bioengineering, Arizona State Univ., Tempe, AZ

Abstract: Gene silencing techniques such as RNA interference (RNAi) are rapidly being adopted for treatment of neurological disorders such as Alzheimer's disease (AD), Parkinson's disease, etc. However, a major challenge in RNAi based studies is the efficient delivery of siRNA molecules to targeted neurons. Current non-viral techniques, such as chemical transfection or gene injection, do not offer the required spatial and temporal control or require tedious manual manipulation and are inefficient. In this study we present a novel biochip that can spatially target and transfect a population of primary neuronal cells in vitro in a high throughput fashion using electroporation. In addition to precisely controlled spatial and temporal transfection, the biochip also allows simultaneous assessment of a) neuronal morphology, b) specific proteins using

fluorescent immunohistochemistry and c) electrical activity of single neurons and neuronal networks. The biochip utilizes a MEA (microelectrode array) that can induce electroporation in a targeted group of cells in a culture by controlling the electric field intensities associated with the electrodes and simultaneously monitor electrical activity of single neurons. An optically transparent MEA was micro-patterned on a glass substrate using standard photolithography procedure. The technology was tested for site-specific and controlled transfection of primary neurons and 3T3 fibroblasts with siRNA and PI, a cell impermeant dye and transfection efficiencies >60% and cell viabilities >80% were achieved. The obtained transfection efficiencies were considerably higher when compared with chemical transfection. siRNA based silencing of GAPDH (a house keeping gene) and monitoring of electrical activity of targeted neurons both before and after transfection using the biochip is currently being investigated. Independent control of the individual electrodes allowed for 32 independent transfection based experiments to be performed on the same culture. Development of this technology offers a novel method for low cost, efficient and high throughput RNAi based studies and controlled delivery of nucleic acids to targeted population of neurons.

Disclosures: C. Patel, None; **A. Sridharan**, None; **J. Muthuswamy**, None.

Nanosymposium

729. Gene Therapy For Diseases of the Brain

Location: Room 5B

Time: Wednesday, November 17, 2010, 8:00 am - 9:45 am

Program Number: 729.7

Topic: C.14. Gene Therapy

Support: CIRM RT1--01107-1

Title: Genetic correction of SOD1 point mutations in ALS patient derived iPS cells

Authors: *Y. LIU[1,2], H. XUE[1], I. GARITAONANDIA[2], H. TRAN[2], S. PAPADEAS[3], P. JIANG[4], P. LIEU[5], L. LAURENT[1], W. DENG[4], N. MARAGAKIS[3], M. RAO[5], J. LORING[2];
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Abstract: Human induced pluripotent stem cells (iPSCs) derived from patients are a powerful tool to model diseases in vitro. Identification and correction of the genetic

defects in a culture dish will provide proof of principles for future personalized medicine. We have derived iPSCs from the dermal fibroblasts of two cases of familial Amyotrophic Lateral Sclerosis (ALS) patients with different point mutations at the Cu-Zn superoxide dismutase 1 (SOD1) gene. Our goals are to perform genetic repair at the SOD1 locus using homologous recombination and to evaluate motoneurons and astrocytes derived from lines both before and after genetic correction for cell growth, survival, apoptosis, and differentiation behavior in normal culture conditions as well as under stress. We have developed a fast and precise method for building targeting vectors by recombineering-based technology and streamlined gene targeting procedure in human iPSCs. Results from this work will offer proof-of-concept that iPSCs can be used for the generation of disease-corrected, patient-specific cells with potential values for cell therapy application. This work will also facilitate functional studies and drug discovery aimed at treating neurodegenerative diseases.

Disclosures: Y. Liu, None; **H. Xue**, None; **I. Garitaonandia**, None; **H. Tran**, None; **S. Papadeas**, None; **P. Jiang**, None; **P. Lieu**, None; **L. Laurent**, None; **W. Deng**, None; **N. Maragakis**, None; **M. Rao**, None; **J. Loring**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.1

Topic: D.01. Chemical Senses

Support: R01 DC005964

R01 NS068409

DP1 OD006437

Title: Population level functional characterization of sulfated-steroid responsive vomeronasal sensory neurons

Authors: D. TURAGA[1], P. S. XU[1], *T. E. HOLY[2];
[1]Anat. & Neurobio., Washington Univ. Sch. of Med., Saint louis, MO; [2]Anat. & Neurobio., Washington Univ. Sch. of Med., SAINT LOUIS, MO

Abstract: In mice, the accessory olfactory system (AOS) plays an important role in

detecting social odors and producing characteristic behaviors. The vomeronasal organ (VNO) contains the primary sensory neurons (vomeronasal sensory neurons, VSNs) of the AOS. Due to experimental constraints, full functional characterization of VSN response types has proven to be difficult.
Recently we have developed a light-sheet based optical sectioning technique called objective coupled planar illumination microscopy (OCPI) which allows for simultaneous measurement of activities of thousands of neurons. Here we use OCPI microscopy to image VSNs expressing GCaMP2 (a genetically encoded calcium sensitive fluorescent protein) and measure their activity in response to ligands. In particular we use sulfated-steroids - a recently identified class of ligands activating the VNO - to functionally characterize the VSNs. 12 structurally distinct sulfated-steroids were applied to the tissue in a random interleaved order multiple times and the resultant activity was measured.
In a single experiment we were able to measure the simultaneous activities of ~2500 VSNs from a single female VNO. ~30% of the measured VSNs were responsive to the set of sulfated-steroids. We have tabulated the proportion of VSNs activated by each of the ligands. For example, 17-beta-estradiol sulfate produced the maximum activity (411/2480 VSNs) while testosterone sulfate produced comparatively lower activity (31/2480). Clustering algorithms identified distinct patterns from the neural responses to these ligands. For example, corticosterone 21-sulfate and hydrocortisone 21-sulfate activate mostly the same VSN populations (70/2480). In addition to functional characterization, we were also able to study the spatial localization of each of the functional types. Based on the distance from the surface of the epithelium, most of the functional types are in the apical regions of the VNO. There was one exception - a functional cluster which represents VSNs activated by epipregnanolone sulfate, allopregnanolone sulfate and epitestosterone sulfate (36/2480) that appears to be in the basal layer of the VNO.
Our experiments allow for a comprehensive population level characterization of the sulfated-steroids responsive VSNs and will enable a detailed examination of sensory differences between sexes and individuals.

Disclosures: D. Turaga, None; **T.E. Holy**, None; **P.S. Xu**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.2

Topic: D.01. Chemical Senses

Support: NIH R01 DC005964

NSF IGERT grant 0548890

Title: Robust encoding of concentration in the accessory olfactory system

Authors: *H. A. ARNSON[1], T. E. HOLY[2];
[1]Anat. and Neurobio., Washington Univ. In St. Louis, SAINT LOUIS, MO; [2]Anat. and Neurobio., Washington Univ. In St. Louis, Saint louis, MO

Abstract: The accessory olfactory system (AOS) is specialized for detecting social cues, a major source of which is urine. Evidence suggests that the levels and identities of specific compounds found in urine, such as sulfated steroids, can be used to communicate relevant information. However, the perception of concentration can be confounded by environmental uncertainty - naturalistic stimuli such as urine are subject to variable dilution or evaporation in ways that are not necessarily biologically relevant. One way to achieve a stable representation of concentration is through ratio encoding. While the absolute concentration of compounds will change following urine dilution or evaporation, the relative concentration of two compounds will not. A plausible mechanism to compute ratios is through logarithmic encoding of concentration, based on the principle that $\log(X) - \log(Y) = \log(X/Y)$. This scheme requires that neurons represent concentration linearly with respect to the log-concentration, a task that is complicated by receptor saturation. A potential solution involves representing log-concentration at the population level, which requires receptor neurons with a range of sensitivities to the same compounds and the pooling of information across cells.
We set out to investigate whether it is possible to extract log-concentration from populations of sensory neurons in the vomeronasal organ and if this information could be used to compute concentration ratios. Using multielectrode array recordings and calcium imaging, we found that neurons responded with selectivity to a diverse battery of sulfated steroids and displayed differing sensitivities across a range of stimulus concentrations. We found that sensory neuron population activity could be used to encode log-concentration. Using a linear model inspired by the anatomy of the AOS, we found that sensory neuron information could be used to represent the log-ratio of concentrations under specific parameters such as particular synaptic weights and numbers of neurons. This provides evidence that the AOS, in principle, is capable of stably encoding concentration information based on sensory neuron activity and anatomical properties.

Disclosures: H.A. Arnson, None; **T.E. Holy**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.3

Topic: D.01. Chemical Senses

Support: Heidelberger Akademie der Wissenschaften

EMBO

BBSRC

DFG

MPG

Title: Slow evolution of synaptic Ca2+ signals in adult mouse olfactory bulb granule cell spines

Authors: *V. EGGER[1], N. M. ABRAHAM[2], A. T. SCHAEFER[3], T. KUNER[2];
[1]Inst. Physiologie, LMU, Muenchen, Germany; [2]Inst. for Anat. and Cell Biol., Heidelberg, Germany; [3]MPI for medical research, Heidelberg, Germany

Abstract: In the vertebrate olfactory bulb, axonless granule cells (GC) mediate self- and lateral inhibitory interactions between mitral/tufted cells via reciprocal dendrodendritic synapses. Synaptic GC output occurs on both fast and slow time scales, allowing for multiple GC functions during olfactory processing. Here we describe a possible mechanism for delayed GC output. We used current-clamp recordings and two-photon imaging in acute brain slices from adult mice (PND 36-65; dye: 100 µM OGB-1; room temperature) and sensory-like stimulation, i.e. extracellular activation of a glomerulus. We also studied responses in GCs where either GluRN1 or GluRA2 were deleted via viral transfection.
We observed 16 evoked responses in 13 spines with an average amplitude of 52 ± 47 % ΔF/F. The average release probability was estimated as p = 0.11 ± 0.06. While the onset of the evoked synaptic responses was tightly correlated to the stimulus, in most cases the peak amplitude occurred several 100 ms later (average time to peak 410 ± 460 ms, n = 12; 2 events with peak past the duration of the scan, i.e. > ~ 1000 ms).
This slow rise was independent of Ca^{2+} entry via NMDARs, since similar signals occurred in GluN1 knockout GCs with reduced Ca^{2+} entry (34 ± 10%, n = 12 spines; average time to peak 550 ± 300 ms, n = 13 events; 4 more with peak > ~ 1000 ms). Additional Ca^{2+} entry due to deletion of GluA2, however, resulted in larger ΔF/Fs that rose even more slowly (73 ± 17%, n = 8 spines; ΔF/F time to peak 700 ± 250 ms, n = 7 events; 13 more with peak >~ 1000 ms; P = 0.03 vs WT for all responses, Mann-Whitney test). We also observed 6 spontaneous events in the WT spines, with a similar amplitude as for the evoked responses (57 ± 26 % ΔF/F) and a faster rise (145 ± 65 ms) which could

be explained by the frequent occurrence of EPSP barrages in response to glomerular stimulation.
Thus the slowly evolving events constituted the majority of synaptic responses; they may allow for asynchronous, long latency GC output. Since GluA2$^{\Delta GCL}$ animals performed significantly faster in a difficult odor discrimination task than WT animals, the observed increase in rise times apparently does not interfere with fast odor discrimination.

Disclosures: V. Egger, None; **N.M. Abraham**, None; **A.T. Schaefer**, None; **T. Kuner**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.4

Topic: D.01. Chemical Senses

Support: DC00566

DC04657

DC008066

DC002994

Title: Associative cortex in the first olfactory brain relay station?

Authors: *D. RESTREPO[1], M. T. LUCERO[2], D. H. GIRE[1], W. DOUCETTE[1];
[1]Cell Dev. Biol & Neurosci Prgm, Univ. Colorado Denver, Aurora, CO; [2]Univ. of Utah, Salt Lake City, UT

Abstract: Synchronized firing of mitral cells in the olfactory bulb, the first relay station in the olfactory system, has been hypothesized to help bind information together in olfactory cortex. In this first survey of synchronized firing by mitral cells in awake-behaving vertebrates, we find sparse divergent odor responses during go-no go odor discrimination tasks. Surprisingly, when synchronized mitral cell firing is measured in the odor discrimination task the rewarded odor elicits an increase in firing rate, and the unrewarded odor elicits a decrease regardless of the odor used, and even when rewarded/unrewarded odors are reversed. Simultaneous addition of α and β adrenergic

blockers to the olfactory bulb results in marked diminution of the magnitude of the differential synchronized firing response to rewarded and unrewarded odors (but not in regular mitral cell firing). Our study indicates that synchronized firing conveys information on odor value (is it rewarded?) rather than odor quality (what is the odor?). These data suggest that mitral cells contribute to decision-making through adrenergic-modulated synchronized firing.

Disclosures: D. Restrepo, None; **W. Doucette**, None; **D.H. Gire**, None; **M.T. Lucero**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.5

Topic: D.01. Chemical Senses

Title: The role of interneurons in noradrenergic plasticity in the main olfactory bulb

Authors: H. S. DEMMER[1], *S. D. SHEA[2];
[1]Cold Spring Harbor Lab., Cold spg hbr, NY; [2]Cold Spring Harbor Lab., COLD SPG HBR, NY

Abstract: Noradrenaline (NA) release in the main olfactory bulb (MOB) plays an especially critical role in certain forms of mammalian social memory, as well as in other forms of associative odor learning. NA levels are dramatically increased in the MOB during social encounters, very likely due to release from axons originating in locus coeruleus (LC). Odor learning is associated with changes in transmitter release and neuronal firing in the MOB, and blockage of NA transmission in the MOB using a variety of methods consistently disrupts odor learning. These finding strongly suggest that NA induces some form of neural plasticity in the MOB. Indeed, in anesthetized mice, experimentally-induced release of NA combined with odor exposure is sufficient to induce stimulus-specific long-term changes in odor responses of MOB mitral cells (Shea et al., 2008). Moreover, the changes in mitral cell firing were mirrored by a corresponding change in behavior: after pairing a female odor with stimulation of NA release, the mice showed a highly specific reduction of attraction to the paired odor. Thus mice treated the stimulus like a familiar, remembered odor. These changes in mitral cell firing may be achieved by at least two different circuit mechanisms. First, the direct input from the olfactory sensory neurons may be modified via increased feedforward

presynaptic inhibition via periglomerular (PG) cells. Alternatively, the feedback inhibitory influence of granule cells (GC) on mitral cell output may be enhanced by NA, thereby suppressing the firing of the mitral cells. Here we examine the dynamics in odor-driven spiking properties of these two types of interneurons during the induction of NA-dependent neural plasticity. We are making extracellular recordings in vivo in the MOB using a loose seal, cell attached configuration. We are acquiring the cells 'blindly' using depth measurements to target each cell type and using juxtacellular dye filling to confirm the cell identity. We are currently measuring changes in odor-evoked activity of these cells before and after stimulation of LC. By comparing the odor responses of the cells before and after pairing an odor with NA release we will be able to clarify some of the cellular targets of NA and the participation of feedforward and feedback inhibitory circuits in olfactory learning.

Disclosures: H.S. Demmer, None; **S.D. Shea**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.6

Topic: D.01. Chemical Senses

Support: NIH NIDCD R01DC000086

NIH NIDCD R01DC009977

NIH NIDCD R01DC009994

Title: Controlling the stimulus: A microscope design for awake-behaving channelrhodopsin activation

Authors: *D. C. WILLHITE[1], M. E. PHILLIPS[2], J. ZINTER[2], E. OLSEN[2], J. V. VERHAGEN[3];
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Abstract: Activation or suppression of neurons with light stimulation has recently become possible, with a number of transgenic mouse models and viral vectors expressing channelrhodopsin or halorhodopsin variants available. The ability to manipulate the

pattern of light-mediated neuronal excitation is particularly attractive as a means to study the olfactory system, where the complexity of the odor stimulus presents unique challenges. We have modified an Olympus BX50WI microscope to present any desired excitation pattern by integrating a digital micromirror device (DMD). The DMD and water rewards are controlled by custom software written in LabVIEW. We show that mice expressing ChR2 under the Thy1 promoter (Arenkiel et al., Neuron 2007) are able to discriminate arbitrary patterns as assayed by behavioral response to water reward when light is presented directly to the dorsal olfactory bulbs through thinned bone.

Disclosures: D.C. Willhite, None; **M.E. Phillips**, None; **J. Zinter**, None; **E. Olsen**, None; **J.V. Verhagen**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.7

Topic: D.01. Chemical Senses

Title: Millisecond temporal precision in olfactory coding

Authors: *R. SHUSTERMAN[1], M. SMEAR[1], A. A. KOULAKOV[2], D. RINBERG[1]; [1]Janelia Farm Res. Campus, HHMI, ASHBURN, VA; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: In contrast to most sensory systems, mammalian olfaction is generally considered a slow sensory modality. The temporal pattern of the brain activity related to odor processing is defined by sniffing, which is, in mice, at the order of hundreds millisecond time scale. To investigate the sub-sniff temporal precision of olfactory coding we aligned neuronal activity of mitral/tufted (M/T)cells in olfactory bulb of the awake mouse in response to odor stimulation with sniffing signal recorded simultaneously.
We show that temporally aligning M/T cell activity to inhalation reveals vigorous single cell responses with 10 ms trial-to-trial jitter, rivals that of other sensory systems. Many cells give brief and inhalation-locked odor responses that can only be detected after sniff alignment. Responses are more tightly locked to sniff phase than to time after inhalation onset. Further, temporal precision of the responses can be regulated dynamically by variations in the inhalation duration. In addition, individual cells respond with diverse, odor-specific activity patterns, and individual odorants evoke diversely timed responses

across cells. Response phase thus contains information about odor stimuli, which may be read by downstream neurons. The precision with which sniffing entrains odor responses points toward a model of olfactory coding that utilizes synchronization within odorant-specific ensembles of M/T cells.

Disclosures: R. Shusterman, None; **M. Smear**, None; **A.A. Koulakov**, None; **D. Rinberg**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.8

Topic: D.01. Chemical Senses

Support: HHMI JFRC Visiting Scientist Program

Title: Mice can smell time: Olfactory discrimination of purely temporal cues

Authors: *M. SMEAR[1,2], R. SHUSTERMAN[1], D. HUBER[1], D. RINBERG[1], T. BOZZA[3];
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Abstract: Responses of olfactory neurons to odor stimulation are rich in temporal structure, which varies in a stimulus-dependent manner. In air-breathing vertebrates, olfactory system activity is modulated by breathing. Neurons often respond to odor stimuli not only by changing firing rate, but by changing the phase of the sniff cycle in which they fire. Does the respiration phase of neuronal activation encode olfactory information that the animal can use? To answer this question, one would like to ask whether an animal can distinguish fixed odor stimuli applied at different phases of the sniff. Testing this idea requires decoupling olfactory stimulation from airflow in the nose, which may be impossible with odorants. To circumvent this issue, we have taken an optogenetic approach to stimulate olfactory inputs independently of respiration.
We have generated transgenic mice that express Channelrhodopsin2 (ChR2) in olfactory sensory neurons (OSNs). One mouse line ChR2 expresses in all OSNs. In another, light-sensitivity is genetically restricted to a single glomerulus. We chronically implant an optical fiber to deliver light stimuli to the nose, and a sniff cannula to measure breathing. Mice are trained in a head-fixed, go-no go discrimination paradigm. All mice first learn

to report detection of odors, after which we test them on detection of light stimuli. OSN-ChR2 mice rapidly learn to report detection of light stimuli, under conditions in which identically-prepared and trained wild-type mice fail to do so. After OSN-ChR2 mice learn to report light detection, we next train them to discriminate identical light pulses solely on the basis of when in the sniff cycle they occur. When asked to report whether a light stimulus occurs at the onset of inhalation or exhalation, OSN-ChR2 mice quickly acquire high performance. Further, these mice are capable of exquisitely precise temporal discriminations -- light stimuli occurring at inhalation onset can be distinguished from stimuli at 10 ms after the onset of inhalation. We conclude that mice can discriminate spatially identical patterns of ORN activation solely on the basis of a temporal cue - timing relative to sniff cycle, even when stimulation is restricted to a single glomerulus. Is this sniff-timing unique to olfactory input, or can the animal report the sniff timing of other modalities? We are testing whether mice can sniff-time auditory stimuli or optogenetic stimuli to barrel cortex . In addition, we are recording in olfactory bulb (OB) to ask whether sniff phase preferences are present at this level of processing.

Disclosures: M. Smear, None; **R. Shusterman**, None; **D. Huber**, None; **D. Rinberg**, None; **T. Bozza**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.9

Topic: D.01. Chemical Senses

Support: Travel Grant from International Society for Neurochemistry

Travel Grant from Sarojini Damodaran fellowship, TIFR

Title: Non-redundant odor coding by sister mitral cells revealed by light addressable glomeruli in the mouse

Authors: *A. K. DHAWALE[1,2], A. HAGIWARA[3], U. S. BHALLA[1], V. N. MURTHY[3], D. F. ALBEANU[2];
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Abstract: Spatially organized convergence of sensory inputs in the brain often leads to

similar response properties in target neurons that are in close vicinity. Whether their individual information content is redundant or independent depends on the circuit architecture (the interplay between common input, lateral signals and feedback from other brain areas) and the computational goals of the network.
In the mammalian olfactory bulb (OB), sensory neurons expressing the same type of olfactory receptor (~10,000) converge in tight focus, forming clusters of synapses called glomeruli (~2,000). From each glomerulus, a few dozen mitral/tufted (M/T) cells carry the output further to the cortex. The M/T cells have one primary dendrite that projects to a single glomerulus, but can sample inputs on their primary and secondary dendrites from functionally diverse glomeruli via several types of interneurons. Thus, a few dozen M/T cells share input from the same parent glomerulus, but may have different inhibitory surrounds.
We probed the odor response properties of M/T cells using extracellular recordings and an optogenetic approach to ask whether the OB is more than a relay station. Do M/T cells receiving common input from the same parent glomerulus (sister cells) carry redundant information about odors to cortex?
We generated transgenic mice expressing channelrhodopsin-2 in all olfactory sensory neurons and adapted a digital micro-mirror device (DMD) to selectively stimulate individual glomeruli in order to identify sister cells. We find that sister cells have highly correlated responses to odors as measured by average spike rates, but differ significantly in spike timings (with respect to the respiration phase). In contrast, non-sister cells correlate poorly on both these measures. We suggest that sister M/T cells carry two independent channels of information: average activity representing shared glomerular input, and phase-specific information that refines odor representations and is substantially independent for sister cells.
We are currently investigating the mechanisms underlying the modulation of phase response properties of M/T cells. Using intersectional Cre/lox strategies, we control the activity of genetically defined interneuron subpopulation via optogenetic molecular switches. We are also exploring how M/T cells phase information is altered by the state of the circuit (anesthetized versus awake) and processed by the olfactory cortex.

Disclosures: A.K. Dhawale, None; **D.F. Albeanu**, None; **U.S. Bhalla**, None; **V.N. Murthy**, None; **A. Hagiwara**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.10

Topic: D.01. Chemical Senses

Title: Mitral cell response latencies reliably predict odor identity

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Abstract: At the level of the olfactory bulb (OB) odor information is contained in the spike patterns of mitral/tufted (M/T) cells. In addition to the identity of the activated M/T cells, the temporal patterns of their responses are important for olfactory coding [1]. However, it is still unclear as to which aspects of these spatio-temporal patterns contain the odor-specific information that is subsequently decoded by the OB's target areas. Behavioral studies in vertebrates show that odor discrimination and recognition can be accomplished within 500 ms or even less depending on the task and the species. Attention has thus been directed towards activity features on short time scales, such as instantaneous firing rates, the average firing phase and response latencies [2, 3, 4]. The latter have been shown to be important coding parameters in other sensory systems. While odor- and concentration-dependent latencies have also been described in the olfactory system, a potential role for odor coding has been discussed controversially [2, 3].
In order to elucidate the specificity and reproducibility of odor response latencies, one has to meet two crucial requirements: (1) M/T cell responses need to be measured at a sufficiently high temporal resolution and simultaneously, since only simultaneous recordings can reveal the relative response latencies which may contain the odor-specific information. (2) The similarity of the latency patterns of the M/T cell population under investigation has to be quantified in order to assess the patterns' reproducibility and specificity under different experimental conditions. Since studies satisfying both requirements are lacking, the role of response latencies in olfactory coding has remained unclear thus far.
We solve this issue by using a fast confocal line illumination microscope and ensemble correlation techniques. The resulting evidence reveals that latency vectors of M/T cells are reproducible and odor-specific. They accurately predict the odor identity on a single trial basis and on short time scales. Latency patterns thus appear to contain all the information higher brain centers would need to identify odors and their concentration.
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[3] Bathellier, B., Buhl, D. L., Accolla, R., and Carleton, A. Neuron 57(4), 586 - 598 Feb (2008).
[4] Lehmkuhle, M. J., Normann, R. A., and Maynard, E. M. J Neurophysiol 95(3), 1369 - 1379 Mar (2006).

Disclosures: S. Junek, None; **E. Kludt**, None; **F. Wolf**, None; **D. Schild**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.11

Topic: D.01. Chemical Senses

Support: NIH NS02694 (G.L.W.)

Title: Synaptic clustering within olfactory bulb glomeruli controls patterns of neuronal activity

Authors: *M. BORISOVSKA, M. J. MCGINLEY, A. BENSEN, G. L. WESTBROOK; Oregon Hlth. Sci. Univ., PORTLAND, OR

Abstract: Odors generate activity in olfactory receptor neurons whose axons form axodendritic synapses within olfactory bulb glomeruli. These afferent inputs are anatomically segregated from dendrodendritic synapses within each glomerulus. However, little is known how synaptic compartmentalization contributes to glomerular signaling. To investigate the dependence of network activity on the segregation of axodendritic and dendrodendritic synapses, we examined mice lacking the olfactory cell adhesion molecule (OCAM). Consistent with their altered intraglomerular (Walz et al., 2006), glomeruli in OCAM knockout mice had a more heterogenous distribution of axodendritic compartments as measured by immunolabeling with olfactory marker protein (OMP). However, the total area occupied by axonal inputs was similar to wild-type mice, indicating that there was not a loss of axons, but rather a breakdown of segregated axodendritic and dendrodendritic compartments.
We examined network activity in olfactory bulb slices from OCAM knockouts (P21-29) using whole cell recording from pairs of mitral cells. Mitral cells projecting their apical dendrite to the same glomerulus show highly synchronized activity (Schoppa and Westbrook, 2001). However, correlated spiking was significantly reduced in OCAM knockout mice, as was electrical coupling between dendrites in the glomerulus. Evoked and spontaneous slow oscillations in mitral cells and external tufted were also broader and had multiple peaks in OCAM knockout mice, indicating that intraglomerular activity was desynchronized on both slow and fast time scales. To assess the degree of interaction between mitral cells projecting to the same glomerulus, we used coherence methods to analyze spontaneous sub-threshold voltage oscillations. Coherent activity was markedly reduced across a broad range of frequencies in mitral cells from OCAM knockout mice. Our results indicate that synchronous activity within each glomerulus is dependent on segregation of synaptic compartments. We speculate that desynchronization of activity within glomeruli might be expected to reduce olfactory discrimination in these mice,

which have been reported to show an increase in olfactory 'acuity' (Walz et al., 2006).
This work was supported by National Institutes of Health grant NS02694 (G.L.W.)

Disclosures: M. Borisovska, None; **M.J. McGinley**, None; **A. Bensen**, None; **G.L. Westbrook**, None.

Nanosymposium

730. Coding and Processing of Olfactory Signals

Location: Room 1B

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 730.12

Topic: D.01. Chemical Senses

Support: NIH Grant RO1-DC006640

NIH Grant 5T32NS007083-28

Title: Lateral inhibition between olfactory bulb glomeruli mediated by GABAergic inputs targeted on external tufted cells

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Abstract: An individual odor activates a distinct pattern of glomeruli within the olfactory bulb (OB), reflecting the different odorant receptors stimulated in the nose. The OB includes dense networks of GABAergic interneurons, suggesting that lateral inhibitory interactions between glomeruli could impact the relationship between glomerular input and output patterns. Using patch clamp recordings from rat OB slices, we tested for the mechanisms of lateral inhibition that occur within the glomerular layer by examining the responsiveness of external tufted (ET) cells. ET cells were chosen for this analysis because they may mediate feed-forward excitation of output mitral cells from ORN inputs (de Saint Jan et al., 2009). In addition, ET cells are part of a glomerulus-specific network of cells that, together with mitral cells, engage in the synchronized long-lasting depolarization (LLD) that dominates bulbar responses to ON stimulation (Gire and Schoppa, 2009); thus the ET cell LLD provides a convenient marker of the output of an entire glomerulus. Upon electrical stimulation of a "conditioning" glomerulus (50-500 μA) that was 60-550 μm away from the "target" glomerulus with which the ET cell was affiliated, we found strong evidence for lateral inhibition. This inhibition was observed as

a significant inhibitory post-synaptic current (IPSC) in voltage-clamped ET cells (52±8 pA at V_{hold} = 0 mV, n = 22), presumably arising from periglomerular cells, as well as a marked reduction in the size of the LLD evoked by stimulation of the target glomerulus (39±10% reduction in charge integral, n = 5, $p < 0.05$). There was a close relationship between the duration of ET cell inhibition (half-width = 28±5 ms, n = 22) and the time-interval between conditioning and target glomeruli stimulation (20 ms) that was effective for LLD suppression, suggesting that LLD suppression was linked to ET cell inhibition. Dual stimulation of the target glomerulus did not inhibit the LLD (n = 5). Amongst the factors that could impact the magnitude of lateral inhibition, one is the activation-history of a target glomerulus. Strong prior activation could result in glutamate transients that reduce lateral inhibition by suppressing GABA release onto ET cells. Consistent with this hypothesis, we found that application of the group II metabotropic glutamate receptor agonist DCG-IV (3 μM) reversibly decreased the lateral IPSC in ET cells (77±6% reduction in charge integral, n = 5, $p < 0.05$). Taken together, these results suggest that local lateral interactions within the glomerular layer of the bulb may impact the OB's input-output relationship through mechanisms that could be regulated by prior odor exposure.

Disclosures: J.D. Whitesell, None; **N.E. Schoppa**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.1

Topic: D.04. Vision

Support: NEI EY019273

McKnight Foundation

Klingenstein Foundation

Alfred P.Sloan Foundation

Title: Evidence for limited peri-saccadic remapping in area MT

Authors: *W. S. ONG[1], J. W. BISLEY[2];
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Abstract: We asked whether MT neurons had retinotopic or spatiotopic receptive fields and whether they displayed peri-saccadic remapping. We recorded the responses from single MT neurons in animals performing visually guided saccades during which a moving dot stimulus (100% coherence) or a circular stimulus was presented. The dot stimulus moved in the preferred direction of the recorded neuron in the pre-saccadic or post-saccadic receptive field for 500 ms; its onset occurring 80 ms before the saccade target appeared. In other trials, a luminance-matched circle was flashed for 50 ms in the pre- or post-saccadic receptive field at random time intervals between 100ms before the saccade target appeared to 350 ms after. Mean saccadic latency was 192 ± 35 ms.
We recorded from 73 MT neurons. All of them had retinotopic receptive fields and none of them showed clear pre-saccadic remapping with either stimulus. However, with the flashed circle, almost all of the neurons showed late post-saccadic remapping; when a stimulus was flashed on and off shortly before the beginning of the saccade we found a small but significant neural response after the saccade in the post-saccadic receptive field ($p<<0.001$).
As a comparison, we also recorded from 22 LIP neurons and showed that they have strong post-saccadic remapping, with a remapped response not different to a pure visual response.
Although no neurons had spatiotopic receptive fields nor exhibited pre-saccadic remapping, the presence of the late post-saccadic response to a stimulus flashed entirely prior to the saccade indicates that a remapping mechanism may act on MT neurons. This, together with gain fields, could explain results showing spatiotopic processing in area MT.

Disclosures: W.S. Ong, None; **J.W. Bisley**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.2

Topic: D.04. Vision

Support: NIH EY016178

NIH EY013644

Title: Estimation of heading in the presence of moving objects: A functional role for 'opposite' cells in area MSTd?

Authors: *H. KIM, G. C. DEANGELIS;
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Abstract: Previous studies (Gu et al., 2006) have described two basic types of visual-vestibular neurons in area MSTd. 'Congruent cells' have the same heading preference for visual and vestibular self-motion cues, whereas 'opposite cells' prefer nearly opposite directions. Congruent cells contribute to improved heading discriminating when visual and vestibular cues are combined, whereas opposite cells do not (Gu et al., 2008). What, then, is the functional role of opposite cells? Based on simulations, we propose that a mixed population of congruent and opposite cells allows accurate decoding of heading in the presence of moving objects.

Moving objects distort the optic flow field that arises due to self-motion, and this greatly complicates the task of estimating heading solely from optic flow. Because opposite cells are never optimally stimulated during self-motion through a static environment, strong activation of opposite cells may signal visual motion that is inconsistent with self-motion. Thus, the key intuition is that the relative activity of congruent and opposite cells may allow a simple population decoder to dissociate self-motion and object motion.

Each model neuron performed a weighted linear sum of visual and vestibular inputs. The visual input had two components: one representing background optic flow due to self-motion and the other representing object motion. Background and object motion could interact in two ways to determine visual tuning: a scalar sum or a vector sum. The vector sum represents a worst-case scenario because the visual response inherently confounds the directions of background and object motion. We used maximum likelihood (ML) decoding to estimate heading from the population response in the presence and absence of object motion. When a mixed population of congruent and opposite cells was decoded according to the vestibular heading preference of each neuron, heading estimates were virtually unaffected by object motion. This was not true when only congruent or opposite cells were decoded, or when all neurons were decoded according to their visual heading preference. To explore the origin of this decoding strategy, we used a reinforcement learning rule to train a network of model neurons to estimate heading in the presence of random directions of object motion. This network learned to decode model MSTd neurons according to their vestibular heading preferences.

Our findings suggest that opposite cells may exist in MSTd to allow robust heading estimation in the presence of moving objects, and that a successful strategy of decoding MSTd neurons according to their vestibular heading preference may arise from a simple learning rule.

Disclosures: H. Kim, None; **G.C. DeAngelis**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.3

Topic: D.04. Vision

Support: NWO Veni 016.108.047 to BR

Center for Perceptual Systems to JWP

Title: A Bayesian explanation of biased 3D motion perception

Authors: *B. ROKERS[1], J. W. PILLOW[2];
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Abstract: Organisms make estimates of object motion from limited sensory input. In binocular vision, the information needed to infer an object's 3D motion trajectory is carried by temporal changes in the two retinal images. Curiously, human observers systematically misperceive the 3D trajectory of an object moving through depth, reporting motion directed toward an observer as biased to more lateral directions (Harris & Dean 2003). Here we show that this bias may arise as a natural consequence of optimal inference under sensory uncertainty and a prior for slow speeds.

Previous work on 2D motion perception has shown that a Bayesian model with a prior for slow speeds can account for biases in 2D speed and direction percepts (Weiss et al. 2002; Stocker & Simoncelli 2006). Recent work has sought to extend this paradigm to 3D motion, arguing that 3D biases can be explained by biased estimation of the cues for retinal motion and disparity (Lages 2006), or a fixed difference in the reliability of translational and disparity-based motion signals (Welchman et al. 2008).

We revisit the geometry of the 3D motion inference problem and develop a Bayesian ideal observer model that captures the fundamental constraints on performance. We show that for an observer making noisy retinal measurements, the relative uncertainty in an object's Z (toward/away) velocity and its X (lateral) velocity is a simple function of object distance. Specifically, uncertainty in retinal motion maps linearly to uncertainty in 3D velocity, meaning that Gaussian error in measuring retinal motion leads to a Gaussian-shaped likelihood function for 3D velocity. For normal viewing distances, this likelihood has highly elliptical contours, with much greater uncertainty in Z than in X. Biases in 3D motion perception can be explained by combining this likelihood with a prior for slow speeds.

Experimentally, we presented a plane of dots viewed through a circular aperture. On each trial, the plane moved along a particular motion trajectory, and observers reported an estimate of the plane's trajectory. We varied the contrast of the visual stimulus, which

effectively varied the noise in retinal measurements. Consistent with the predictions of the Bayesian model, lower contrast increased the bias in estimates of motion direction. For each observer, we estimated a prior distribution over 3D motion that was consistent with the entire distribution of motion estimates. These results extend previous Bayesian models of motion perception, illustrate how limited sensory information leads to biased estimation of object motion, and make predictions about the prior distribution of 3D velocities that occur in natural vision.

Disclosures: B. Rokers, None; **J.W. Pillow**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.4

Topic: D.04. Vision

Support: DFG (SFB 550)

EC FP7 project SEARISE

Hermann Lilly Schilling Foundation

Title: Physiologically-inspired neural model accounting for the visual tuning properties of action-selective neurons

Authors: *M. A. GIESE, V. CAGGIANO, P. THIER, F. FLEISCHER;
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Abstract: Neurons with visual selectivity for goal-directed hand actions have been found in multiple cortical regions, including the superior temporal sulcus, the inferior parietal lobule and the premotor cortex. Despite a strong interest in action recognition and the mirror neurons system, the computational mechanisms of action recognition and their neural implementation remain largely unknown. Many existing models of the mirror neuron systems assume that action vision is essentially based on the internal simulation of observed motor behavior exploiting motor representations. However, many of these models do not specify how the required motor-relevant input variables can be extracted robustly from image sequences, exploiting physiologically plausible neural principles. We propose a physiologically plausible model for the recognition of goal-directed hand

actions. It accomplishes recognition from real videos and reproduces a variety of properties of action-selective neurons observed in single-cell recordings. It assumes recognition from learned example views and does not require the solution of difficult computational problems, such as the reconstruction of the 3D effector geometry form image data.
Model architecture: Two major components: 1) A hierarchical neural system for the recognition of object and effector shapes. This part is consistent with many other neural models for object recognition, where the highest hierarchy level accomplishes only partial position invariance. 2) A simple network that integrates the information from recognized effector and goal object shapes. The core of this circuit is a neural map, computed by a gain field, that represents the 2D effector position in an object-centered coordinate frame. The action-selective model neurons integrate the output of this map with outputs from neurons that are selective for the grip type in a multiplicative manner.
Results: The performance of the model was validated with real video sequences showing different types of grips and goal objects. The model reproduces key properties of action-selective neurons in area F5 and the STS, including position invariance, selectivity for sequential order, critical dependence on presence and position of the goal object, reduced responses for 'mimicked actions'.
Conclusions: Visual properties of action-selective neurons, and specifically of F5 mirror neurons, can be accounted for by simple, physiologically plausible neural mechanisms. Most visual properties can be explained by well-established visual processes, without a strong involvement of motor representations.

Disclosures: M.A. Giese: Research Grant; Supported by DFG (SFB 550), the EC FP7 project SEARISE, and the Hermann und Lilly Schilling Foundation. **V. Caggiano:** DFG (SFB 550), Hermann und Lilly Schilling Foundation. **P. Thier:** DFG (SFB 550) and hermann Lilly Schilling Foundation. **F. Fleischer:** DFG (SFB 550), the EC FP7 project SEARISE, and the Hermann und Lilly Schilling Foundation..

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.5

Topic: D.04. Vision

Support: FRSQ Vision Network to RFH and CC

CIHR to CC

CIHR #MOP53346 to RFH

Title: Pattern motion responses in the visual cortex of humans with amblyopia

Authors: *B. THOMPSON[1], M. Y. VILLENEUVE[2], C. CASANOVA[3], R. F. HESS[4]; [1]Optometry & Vision Sci., Univ. of Auckland, Auckland, New Zealand; [2]Martinos Center, Massachusetts Gen. Hosp., Harvard Med. Sch., Boston, MA; [3]Sch. of Optometry, Univ. of Montreal, Monteal, QC, Canada; [4]Ophthalmology, Mcgill Univ., Montreal, QC, Canada

Abstract: There is strong psychophysical evidence that amblyopes have a specific deficit for motion tasks which target extrastriate visual areas such as hMT+/V5 (e.g. Simmers et al., 2006). We used fMRI to test the hypothesis that amblyopia is associated with abnormal function of motion sensitive areas in extrastriate visual cortex. During scanning, participants viewed plaid stimuli composed of two superimposed square-wave gratings, differing in orientation by 120°, that were either perceived to move independently of one another (component motion) or were seen to cohere and move as one (pattern motion). Component motion was perceived when the two drifting gratings had different spatial frequencies (0.2 vs. 0.5cpd), whereas pattern motion occurred when the spatial frequencies were the same. Plaid stimuli were chosen on the basis of previous neuroimaging studies (Huk et al, 2002, Villeneuve et al., 2005) showing that V1 does not differentiate between component and pattern motion, whereas hMT+ shows a differential response that is indicative of higher-level motion integration. Controls (n = 7) and the fellow eyes of amblyopes (n = 6) showed increased activation for component motion in hMT+ compared to pattern motion. This is consistent with previous findings suggesting that component motion activates two neural assemblies in hMT+ whereas pattern motion only actives one (Castelo-Branco et al., 2002). For amblyopic eye stimulation however, there was a selective reduction in the response of hMT+ to the component motion stimulus. Amblyopic, fellow and control eye stimulation produced similar relative responses to the different motion stimuli in all other extrastriate visual areas localized. However, amblyopic eye activation was generally reduced. In addition, the pulvinar was differentially activated by the amblyopic and fellow eyes. Our results support the hypothesis that hMT+ function is abnormal in amblyopia. More specifically, the reduced response of hMT+ to component motion suggests that the abnormal visual development associated with amblyopia leads to an over-integration of motion information. This is consistent with psychophysical studies of motion perception in amblyopia that have found elevated global motion thresholds and an increased tendency to perceive pattern motion in plaids.

Disclosures: B. Thompson, None; **M.Y. Villeneuve**, None; **C. Casanova**, None; **R.F. Hess**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.6

Topic: D.04. Vision

Support: Wellcome Trust

Title: Insights from a unified framework for developing and comparing circuit-based models of direction selectivity: iModel.org

Authors: *P. M. BAKER, W. BAIR;
Dept of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Visual motion perception is believed to arise from outputs of direction selective (DS) neurons in primary visual cortex. Many models have been proposed for DS neurons, but the models vary widely in their fundamental architecture, their level of physiological detail, the nature of their inputs, and the types of responses that they generate. These differences make it difficult or impossible to directly test the models with relevant visual stimuli, to make direct comparisons between models, or to compare model output with the wealth of experimental data.
We developed a set of population models of DS neurons using a common framework with several key features that address these limitations. Our model building blocks are physiological cell classes connected by realistic synapses. The model outputs are spike trains, membrane voltages, and synaptic conductances. Cells in LGN and cortex are organized topographically for receptive field position. Such detail facilitates direct comparison between simulated and experimental protocols and results. In this framework, we built circuit models of DS cells using neuronal populations with linear and nonlinear mechanisms. Our approach puts disparate DS models on equal footing for the first time, allowing comparisons within the same simulation environment. The framework provides the ability to explore model architectures online (iModel.org), for example, visualizing synaptic input patterns. Models can be tested with any visual stimuli, and simulation output can be displayed and analyzed online. These capabilities provide an unprecedented level of transparency, allowing users to easily probe and execute different models, thereby avoiding limitations of investigator-selected results.
We present a set of circuits based on a range of previously published models and novel networks we built for generating DS cells. We show how the framework can be used, not only to assess which circuits are consistent with existing data, but also to develop visual stimulus and analysis paradigms for distinguishing models for future experimental work. We will present novel insights into open problems in visual motion processing obtained using the framework: distinguishing between plausible DS circuits using cross-

correlation, relating subunits of DS neurons to 2D displacement maps, and demonstrating circuits with adaptive temporal integration. Using these examples, we will highlight the utility and flexibility of the modeling framework and the iModel website, including interfaces for customizing visual stimuli, exploring model architectures, and analyzing responses.

Disclosures: P.M. Baker, None; **W. Bair**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.7

Topic: D.04. Vision

Support: Swartz Foundation

NIH 5R01-EY007605

Title: Optimal sensory adaptation without prior representation of the environment

Authors: *S. GEPSHTEIN[1], P. JURICA[2], I. TYUKIN[3], C. VAN LEEUWEN[2], T. D. ALBRIGHT[1];
[1]Vision Ctr. (VCL), Salk Inst., LA JOLLA, CA; [2]Perceptual Dynamics Lab., RIKEN Brain Sci. Inst., Wakoushi, Japan; [3]Dept. of Mathematics, Univ. of Leicester, Leicester, United Kingdom

Abstract: Sensory adaptation is expected to improve sensitivity to the prevailing properties of the environment. But sensitivity to adapting stimuli is found sometimes to increase, sometimes to decrease, and sometimes not to change at all. In addition, large changes in sensitivity sometimes occur to stimuli very different from the adapting ones. We recently advanced a normative theory of visual adaptation that explains these paradoxical results. The theory prescribes how visual systems ought to allocate their limited computational resources such that more resources are used where the measurements are more efficient, and where the resources are more likely to be used because of the statistics of stimulation. The theory predicts that motion adaptation improves the sensitivity to motion in new environments, but the improvement of sensitivity to some stimuli must be accompanied by a reduction of sensitivity to other stimuli. We confirmed these predictions by measuring human contrast sensitivity over the

entire range of visible spatiotemporal modulations of luminance, under different statistics of stimulation. We found that changes of stimulation caused global changes in sensitivity, forming foci of increased and decreased sensitivity in the stimulus space as predicted by the theory.
Now we show that optimal adaptation to changes in the environment does not require the visual system to represent stimulus statistics, in contrast to a large class of probabilistic (Bayesian) models of sensation which presume that sensory systems represent statistical regularities of natural scenes. We model a population of motion-sensitive cells, each tuned to spatial frequency, temporal frequency, and speed of stimuli. The tuning of each cell is noisy: it fluctuates according to a stochastic process independent of other cells and with no feedback about system performance. The amplitude of fluctuations depends on how reliably individual cells measure stimulation: the higher the reliability the smaller the fluctuations. Local fluctuations steer the tuning of individual cells such that the distribution of tuning in the system is in accordance with (1) the theoretically optimal distribution and (2) the distributions of sensitivity observed in our studies of human spatiotemporal sensitivity. In other words, the optimal adaptive behavior emerges from the local stochastic processes bottom-up: without a (prior) representation of stimulation. The optimal behavior is observed when cells conserve their speed tuning, in agreement with a labeled line hypothesis.

Disclosures: S. Gepshtein, None; **T.D. Albright**, None; **P. Jurica**, None; **C. van Leeuwen**, None; **I. Tyukin**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.8

Topic: D.04. Vision

Support: Wellcome Trust

Neuroinformatics Doctoral Training Center (EPSRC)

Title: Direction opponency, not quadrature, is key to the 1/4 cycle preference for apparent motion in the motion energy model

Authors: *N. HEESS[1], W. BAIR[2];
[1]Sch. of Informatics, Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Dept. of

Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Sensitivity to the direction of visual motion is a fundamental property of neurons in the primary visual cortex (V1) and has received wide attention in terms of mathematical models. A key feature of many popular models for cortical motion sensors is the use of pairs of functions that are related by a 90 degree phase shift. This phase relationship, known as quadrature, is the hallmark of the motion energy model and played an important role in the development of a class of model dubbed elaborated Reichardt detectors.
One stimulus thought to provide evidence for quadrature models is the two-flash sinusoidal grating apparent motion stimulus where two sinusoidal gratings differing in spatial phase are presented briefly in close temporal succession. For decades, the literature has supported a link between quadrature and the observation that motion detector models, direction selective neurons, and human observers often prefer a 1/4 cycle displacement (phase difference) for this stimulus. Here we show that this link largely does not exist.
We tested the displacement tuning of the motion energy model in simulations and verified analytically that quadrature does not lead to a preference for a 1/4 cycle step, and in particular that motion energy is not maximized for a 1/4 cycle step. Furthermore, a simple Reichardt model, which prefers 1/4 cycle step, is not a quadrature model, in spite of its mathematical links to some versions of a motion energy model. Thus, quadrature is neither necessary nor sufficient for a motion sensor to prefer 1/4 cycle displacement. Other properties of motion sensors are the key: the opponent subtraction of two oppositely tuned stages that individually have sinusoidal displacement tuning curves. In fact, nearly any linear filter followed by squaring is sufficient to construct an opponent motion model that prefers 1/4 cycle step for the 2-flash grating stimulus, regardless of the spatial frequency of the grating. Thus, psychophysical and neurophysiological data revealing a preference at or near 1/4 cycle displacement do not offer specific support for common quadrature or energy-based motion models. Instead, they point to a broader class of model. To advance the understanding of the relevant models and facilitate their use in experimental design and data interpretation, we make them available www.imodel.org under the topic: Opponency, Quadrature and Displacement Tuning for Two-Frame Motion.

Disclosures: N. Heess, None; **W. Bair**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.9

Topic: D.04. Vision

Support: Penn State SLEIC; SSRI

Title: Asymmetries in cortical responses to temporal modulations of motion coherence and motion-contrast defined forms

Authors: J. D. FESI[1], *R. O. GILMORE[2];
[1]Penn State Univ., University Park, PA; [2]Penn State Univ., UNIVERSITY PK, PA

Abstract: Area MT neurons in both the monkey and humans show sensitivity to variations in the statistical properties of motion -- specifically the extent of motion coherence. A distinct subpopulation of MT cells shows opponent motion responses that signal a form of local motion contrast. Here we measured cortical motion coherence activity thresholds in human adults using a steady-state visual evoked potential (SSVEP) technique in which motion coherence values varied across time. The goal was to compare motion coherence thresholds to global motion displays both with and without local motion contrast. Binocular responses were recorded over the occipital midline (Oz) and three lateral sites (P07, O1, O2, and P08). Analysis centered on phase- locked responses at the coherence modulation frequency (F1: 1.2 Hz), its 2nd harmonic (2F1: 2.4 Hz) and the dot update frequency (F2: 36 Hz). Results indicate stronger responses to displays that depict increasing global motion coherence levels across time compared with those that depict coherence decreases. Moreover, response curves to displays that show increasing levels of motion contrast are monotonic unlike analogous displays that lack the local motion contrast cue. The data suggest that the dynamics of brain electrical responses differ between displays with uniform motion characteristics and those with local motion contrast -- possibly due to interactions among motion opponent and non-opponent populations in hMT. Moreover, brain responses to coherent global motion adapt quickly, giving rise to asymmetric tuning curves to displays that show decreasing versus increasing global coherence.

Disclosures: J.D. Fesi, None; **R.O. Gilmore**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.10

Topic: D.04. Vision

Support: IRCSET Postdoctoral Fellowship

Title: The timing of coherent motion processing in human visual cortex

Authors: *E. C. LALOR, J. P. BARRETT, R. B. REILLY;
Trinity Col. Dublin, Dublin, Ireland

Abstract: Many recent studies have posited specific dysfunction of the dorsal visual stream in a variety of mental disorders. Thus, the ability to obtain a response from humans with high temporal resolution that originates almost entirely in the dorsal stream would be beneficial. Given that motion is preferentially processed in the dorsal stream, manipulating the motion of a stimulus is one approach for achieving this. Previous EEG studies have used the motion onset VEP to assess the electrophysiology of motion processing with high temporal resolution. However, time-locked averaging to such stimuli undoubtedly produces responses that consist of contributions from several visual areas and not just areas responsible for processing motion. To redress this we created a continuous random dot kinematogram where each dot moved with a speed of 2.4°/s and where every 23 - 141 ms the percentage of dots that moved coherently was modulated by a stochastic control signal. The remaining dots moved at the same speed, but in a random direction different from that of the coherent motion. Thus the contrast and the total amount of motion were constant at all times and the only variation was in the percentage of dots moving coherently. Here we present data from 10 healthy subjects who viewed these stimuli while their EEG was recorded. We assume that the recorded EEG represents a convolution of the control signal and an unknown impulse response of the visual system. Using least-squares estimation we obtain an impulse response which we call the 'Coherent Motion VESPA'. We show a robust response with highly specific spatial and temporal resolution (Fig 1). The response onsets around 100 ms which is 30-40 ms later than standard VEP responses and it displays a single dominant positive peak at around 130 ms. We interpret this as a response from areas specific to global motion processing in the dorsal stream with little or no contribution from other areas and that it indicates the timing of coherent motion processing in human cortex. This response may have utility in research on the visual dysfunction found in a number of mental disorders.
$$graphic_DDB12C4C-89F8-411C-B744-A896BC6954B7$$

Disclosures: E.C. Lalor, None; **J.P. Barrett**, None; **R.B. Reilly**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.11

Topic: D.04. Vision

Support: DARPA (IPTO and DSO)

NSF-0640097

NSF grant #IIS 0827427

NSF grant #SBE 0542013

IIT

Title: Do dorsal stream neurons encode combinations of local motion direction?

Authors: *C. TAN[1], V. YORGAN[2], T. SERRE[3], D. SHEINBERG[2], T. POGGIO[1]; [1]Brain and Cognitive Sci., MIT, CAMBRIDGE, MA; [2]Neurosci., [3]Cognitive and Linguistic Sci., Brown Univ., Providence, RI

Abstract: It has been reported that one-third of neurons in monkey visual area V2 are selective for combinations of orientations (Anzai, Peng and Van Essen 2007), a result that is broadly consistent with a class of hierarchical models of ventral stream visual processing. Here, we explore whether dorsal stream neurons in areas MT/MST similarly encode combinations of local motion direction by performing template-matching, as postulated by dorsal stream analogs of these models (e.g. Jhuang, Serre, Wolf & Poggio 2007), and for which suggestive evidence has been found (Majaj, Carandini & Movshon 2007; Yu, Page, Gaborski & Duffy 2010).

We characterize the higher-level structure of spatiotemporal receptive fields of dorsal stream neurons by mapping their subunit organization. Receptive fields were subdivided along a hexagonal grid, and direction selectivity was measured at each subfield. We then exhaustively measured the response of each cell to simultaneous stimuli at select pairs of locations. We examine these neural recordings to study the various types of potential interactions between subunits (e.g. amplitude reduction, flattening, etc) found in these combination-selective cells, as found in the ventral stream and in good general agreement with Hubel-and-Wiesel type computational models.

In parallel, going beyond casting the neural responses in terms of excitatory and inhibitory mechanisms, we attempt to shed light on the more abstract computational functions of these cells via two approaches. Firstly, following a more qualitative approach, we compare predictions made by several computational models and their variants to see which of these are able to account for the variety of "behaviors" displayed by the cells in response to simultaneous two-subfield stimulation. Secondly, we also

quantitatively compare the ability of the various computational models to reproduce and predict the two-subfield neural data in a cross-validated manner.
We conclude by discussing similarities and differences between neural data and model predictions for both the ventral and dorsal streams, as well as implications for a more unified understanding of shape and motion processing in visual cortex.

Disclosures: C. Tan, None; **V. Yorgan**, None; **T. Serre**, None; **T. Poggio**, None; **D. Sheinberg**, None.

Nanosymposium

731. Visual Motion: Neural Mechanisms II

Location: Room 25A

Time: Wednesday, November 17, 2010, 8:00 am - 11:00 am

Program Number: 731.12

Topic: D.04. Vision

Support: NSF-0640097

NSF-0827427

Title: Computational mechanisms of motion processing in visual area MT

Authors: *T. A. POGGIO[1], H. JHUANG[2], T. SERRE[3];
[1]Dept Brain And Cog Sci., MIT, CAMBRIDGE, MA; [2]MIT, Cambridge, MA; [3]Brown Univ., Providence, RI

Abstract: Dorsal stream is thought to critically involved in the motion processing in the visual cortex. Cortical processing of motion information starts in the primary visual cortex (V1) where neurons have relatively small receptive fields and are selective for specific directions of motion and orientations of stimuli. These motion signals are then integrated in area MT, where neurons tend to have larger receptive fields (about 2-3 times larger) and exhibit selectivity for the speed and global motion of a stimulus as reported perceptually. In previous work, two main classes of models have been described. While these models have been shown to fit well neurophysiological data from areas V1 and MT (Simoncelli & Heeger, 1997; Rust et al, 2006; Perrone, 2002, 2004, 2006), these models are agnostic about how the observed selectivity of neurons in the dorsal stream could be shaped by visual experience.
In previous work, we developed a computational model of the dorsal stream of the visual cortex. We showed that the resulting model could learn, from training video sequences,

motion features that are useful for the recognition of both human actions (Jhuang et al, 2007) and mouse behaviors (Jhuang et al, SFN 2009). The model is based on a hierarchical architecture and assumes two functional classes of cells: Simple units pool over spatial frequencies and motion directions locally to increase the complexity of the preferred stimulus of the neurons. Complex units pool together the activity of neighboring afferent units with the same preferred stimulus but at slightly different positions and scales to increase the tolerance to 2D transformations. In this work, we compare the response of model units to various motion stimuli with that of V1 and MT neurons. We show that learning from natural video sequences (Einhauser et al, 2002) leads to a dictionary of motion selective units in area MT that agree well with the distribution of tunings obtained in area MT. In particular, we find a good quantitative agreement between the distributions of tuning selectivity for plaid stimuli for model vs. cortical cells in area MT (Rust et al, 2006). The proposed model integrates spatial frequencies and directions of motion over a localized region, and could possibly explain recent findings from several groups (Priebe et al, 2003, 2006; Majaj et al, 2007).

Disclosures: T.A. Poggio, None; **H. Jhuang**, None; **T. Serre**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.1

Topic: D.17. Voluntary movements

Support: NIH Research Grant NS011862 from NINDS

Title: Spike trains in posterior parietal cortex (PPC) encode trained and natural grasping behaviors

Authors: *E. P. GARDNER, J. CHEN;
Dept Physiol., New York Univ. Sch. Med., NEW YORK, NY

Abstract: To investigate the role of somatosensory and motor information during prehension, we used digital video and burst analysis of simultaneously recorded spike trains to define epochs when neuronal firing rates in PPC areas 5 and 7b/AIP exceeded 1 SD above the mean. We reconstructed the trajectory of hand movements during each burst from successive digital video images as three macaques grasped and manipulated objects in a trained prehension task, and when engaged in natural grasping behaviors to

acquire pieces of fruit. In the task, PPC neurons respond more vigorously during object acquisition than to manipulation. Firing rates rise 250-500 ms before touch, and peak as the hand is preshaped during reach, or at initial contact with the object. Firing rates decline as grasp is secured, and return to baseline or are inhibited during subsequent actions. Some neurons responded to grasping actions of the right and left hands (bilateral neurons), suggesting that their firing patterns reflect grasp intentions, or the internal motor commands for execution of these behaviors.
Acquisition-sensitive firing patterns were also observed when the animal grasped food morsels at various workspace locations. Firing began as the animal projected the hand towards the food, and continued as the hand tracked it. Firing peaked as the fingertips contacted the food, and ended when it was secured in the hand. High firing was elicited when food morsels were plucked from a tray, with the fingers preshaped for precision grip, or during tracking actions when the fingers were spread apart to maximize surface area. As in the task, bilateral neurons responded to prehensile actions performed unilaterally by either hand. A second, weaker burst often occurred when food was placed in the mouth. Other neurons responded vigorously to acquisition by the contralateral hand, but fired at highest rates when bilateral actions were coordinated between the left and right hands, as when food morsels were transferred between them. These intrapersonal-coordinated neurons did not just encode equivalent tactile information from either side, but preferentially signaled coincident somatosensory data shared between hemispheres during synergistic hand actions. The two classes of bilateral neurons thus provide somesthetic feedback from both limbs, and encode whether they are acting independently or in concert.
Our findings support hypotheses that PPC firing patterns reflect the animal's intentions to accomplish task goals in motor coordinates. They suggest that actions preceding contact reinforce subsequent neural responses, allowing subjects to acquire and manipulate objects in a continuous, smooth sequence.

Disclosures: E.P. Gardner, None; **J. Chen**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.2

Topic: D.17. Voluntary movements

Support: NSF

NIH-NINDS-CRCNS-R01

NIH Director's Pioneer Award

BWF

Title: Lack of evidence for inhibitory gating in monkey M1

Authors: *M. T. KAUFMAN[1], M. M. CHURCHLAND[2,1], K. V. SHENOY[2,1,3];
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Abstract: M1 is thought to be largely responsible for movement execution. Given that premotor cortex projects heavily to M1, and that it exhibits firing rate modulation during the preparation of movement, how does the brain prevent preparatory activity from driving movement?
One possibility is that preparatory activity never escapes premotor cortex, perhaps because premotor output neurons remain strongly inhibited during the preparatory period. If this were true, we would expect that firing rates of premotor inhibitory neurons would be high during preparation then low during movement, to correctly time output to downstream areas (including M1). However, we have previously shown evidence inconsistent with this hypothesis in dorsal premotor cortex (PMd; Kaufman et al, SFN 2008).
An alternative hypothesis is that there is strong inhibition within M1 during movement preparation, and this serves to prevent M1 from acting on premotor input. To test this hypothesis, we performed single-unit neural recordings in M1 of two monkeys performing a delayed-reach task. In this task, a target appeared while monkeys fixated. The monkeys were required to withhold movement until a go cue, then reach to the target. We used the recorded waveform shape to distinguish putative inhibitory interneurons from pyramidal cells (25/36 putative inhibitory/excitatory neurons for monkey J; 17/19 for monkey N). The M1 gating hypothesis predicts that inhibitory activity should be high during the preparatory period (to prevent motor output) and low during movement (to permit output). We instead found the opposite pattern: M1 inhibitory activity was on average near baseline during the preparatory period, and increased during the movement (mean 17 spk/s during baseline to 44 spk/s peak for monkey J; 18 to 31 for monkey N). Excitatory activity rose less from baseline to movement (from 13 to 19 for J; 18 to 29 for N). Peak inhibitory activity occurred near movement onset, similar to PMd. Nor did we find a distinct subset of inhibitory neurons that exhibited the hypothesized pattern of activity. These results suggest that movements may be gated further downstream, such as in the spinal cord. Alternatively, some fundamentally different mechanism may prevent preparatory activity from producing movement. One possibility is that the brain exploits the fact that the neuron to muscle projection is many-to-one. This could allow neural activity to change during preparation without causing an immediate change in muscle activity.

Disclosures: M.T. Kaufman, None; **M.M. Churchland**, None; **K.V. Shenoy**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.3

Topic: D.17. Voluntary movements

Support: Australian Neuromuscular Research Institute

Title: Differences in task related and coincident spike activity between regular and fast spiking neurons in the cat motor cortex

Authors: *S. GHOSH[1], D. PUTRINO[2];
[1]Ctr. Neuromuscular Neurolog. Disorders, Univ. of Western Australia, Perth, WA, Australia; [2]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Combined morphological and physiological studies have revealed that at least 2 types of cortical neurons can be identified based on extracellular spike characteristics: regular spiking (RS), putatively excitatory neurons, and fast spiking (FS), putatively inhibitory interneurons. In order to study the function of RS and FS neurons in the motor cortex, we recorded spike activity simultaneously from up to 24 chronically implanted microelectrodes in the primary motor cortex (MI) of 4 adult cats of either sex, while they performed a learned skilled reaching and retrieving task using either forelimb. Video recording (synchronized to neural activity records) during task performance was used to subdivide the task into multiple stages (background, preparatory or reaction time, reach, withdraw and feed) and relate spike activities to each task trial. Intracortical microstimulation at the recording sites evoked contraction of contralateral forelimb or hindlimb muscles. Isolated single units that significantly modulated their spike activity during the task were classified as either RS (65%) or FS (35%) depending on their baseline firing rate and spike width. Patterns of task-related spiking modulation was assessed using peri-stimulus time histograms and activity classified as either broadly-tuned (BT, modulated during more than 1 task stage) or narrowly-tuned (NT, modulated during 1 task stage). The majority of RS neurons (90%) in this study showed BT activity during task performance, while FS neurons were equally likely to show BT (48%) or NT (52%) task-related activity. Most NT neurons showed modulation during the reach stage, while most BT neurons showed modulation during the reach, withdraw and feed stages. Coincident spike activity of neural pairs was evaluated during task performance by constructing shuffle subtracted cross-correlograms. Coincident activity was frequently

observed between forelimb related neurons but rarely between hindlimb neurons. Among forelimb related neurons coincident activity was observed more frequently between FS-FS (36% of tested pairs), than RS-FS (14%) or RS-RS (9%) pairs. The study shows that spike activity in inhibitory neurons is more likely to be tuned to specific stages of a multistage task involving many motion elements and limbs. In addition, inhibitory neuronal pairs are also more likely to show coincident spike activity during task performance. Synchronized spiking in FS neurons during particular task stages may play an effective role in controlling the temporal activity profile of excitatory projection neurons.

Disclosures: S. Ghosh, None; **D. Putrino**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.4

Topic: D.17. Voluntary movements

Support: CONACYT, DARE, SGF, BioX, Christopher and Dana Reeve Foundation

NIH RO1-NS054283

NIH Director's Pioneer Award IDP1OD006409

Title: Low dimensional neural features predict specific muscle EMG signals

Authors: *Z. RIVERA ALVIDREZ[1], R. S. KALMAR[2], S. I. RYU[3,1], K. V. SHENOY[1,2];
[1]Electrical Engin., [2]Neurosci., Stanford Univ., Stanford, CA; [3]Neurosurg., Palo Alto Med. Fndn., Palo Alto, CA

Abstract: Understanding the relationship between neural activity in motor cortex and muscle activity during movements is important both for basic science and for the design of neural prostheses. While there has been significant work in decoding muscle EMG from neural data, decoders often require many parameters, making the analysis susceptible to overfitting. Overfitting reduces generalizability and can make the results difficult to interpret scientifically. To address this issue, we recorded simultaneous neural activity from the motor cortices (M1/PMd) of two rhesus monkeys performing a non-

delayed arm-reaching task to a grid of targets arranged in concentric rings (max radius = 12 cm) while recording EMG from arm muscles. In this work, we focused on relating the mean neural activity (averaged across all reach trials to one target) to the corresponding mean EMG. In order to obtain a more compact representation of the neural population, we used dimensionality reduction. We reduced the dimensionality of the neural data using factor analysis and found that salient features of the low-dimensional (low-D) neural activity could be matched to to salient features of the EMG data. Using these features as a signature of muscle activity, we derived low-D neural measures based on reaches to only one reach target (<5% of the data) that could explain EMG for reaches across multiple targets (average R^2 = 0.65). Because we did not directly fit the EMG data to the neural activity, our method is unlikely to overfit and the neuron-to-EMG relationship found has implications for the mechanisms of motor control. Our results suggest that the population activity of cortical neurons of unidentified type is tightly related to muscle EMG measurements, predicting a lag between cortical activity and movement generation of 48 ms. This lag is longer than the estimated delay between corticomotoneuronal single-cell firing and postspike EMG facilitation (McKiernan, et al., 1998), but in agreement with lag estimations based on optimal correlations between M1 cell firing and EMG (Miller, et al., 2003). Furthermore, our ability to predict EMG features across different kinds of movements suggest that there are signatures in low-D neural space that correspond to activation of particular muscles.

Disclosures: Z. Rivera Alvidrez, None; **R.S. Kalmar**, None; **S.I. Ryu**, None; **K.V. Shenoy**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.5

Topic: D.17. Voluntary movements

Support: NIH Grant DP1-OD003646

NIH Grant R01-EB006385

Title: Evaluation of excitatory and inhibitory spiking associations in the cat motor cortex related to a skilled movement task using a point process framework

Authors: D. PUTRINO[1], *S. KIM[2], S. GHOSH[3], E. N. BROWN[1,2];

[1]Anesthesia and Critical Care, Massachusetts Gen. Hosp., Boston, MA; [2]Brain and cognitive sciences, MIT, Cambridge, MA; [3]Ctr. for Neuromuscular & Neurolog. Disorders, Univ. of Western Australia, Perth, Australia

Abstract: An understanding of how different neuronal cell types in the primary motor cortex (MI) interact with one another is an essential aspect of studying voluntary movement production in the brain. Recent advances in statistical modeling procedures have allowed for the evaluation of associations that occur between simultaneously recorded neurons in unprecedented detail. Microwires were implanted into the forelimb (FL) and hindlimb (HL) representations of MI in two adult cats of either sex, and used to record spiking activity from multiple neurons during the performance of a reaching task. The extracellular spiking features of isolated units were used to classify them as either regular spiking (RS; putatively excitatory) or fast spiking (FS; putatively inhibitory interneuron) neurons, and firing associations between simultaneously recorded neurons were evaluated using a novel Granger causality measure applied to point process data. A population of 264 neurons (171 RS, 93 FS) were analyzed, and 1794 simultaneously recorded pairs including different combinations of neuronal subtypes (RS-RS, RS-FS, FS-RS and FS-FS) were assessed for evidence of significant spiking associations during performance of the movement task. Spiking associations between FS-FS neurons were the most commonly seen (15.71% of pairs tested) followed by RS-RS (7.14%), FS-RS (7.01%), RS-FS (6.29%). Excitatory associations were significantly more likely to occur in RS-RS and FS-RS pairings, but excitatory and inhibitory associations were equally likely in FS-FS and RS-RS pairings. Neurons were also paired based upon whether they were recorded from HL or FL regions of MI. Spiking associations were most commonly seen between HL-HL pairs (23.11% of pairs tested), followed by FL-FL (16.43%) and FL-HL (2.21%) pairs. However, while the detected FL-FL and FL-HL associations were significantly more likely to be excitatory in nature, HL-HL (91.3%) associations were almost exclusively inhibitory. The results of this study indicate that during the performance of a skilled reaching task, pairings of different neuronal subtypes in the FL representation of MI synchronize their firing activity, while neuronal pairs in HL regions undergo significant desynchronization.

Disclosures: D. Putrino, None; **S. Kim**, None; **S. Ghosh**, None; **E.N. Brown**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.6

Topic: D.17. Voluntary movements

Support: NIH NINDS R01 NS048845

PVA Research Foundation 114338

Title: Differential modulation of beta local field potentials in motor and premotor cortex during multi-sensory action observation

Authors: *A. J. SUMINSKI, K. TAKAHASHI, N. G. HATSOPOULOS;
Organismal Biol. and Anat., Univ. Chicago, CHICAGO, IL

Abstract: Experimental evidence has shown greater diversity in the responses of neurons in primary motor cortex (MI) than is typically assumed. In addition to driving overt movement, neurons in MI discharge in response to passive visual observation of action, kinesthetic illusion, and passive joint motion. We have recently shown that the presence of congruent multi-sensory feedback during observation evokes responses in MI that are nearly indistinguishable from those measured during overt movement. Are local field potentials (LFPs) in motor cortex similarly modulated by multi-sensory feedback?
Two rhesus monkeys were trained to perform a random target pursuit task in the horizontal plane at the level of their chest. They moved a visual cursor aligned with their hand location to hit a sequence of targets using a two-link robotic exoskeleton. Immediately following each target hit, a new target appeared in a random location. A chronically implanted multi-electrode array was used to record LFPs in MI and premotor cortex (PM) while the monkeys performed the task. Each experimental session consisted of two phases. In the active movement phase (AM), the animal controlled the cursor via the exoskeleton. During the passive playback phase, target positions and cursor movements generated during the active movement phase were replayed to the monkey in three observation conditions. In the Visual Playback (V) condition both the cursor and the target were visible to the animal during playback while the animal voluntarily maintained a static arm posture. In the Proprioceptive Playback (P) condition both the cursor and target were invisible and the monkey's hand was moved through the replayed trajectory of the invisible cursor by the robotic exoskeleton. In the Visual+Proprioceptive Playback (V+P) condition both the target and the cursor were visible and the monkey's hand was moved through the replayed cursor trajectories by the exoskeleton.
We observed a significant increase in beta frequency (10 - 45Hz) LFP power in MI and PM during both active movement and action observation. The observation conditions evoked stronger beta responses than the AM condition, with the greatest beta power being found in the P condition. Beta oscillations were most strongly phase locked to each target hit in the P condition as well. Furthermore, we found that beta activity propagated as traveling waves in each condition. The propagation speed of waves sustained for longer than 20ms ranged from 20 - 40 cm/s and their propagation directions were bimodally distributed forming an axis aligned parallel to the rostral-to-caudal axis over the arm area of MI.

Disclosures: A.J. Suminski, None; **K. Takahashi**, None; **N.G. Hatsopoulos**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.7

Topic: D.17. Voluntary movements

Support: NIH grant MH60246-03

Title: Thresholds and locations of excitation and inhibition in primary motor cortex: Evidence from electromyography and imaging

Authors: *S. NARAYANA[1,2], C. RÁBAGO[2], W. ZHANG[2], C. STRICKLAND[2], C. FRANKLIN[2], P. FOX[2], J. LANCASTER[2];
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Abstract: The neuronal origins of cortical excitation and inhibition induced by Transcranial magnetic stimulation (TMS), the thresholds or the cortical locations of these phenomena are largely unknown. This study characterizes the excitation and inhibition at the site of stimulation as well as inhibition at remote sites in the cortex using electromyography and cerebral blood flow (CBF) measurements.
Fourteen healthy adult participants (7 males; mean age 29.6) performed bi-manual isometric contractions of the first dorsal interosseous (FDI) muscles at 25% of maximum voluntary contraction (MVC) in a TMS/electromyography (TMS/EMG) study. In a separate study, twelve, healthy adults (6 males, mean age 35) underwent CBF measurement using H215O-PET imaging during TMS (TMS/PET). In the TMS/EMG study, the incidence, onset and duration of Cortical silent period (CSP), ipsilateral silent period (ISP), as well as the onset and amplitude of motor evoked potential (MEP) were calculated using the automated parameterization method (Rabago et al., 2009). During TMS/PET study each subject underwent trials of 3 Hz TMS at 75%, 100% and 125% resting motor threshold (rMT), and sham TMS. Significant increases and decreases in CBF in the group statistical parametric images (SPI) of voxel-wise computation of z-scores contrasting TMS conditions with sham TMS were identified.
The incidence of CSP and ISP data showed that similar to MT, CSP and ISP thresholds could be defined as the minimum stimulus intensity to yield each phenomenon in 50% of the epochs. The average motor threshold (MT) for the group was 55±16.9% machine output. The threshold for CSP was 73.1±10.7% MT, and the threshold for ISP was

111.0±26%MT. Excitatory effects of TMS were evidenced by increasing Z-scores and volumes of activation with increasing TMS intensity at LM1-hand. The peak locations of activations were observed to be sulcal. The inhibitory effects of TMS, seen as decreased CBF, were evident at the site of stimulation (LM1-hand) as well as in the contra-lateral M1-hand (RM1-hand) and were more superior and anterior. The co-ordinates of decreased CBF were predominantly seen at the gyral crown especially at the site of stimulation.
We demonstrate here differences in thresholds, onsets, and locations of excitatory and inhibitory effects of TMS that support the idea of different neuronal origins of these phenomena. Increases in the excitatory drive at the site of stimulation were evident in imaging as increasing CBF. Peak activations were located in the central sulcus. The inhibitory effects of TMS were seen at multiple sites mainly at the gyral crown, perhaps indicating to the origin of inhibition to be interneurons.

Disclosures: S. Narayana, None; **C. Rábago**, None; **P. Fox**, None; **W. Zhang**, None; **C. Strickland**, None; **C. Franklin**, None; **J. Lancaster**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.8

Topic: D.17. Voluntary movements

Support: NSF-CDI type 1 (ET)

AHA (KG)

NSF-CDI type 1 (JC)

NSF-CAREER (JC)

Title: Directional and temporal selectivity in motor cortex

Authors: E. B. TORRES[1], K. GANGULY[2], J. V. JOSE[3], *J. M. CARMENA[4];
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Abstract: It is generally believed that the planning of voluntary reaches primarily

engages the Pre-Motor and Parietal cortices while their execution is controlled by the Primary Motor cortex. Primary Motor cortex has been involved in different force learning patterns during reaching motions. Mastering the arm dynamics to control such hand motions requires the distinction and the coordination of different acceleration patterns internally produced with different time scales. Given that intentional planning is thought to occur in Pre-motor and Parietal areas, here we ask if movement activity does contribute to movement planning within motor cortex. We found indeed that movement activity increases the information transmission of preparatory activity in motor cortex and yields a mutual-information oscillatory pattern which allows a precise selection of the direction and the timings of maximal acceleration relative to maximal speed. Movement activity in motor cortex specifically contributes to the update of movement preparation for selection of multiple temporal dynamic time-scales.

Disclosures: E.B. Torres, None; **K. Ganguly**, None; **J.M. Carmena**, None; **J.V. Jose**, None.

Nanosymposium

732. Cortical Neurophysiology for Movement Control

Location: Room 33C

Time: Wednesday, November 17, 2010, 8:00 am - 10:15 am

Program Number: 732.9

Topic: D.17. Voluntary movements

Support: NIH grant NS37422

Belgian American Educational Foundation, Belgium

Fondation pour la Vocation, Belgium

Title: Generalization patterns of motor learning depend on M1 plasticity

Authors: *J.-J. ORBAN DE XIVRY[1], S. E. PEKNY[1], M. K. MARKO[1], D. PASTOR[1,2], J. IZAWA[1], P. CELNIK[3], R. SHADMEHR[1];
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Abstract: During adaptation, the brain not only learns to produce motor commands that reduce errors in the trained workspace, but also generalizes this training to novel areas.

The generalization pattern is thought to be a signature of the brain region most responsible for the formation of motor memories. For example, in force field adaptation, the generalization pattern suggests that the motor memories are formed via computational elements that are tuned to the direction of movement, but modulated very broadly by position of the limb. This encoding appears to be in proprioceptive coordinates, consistent with the coding found in the cells of the primary motor cortex. If so, then altering mechanisms of plasticity in the motor cortex should alter the patterns of generalization. Here, we tested this idea in humans. We trained subject to reach to a target (target 1) that was place in the left workspace under force-field perturbations. We tested the generalization patterns by asking the subjects to reach to two other targets in the right workspace (these movements were in channel trials in which errors were effectively eliminated). These targets were arranged to be identical to the trained target in extrinsic space (same hand displacement), or intrinsic space (same joint displacement). We applied transcranial direct current stimulation (tDCS) over the primary motor cortex during adaptation. Earlier work had demonstrated that tDCS does alter cortical plasticity. We found no effect of stimulation in the trained workspace, but only in the patterns of generalization that accompanied this training: application of tDCS to M1 produced greater generalization in intrinsic coordinates but had no effect on generalization patterns in extrinsic coordinates. The intrinsic space generalization patterns that accompany adaptation to force fields appear to depend on plasticity in M1.

Disclosures: J. Orban de Xivry, None; **S.E. Pekny**, None; **M.K. Marko**, None; **D. Pastor**, None; **J. Izawa**, None; **P. Celnik**, None; **R. Shadmehr**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.1

Topic: E.08. Biological Rhythms and Sleep

Support: CHP Innovation Award, Children's Hospital of Pittsburgh of UPMC (to Dr. Robert D. Nicholls)

RAC graduate fellowship, Children's Hospital of Pittsburgh of UPMC (to Wan Zhu)

Title: A novel transcriptional regulator of the mammalian circadian system

Authors: *W. ZHU[1,2], B. J. HENSON[1], J. WETZEL[1], R. D. NICHOLLS[1,2];
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Abstract: Circadian rhythms provide an internal body clock that governs metabolic, physiological, and behavioral functions linked to 24 hour light-dark cycles. A cell-autonomous master clock in the suprachiasmatic nucleus (SCN) synchronizes body clocks; light entrains the master clock and food can entrain peripheral clocks. Cellular clocks are driven initially by a positive feedback loop comprised of heterodimers of CLOCK and BMAL1, transcription factors that bind "E-box" motifs and activate transcription of circadian core, input, and output genes, including *PER* and *CRY*. PER/CRY heterodimers then inhibit CLOCK/BMAL1 function, forming a negative feedback loop. Oscillations driven by these loops are fine-tuned by ancillary regulatory loops (eg., via REV-ERBα) and post-transcriptional regulatory events. Here, we present evidence that nuclear respiratory factor-1 (NRF1) participates in circadian regulation. Bioinformatics analyses of genome sequences with a 10-nt core NRF1 binding motif identified evolutionary conserved motifs in the 5' regulatory regions of 25 of 45 core clock and circadian regulatory genes, including *CLOCK*, *PER1*, *CRY1*, *REV-ERBα*, *DBP*, and *CSNK1E*. Chromatin immunoprecipitation and *NRF1* siRNA assays confirmed NRF1 binds the predicted sites and regulates expression of these genes. Similarly, transient transfection assays for promoter (*CLOCK*, *CRY1*) and promoter-enhancer (*PER1*, *DBP*) reporters indicate that mutations of NRF1 binding sites significantly reduce luciferase activities; intriguingly, mutations of flanking E-boxes at the *DBP* enhancer have no effect in this assay. NRF1 and CLOCK could interact at the *DBP* enhancer, and we confirmed interaction by co-immunoprecipitation, where NRF1 and BMAL1 each primarily interact with a phosphorylated CLOCK isoform. Using serum-induced circadian oscillations in NIH 3T3 cells, *Nrf1* mRNA and protein levels oscillated; intriguingly, short and long *Nrf1* mRNA isoforms oscillate in antiphase patterns corresponding to miR-96 (targets the short form) and miR-182 (targets both forms) expression. Two different experimental assays indicate that miR-96/-182 regulate *NRF1* mRNA and translation, with previous studies suggesting circadian expression of both miRNAs in mouse retina. Finally, preliminary data from the Allen Brain Atlas illustrates enriched *Nrf1* expression in the mouse SCN. NRF1 has developmental and functional roles in central and retinal neurons, in energy balance, and as NRF1 regulates ~6% of genes across the genome but ~55% of circadian regulators (a 9-fold increase), we conclude that NRF1 contributes to circadian gene regulatory networks.

Disclosures: W. Zhu, None; **B.J. Henson**, None; **J. Wetzel**, None; **R.D. Nicholls**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.2

Topic: E.08. Biological Rhythms and Sleep

Support: NIH P01-NS39546

Title: Rhythmic microRNAs targeting Bmal1: Implications for post-transcriptional modulation of circadian rhythms in the SCN pacemaker and in the periphery in mammals

Authors: V. SHENDE[1], M. GOLDRICK[2], S. RAMANI[2], *D. J. EARNEST[3,1];
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[3]Neurosci & Exp. Therapeut., Texas A&M Univ. Hlth. Sci. Ctr., COLLEGE STA, TX

Abstract: MicroRNAs (miRNAs) are small, non-coding RNAs that interact with 3'UTR elements to regulate stability or translation of target mRNAs. miRNAs have been recently implicated in the regulation of a wide array of biological processes including circadian rhythms. Because rhythmicity is a prevalent property among core and regulatory elements of the circadian clock mechanism, we explored possible timekeeping function of miRNAs by first determining whether expression of specific miRNAs predicted to target clock genes oscillate in SCN cells as well as in the periphery. Real-time PCR analysis was used to examine the expression profiles of mature miRNAs that are predicted to target 3'UTR of Bmal1 mRNA because mutations in this clock gene produce behavioral arrhythmicity. Effects of miRNA over-expression on BMAL1 protein levels and 3'UTR activity of this clock gene were also analyzed. In SCN2.2 cells, levels of miR-142-3p were marked by circadian rhythmicity and overexpression of this miRNA decreased BMAL1 protein levels. It is interesting that miRNAs with Bmal1 as a predicted target were also expressed extracellularly in SCN2.2-conditioned medium and in the circulation of mice. Bimodal rhythms in the levels of miR-152, and miR-494 were observed in mouse serum. Luciferase-3'UTR reporter assays indicate that when over-expressed in NIH/3T3 fibroblasts, these miRNAs significantly repress Bmal1 3'UTR-mediated luciferase activity. Our results suggest that miRNAs may play a role in the intracellular and extracellular regulation of the circadian clockworks both within the SCN and the periphery. Supported by NIH P01-NS39546 (DE)

Disclosures: V. Shende: None. **M. Goldrick:** Employment; BIOO Scientific Corp. **S. Ramani:** BIOO Scientific Corp. **D.J. Earnest:** Research Grant; NIH P01-NS39546.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.3

Topic: E.08. Biological Rhythms and Sleep

Title: Analysis of circadian rhythm using a novel SCN-specific Cre transgenic mouse line

Authors: *A. CHANG[1], T. MATSUKI[1], A. SKACH[3], M. YANAGISAWA[3,2];
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Abstract: The neurons that make up the suprachiasmatic nucleus (SCN) temporally organize behavior into circadian cycles of activity and rest. When dissociated, these neurons individually oscillate with various period, phase, and amplitude. These conflicting results can be reconciled if inter-neuronal networking in the SCN is required for a consolidated behavioral circadian rhythm. To test this hypothesis, a novel SCN-specific Cre transgenic mouse line, named NMS-Cre, was developed by inserting a bicistronic Cre expression cassette at the 3'-untranslated region of the Neuromedin S (NMS) gene. By crossing NMS-Cre line to a lox-STOP-lox diphtheria toxin receptor line, behavioral circadian rhythm was disrupted upon intraperitoneal injection of diphtheria toxin. A histological examination showed that diphtheria toxin injection eliminated ~85% of NMS-Cre containing neurons at the SCN. Next, NMS-Cre mediated Bmal1 conditional knockout animals were generated to study behavioral rhythm output when most of the SCN neurons are without a molecular oscillator. The NMS-Cre;Bmal1$^{flox/flox}$ animals have essentially normal circadian rhythm of locomotor activity. Then, we generated NMS-Cre mediated Vesicular GABA transporter (VGAT) conditional knockout animals because GABA has long been suspected to play a role in behavioral circadian rhythm. The NMS-Cre;VGAT$^{flox/flox}$ animals performed normally in behavioral circadian rhythm parameter such as free-running period, robustness, and phase response curve. These in vivo data demonstrated a model that intra-SCN neuronal network is required for behavioral circadian rhythm, and mediates molecular clock outputs from a small number of SCN neurons. Despite the fact that virtually all SCN neurons are GABAergic, GABA is an unlikely transmitter for this intra-SCN networking.

Disclosures: A. Chang, None; **T. Matsuki**, None; **A. Skach**, None; **M. Yanagisawa**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.4

Topic: E.08. Biological Rhythms and Sleep

Title: A mechanism coupling two major subregions of the mammalian central circadian pacemaker

Authors: *Y. SHIGEYOSHI[1], S. KOINUMA[1], M. NAGANO[1], K. YAGITA[2], S.-I. HASHIMOTO[3];
[1]Kinki Univ. Sch. Med., Osaka 589-8511, Japan; [2]Osaka Univ. Grad. Sch. Med., Suita, Japan; [3]Japan Sci. and Technol. Agency, Tokyo, Japan

Abstract: In the present study, we tried to delineate the mechanism that synchronizes subregions of the suprachiasmatic nucleus (SCN), the center of the mammalian circadian rhythm. Morphologically and functionally, the SCN is divided into ventrolateral (VLSCN) and dorsomedial regions (DMSCN). The optic nerve projects to the VLSCN but not DMSCN, suggesting that only VLSCN but not DMSCN directly receives the optical information from the retina. Thus, the functional differences clearly appear when animals are exposed to light during the night. Previously, we demonstrated that the endogenous desynchrony between VLSCN and DMSCN occurs after a rapid light:dark cycle shift in rats. After the LD cycle shift, the VLSCN was reset rapidly while the DMSCN shift slowly. The observation suggests that the jet lag syndrome is caused by a slow shift of DMSCN. As these subregions are synchronized in a usual photic environment and they show resynchronization after the transient desynchrony, it is probable that the subregions also have distinct ligand and receptor systems to different signal transduction pathways that generate the phase shift. We performed comprehensive gene expression analysis of DMSCN and VLSCN by using a micropunch method. As a result, two ligand-receptor pairs that possibly mediate the synchronization between VLSCN and DMSCN were selected. One was VIP and Vpac2 receptor. In situ hybridization study demonstrated that VIP and Vpac2 receptor mRNAs were confined to VLSCN and DMSCN, respectively. We applied VIP and VPAC2 agonist to the SCN and, with separation of DMSCN and VLSCN, the DMSCN alone shows a large shift longer than 10 hours. The findings together suggests that VIP and Vpac2 system possibly a mediator to resynchronization after LD cycle shift.

Disclosures: Y. Shigeyoshi, None; **S. Koinuma**, None; **M. Nagano**, None; **K. Yagita**, None; **S. Hashimoto**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.5

Topic: E.08. Biological Rhythms and Sleep

Title: Glutamatergic neurons in the dorsomedial hypothalamic nucleus regulate circadian and diurnal rhythms of locomotor activity

Authors: *N. VUJOVIC[1], P. M. FULLER[1], Q. TONG[3], B. B. LOWELL[2], C. B. SAPER[1];
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Abstract: Previous studies have shown the dorsomedial nucleus of the hypothalamus (DMH) to be a major relay and integrator in the circuitry through which patterns in arousal state, behavior and endocrine rhythms are temporally organized. The DMH receives both direct and indirect inputs from the suprachiasmatic nucleus (SCN), the master circadian pacemaker. Cell-specific DMH lesions reduce the amplitude of circadian rhythms of wakefulness, corticosteroid secretion, feeding and locomotor activity (LMA), and reduce mean daily LMA, body temperature and wakefulness levels. The DMH sends excitatory (glutamate and thyrotropin releasing hormone) projections to wake-promoting neurons in the lateral hypothalamus (LH) and other structures, and we hypothesize that these projections are critical for driving rhythms in behavior, specifically LMA. To test the importance of glutamatergic efferents of the DMH in vivo, we employed mice in which the second exon of the gene for the vesicular glutamate transporter 2 (VGlut2) was flanked by loxP sites. By stereotaxically injecting an adeno-associated viral (AAV) vector expressing Cre recombinase into the DMH, we were able to selectively eliminate VGlut2 protein, and hence glutamatergic neurotransmission from this region. LMA and body temperature were measured using biotelemetry and control experiments were performed by injecting an AAV expressing only green fluorescent protein in the DMH. Loss of VGlut 2 expression in the DMH correlated with a measurable decrease in the amplitude of LMA rhythms (approximately 40% that of controls) and daily mean activity levels (approximately 50%) for free-running mice in constant dark. This indicates that glutamatergic outputs of the DMH are an important part of the outflow circuit by which the SCN drives circadian rhythms in LMA. Furthermore, when entrained to a 12:12 light dark cycle, mice in which VGlut 2 expression was deleted in the DMH showed similarly lowered mean LMA (roughly 50% of control levels) and even lower amplitude of LMA rhythms relative to controls (only about 20% the amplitude), suggesting that this lesion may also disrupt pathways involved in light masking of LMA. Previous studies have shown that retinohypothalamic projections to the

SCN and/or adjacent hypothalamic areas (which in turn project to the DMH) play an important role in light masking. Our results suggest that glutamatergic efferent neurons in the DMH may also be a “next step” in the circuit by which light suppresses activity in nocturnal rodents. This deletion did not significantly affect body temperature in any of our cohorts.

Disclosures: N. Vujovic, None; **Q. Tong**, None; **P.M. Fuller**, None; **B.B. Lowell**, None; **C.B. Saper**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.6

Topic: E.08. Biological Rhythms and Sleep

Support: DK 040498

DK 081546

Title: Leptin-saporin lesions of the arcuate nucleus induce an arrhythmic circadian feeding pattern

Authors: *M. F. WIATER, M. OOSTROM, R. BARFIELD, T. T. DINH, A.-J. LI, S. RITTER;
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Abstract: The endogenous circadian rhythm of feeding is incompletely understood. The leptin sensitive network within the arcuate nucleus (Arc) of the hypothalamus is important for the control of feeding. Genetic deletion of leptin or leptin receptors results in profound obesity, hyperphagia, and the loss of day/night differences in food intake. Because the Arc is critically involved in control of food intake and contains leptin receptors, we hypothesized that the Arc plays an important role in maintenance of feeding rhythms. To examine this hypothesis, we injected a newly developed targeted toxin, leptin conjugated to saporin (LSAP), into the Arc to lesion leptin receptor-expressing neurons in the vicinity of the injection. Controls were injected saporin conjugated to a peptide with no known action or receptor (blank-saporin, BSAP). We expected the Arc LSAP would disrupt the circadian rhythm of food intake, as seen in rats with genetic deletion of leptin or its receptor. Eating rhythms were monitored continuously (each

minute) over a 60-day period using BioDAQ (Research Diets) automated meal monitoring equipment. Data were analyzed for circadian rhythm using ClockLab (ActiMetrics) software. Eatograms (food intake in actogram format), showing eating times and durations comparable to actograms used for wheel-running activity, and Chi-square periodograms were generated. Feeding was monitored in light:dark, dark:dark, or light:light conditions. The LSAP injection caused profound hyperphagia, weight gain and disrupted circadian feeding patterns. Although LSAP rats remained sensitive to light and dark under certain circumstances and were capable of an apparent rhythm during light:dark conditions, feeding was arrhythmic by all measures when photic cues were removed (i.e., in dark:dark and light:light conditions). At the end of experimentation, lesions were analyzed using immunohistochemistry to detect agouti gene related protein (AGRP) and α-melanocortin stimulating hormone (α-MSH) neurons, both known to express leptin receptors. Cell bodies positive for these peptides were greatly diminished in the Arc. Results indicate that the Arc contributes importantly to the expression of circadian rhythms of food intake. Supported by PHS grant #DK 040498 and DK 081546.

Disclosures: M.F. Wiater, None; **M. Oostrom**, None; **R. Barfield**, None; **T.T. Dinh**, None; **A. Li**, None; **S. Ritter**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.7

Topic: E.08. Biological Rhythms and Sleep

Support: DFN Grant 2004.00.027

Title: Hypothalamic neuropeptides involved in the SCN control of hepatic glucose production

Authors: *A. KALSBEEK[1,2], C.-X. YI[2], E. FLIERS[1];
[1]Amsterdam Med. Ctr. (AMC), Dept Endocrinol. and Metabolism, Amsterdam, Netherlands; [2]Hypothalamic Integration Mechanisms, Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: The hypothalamic paraventricular nucleus (PVN) is an important target area of biological clock output as it harbors the neuro-endocrine neurons that control peripheral hormones such as corticosterone and thyroid-stimulating hormone, as well as the pre-

autonomic neurons that control the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). The master biological clock, located in the hypothalamic suprachiasmatic nuclei (SCN), uses its projections to these neuro-endocrine and pre-autonomic neurons in the hypothalamus to control daily hormone rhythms, e.g. adrenal corticosterone and pineal melatonin release. The SCN also plays an essential role in maintaining daily blood glucose concentrations. Using local intra-hypothalamic administration of GABA and glutamate receptor (ant)agonists we previously demonstrated how changes in ANS activity contribute to the daily control of plasma glucose and plasma insulin concentrations. Selective hepatic denervations evidenced that the ANS is also an important gateway for the SCN to transmit the (phase-shifting) effects of light to the glucoregulatory and clock gene machinery of the liver. Finally, using ICV administration of neuropeptides and/or their (ant)agonists we were able to delineate how the SCN may also "use" the hypothalamic neuropeptide systems to control the daily plasma glucose rhythms. We found that the VIP-containing SCN outputs and the orexin-containing neurons in the lateral hypothalamus are important molecular links to modulate hepatic glucose production. On the other hand, PACAP release in the PVN turned out to be a strong stimulator of hepatic glucose production, but this effect does not seem to part of the circadian timing system. In our most recent experiments we investigated the possible involvement of vasopressin and oxytocin in the circadian timing system. No evidence could be found for an involvement of the vasopressinergic SCN-projections. On the other hand, the oxytocin-containing PVN neurons do seem to be one of the targets for the SCN output. Another possible link between the circadian timing system and glucose homeostasis is the pineal release of melatonin. Despite the recent link of mutations in the melatonin receptor with an increased risk of 2 type 2 diabetes, our investigations so far only found minor effects of pinealectomy, with or without restitution of melatonin, on glucose metabolism. Clearly, further studies combining neuroanatomy and physiology are necessary to reveal the pathways used by the circadian timing system to enforce its rhythmic message on the glucose regulatory system.

Disclosures: A. Kalsbeek, None; **E. Fliers**, None; **C. Yi**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.8

Topic: E.08. Biological Rhythms and Sleep

Support: NSF CAREER Award BES-0547457

Title: Reading the circadian code in Limulus efferent nerve spike train

Authors: *J. S. LIU, C. L. PASSAGLIA;
Biomed. Engin., Boston Univ., Boston, MA

Abstract: The lateral eyes of the horseshoe crab *Limulus polyphemus* show a pronounced circadian rhythm, which is thought to be mediated by efferent nerve fibers from the brain. However, little is known about how the fibers encode and transmit circadian messages. In order to uncover the neural messages that drive circadian changes in lateral eye sensitivity, we recorded efferent spike trains in vivo for several hours and days with an extracellular suction electrode. Statistical features of single and multi-fiber spike trains were characterized using temporal and spectral techniques, including serial correlation and power spectrum analysis. The output of the *Limulus* circadian clock to the eye was highly structured on multiple time scales, consisting of multi-cellular bursts of spikes which were grouped into clusters and packets of clusters that repeated throughout the night and disappeared during the day. The bursts typically occurred around 1 second in clusters of 10-30 bursts separated by several seconds to a minute of quiescence. Clock neurons each contributed only one spike to every burst in a preferred order, and intervals between nearby bursts and clusters were positively correlated in length. The intervals of adjacent clusters followed a gamma distribution in length and were random in order. It was also discovered that the clock output was strongly modulated at night by brief flashes of light. The results provide the first quantitative description of the neural output of the *Limulus* circadian clock. The complex firing pattern may be important for driving circadian rhythms in the eye and other organs. Additional experiments were set up to deliver artificial efferent spike trains to drive retinal activity. Preliminary data showed visible modulation of the ERG amplitude due to the impact of current pulses. Simple non-physiological stimulation routine without burst structure did not produce noticeable changes in ERG amplitude, whereas more naturalistic paradigm increased ERG amplitude within 30 minutes of stimulation onset, and lasted more than 3 hours. In conclusion, the Limulus circadian clock sends complex and highly structured message through the efferent nerve fibers and our results show that the complex firing pattern is essential for effective coding of the circadian rhythm of visual sensitivity.

Disclosures: J.S. Liu, None; **C.L. Passaglia**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.9

Topic: E.08. Biological Rhythms and Sleep

Title: A fearful stimulus alters per2 expression and cfos activity in brain regions involved in fear memory

Authors: *H. PANTAZOPOULOS[1,2], H. DOLATSHAD[2], F. C. DAVIS[2];
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Abstract: Evidence demonstrates that rodents learn to associate a foot shock with time of day, indicating the formation of a fear related time-stamp memory, even in the absence of a functioning SCN. This type of memory may be regulated by circadian pacemakers outside the SCN. As a first step in testing the hypothesis that clock genes are involved in the formation of time-stamp fear memory, we exposed one group of mice to fox urine (TMT) at ZT 0 and one group at ZT 12 for 4 successive days. A separate group with no exposure to TMT was also included as a control. Animals were sacrificed one day after the last exposure to TMT, and per2 and cfos protein were quantified in the SCN, amygdala, hippocampus, and piriform cortex. Exposure to TMT had a strong effect at ZT0, decreasing per2 expression at this time point in all regions except the SCN, and reversing the normal rhythm of per2 expression in the amygdala and piriform cortex. These changes were accompanied by increased cfos expression at ZT0. In contrast, exposure to TMT at ZT 12 abolished the rhythm of per2 expression in the amygdala and piriform cortex, and had no effect on hippocampal per2 expression. In addition, increased cfos expression was only detected in the central nucleus of the amygdala. TMT exposure at either time point did not affect per2 or cfos in the SCN, or wheel-running activity, indicating that under a light-dark cycle, the SCN rhythm is very stable in the presence of repeated exposure to a fearful stimulus. Taken together, these results indicate that exposure to a fearful stimulus has a stronger effect on per2 expression and cell activity during the early subjective day than during the early subjective night, possibly to prepare cells at ZT0 to respond to a fearful stimulus.

Disclosures: H. Pantazopoulos, None; **H. Dolatshad**, None; **F.C. Davis**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.10

Topic: E.08. Biological Rhythms and Sleep

Support: NICHD Grant 36460

Title: Seasonal changes in photoperiod modulate the influence of light on the circadian system

Authors: *G. L. GLICKMAN, J. ELLIOTT, M. R. GORMAN;
Ctr. for Chronobiology and Dept. of Psychology, UCSD, La Jolla, CA

Abstract: Light is the most potent stimulus for regulating the circadian clock. Seasonal changes in photoperiod significantly affect circadian response to light, in terms of photic sensitivity and maximal phase resetting of activity rhythms. While those robust effects were determined under highly controlled lighting conditions, photoperiodic differences in light sampling behavior have yet to be examined and may be critical to understanding the practical relevance of previous findings. In addition, further studies examining photoperiodic modulation of additional light-driven responses are necessary. In this study, male Syrian hamsters were entrained to either a long (LD; LD14:10; n=8) or short (SD; LD10:14; n=8) day for 6 weeks. Subsequently, hamsters were individually housed and video-recorded for the duration of four, 15-minute 480 nm narrowband light pulses, each separated by at least one week. Each animal was observed under two intensities (0.3 and 68.0 $\mu W/cm^2$) at two different circadian phases (ZT14 and ZT19 or ZT22 for LD and SD, respectively). The proportion of time spent in various regions of the cage was determined, and integrated photon counts were calculated. ANOVA assessed differences in the mean proportion of the maximum total exposure between groups (i.e. photoperiod) as well as changes within animals as a function of intensity and circadian phase. Preliminary analyses reveal a trend for a greater mean proportion of maximal photon exposure under LD versus SD during the relatively dimmer light pulse (0.3 $\mu W/cm^2$) ($p<0.059$). In contrast, no significant photoperiod differences were found with the brighter light pulse (68.0 $\mu W/cm^2$). Studies examining the influence of photoperiod on light-induced melatonin suppression are currently underway. Further understanding how photoperiodic history affects various photic responses will be important to the development of optimal light treatment strategies.

Disclosures: G.L. Glickman, None; **J. Elliott**, None; **M.R. Gorman**, None.

Nanosymposium

733. Molecular and Physiological Mechanisms Underlying Circadian Rhythms

Location: Room 4

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 733.11

Topic: E.08. Biological Rhythms and Sleep

Title: Light modulates learning and mood via melanopsin cells

Authors: *C. M. ALTIMUS[1], T. A. LEGATES[2], S. YANG[3], A. KIRKWOOD[3], T. WEBER[4], S. HATTAR[2];
[1]Johns Hopkins Univ., BALTIMORE, MD; [2]Biol., [3]Mind Brain Inst., Johns Hopkins Univ., Baltimore, MD; [4]Neurosci., Rider Univ., Lawrenceville, NJ

Abstract: Short day length and irregular light schedules can lead to mood disorders, cognitive dysfunction and fatigue as observed in seasonal affective disorder (SAD), shift work disorder and transmeridian travel. A common feature of these various light environments is a change in the timing or duration of light input. However, light has only been indirectly attributed to these disorders though its disruption of sleep and circadian rhythms. Here we sought to determine the direct role of light in regulating cognitive functions and mood related behaviors by administering light pulses throughout the circadian cycle in a manner that does not change sleep amounts or abolish circadian rhythmicity. We show that irregular light schedule increases depression like behaviors and reduces hippocampal long term potentiation and learning. Alleviating the depression-like behaviors in mice exposed to the irregular light cycles by administering an antidepressant, desipramine, restores hippocampal dependent learning. In mammals, light is transduced by photoreceptors in the retina into an electrical signal that can be interpreted by the brain. To determine the cells responsible for conveying this light information to areas of the brain that control learning and mood, we used a mouse line lacking melanopsin containing intrinsically photosensitive retinal ganglion cells (ipRGCs). These ipRGCs have been shown to convey light information to modulate circadian rhythms and sleep but have not been linked to light-influenced limbic functions. Learning and mood is unaffected by exposure to light pulses throughout the circadian cycle in mice lacking ipRGCs. These findings reveal that disruptive light conditions can negatively impact learning and mood via light transmission by the melanopsin cells.

Disclosures: C.M. Altimus, None; **T.A. LeGates**, None; **S. Yang**, None; **A. Kirkwood**, None; **T. Weber**, None; **S. Hattar**, None.

Nanosymposium

734. Attention: EEG and TMS

Location: Room 6F

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 734.1

Topic: F.01. Human Cognition and Behavior

Support: NIH 2RO1 MH64043

Title: Transcranial magnetic stimulation over posterior parietal cortex shifts spatial attention biases

Authors: *S. M. SZCZEPANSKI, S. KASTNER;
Dept. of Psychology, Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Using fMRI, we previously described a number of topographically organized areas in frontal (FEF, PreCC/IFS) and posterior parietal cortex (PPC; IPS1-5, SPL1) that carry spatial attention signals (Szczepanski et al, 2010). The neural signals in these topographic areas were spatially-specific, with stronger responses when attention was directed to the contralateral than to the ipsilateral visual field. Based on these data, we have proposed a model of spatial attention control, in which each topographic area contributes to the control of spatial attention across the visual field by generating a spatial bias, or "attentional weight", towards the contralateral hemifield. The sum of the weights within a hemisphere constitutes the overall spatial bias that is exerted over contralateral space. Although these topographic areas work in concert to generate an overall spatial bias towards the contralateral visual field, each individual area most likely contributes a unique component towards this overall bias that may vary considerably among areas. In the current study, we used single-pulse transcranial magnetic stimulation (TMS) to investigate the distinct contribution made by several topographic PPC areas to the overall spatial bias of individual subjects.

In separate sessions, we applied TMS at 200 ms after stimulus onset over three different functional regions of PPC (right IPS1/2, left IPS1/2, and right SPL1) while subjects performed the landmark task, which is a perceptual version of the line bisection task. Neurologically intact subjects show slight biases towards the right or the left visual field while performing this task. We therefore used the landmark task to assess the spatial bias in each of our healthy subjects. As a timing control, subjects also performed the landmark task while right SPL1 was inactivated 100 ms after stimulus onset. Behavioral performance during each of the TMS conditions was compared to performance without TMS.

We found that TMS over both of the right PPC areas shifted subjects' behavioral spatial biases rightward while performing the landmark task, whereas TMS over left PPC shifted subjects' behavioral spatial biases leftward, as compared to performance without TMS. These results were timing specific: shifts in spatial bias were found when TMS was applied to right SPL1 at 200 ms, but not 100 ms, after stimulus onset. Results suggest that each targeted topographic area made its own contribution towards the overall spatial bias exerted over the visual field, as subjects' behavioral bias values were shifted towards the visual field ipsilateral to each of the TMS stimulation sites. These data are consistent with our proposed model.

Disclosures: S.M. Szczepanski, None; **S. Kastner**, None.

Nanosymposium

734. Attention: EEG and TMS

Location: Room 6F

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 734.2

Topic: F.01. Human Cognition and Behavior

Support: Grant of supplies from The Hershey Company

Title: The acute electrocortical (EEG) and blood pressure effects of chocolate

Authors: M. MONTOPOLI[1], *L. C. STEVENS[1], C. SMITH[2], S. PASSINO[2], S. BROWN[2], L. CAMOU[2], K. CARSON[2], S. MAASKE[2], G. MONTOPOLI[2], K. KNIGHTS[2], W. GIBSON[2], J. WU[2];
[1]Psychology, [2]Northern Arizona Univ., Flagstaff, AZ

Abstract: Although many consumers anecdotally report that chocolate has a stimulatory effect on the body and mind, there is a paucity of research on the effect of cocoa bioactive components on electrocortical (ElectroEncephaloGram or EEG) patterns. The present study investigated the effect of consuming chocolate on EEG frequencies. In a between-groups design with 6 conditions, 122 participants consumed either a high cocoa-content chocolate, a low cocoa-content chocolate, a high cocoa-content chocolate +L-theanine, high sugar water, low sugar water, or water. EEGs, blood pressure, and mood were measured before and after a 60-minute digestion period. The results indicated an increase in posterior central beta and a decrease in posterior theta frequencies for the high cocoa-content chocolate condition compared to water. Diastolic blood pressure increased with the consumption of high cocoa-content chocolate when compared to water alone and to high cocoa-content chocolate + L-theanine. Diastolic and systolic blood pressure decreased following consumption of high cocoa-content + L-theanine chocolate relative to high cocoa-content chocolate alone, low cocoa-content chocolate, high sugar water, and low sugar water. Blood pressure changes averaged 4-8 mmHg. No mood changes or gender differences (except for males in general showing more low Alpha) were obtained across the 6 conditions. LORETA neuroimages are presented to identify the locus of EEG changes with consumption of chocolate.

Disclosures: M. Montopoli: None. **L.C. Stevens:** Other Research Support; The Hershey Company. **C. Smith:** None. **S. Passino:** None. **S. Brown:** None. **L. Camou:** None. **K.**

Carson: None. **S. Maaske:** None. **G. Montopoli:** None. **K. Knights:** None. **W. Gibson:** None. **J. Wu:** None.

Nanosymposium

734. Attention: EEG and TMS

Location: Room 6F

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 734.3

Topic: F.01. Human Cognition and Behavior

Support: NARSAD

NIH NIDA Grant DA026452 to A.R.A (P.I.)

NSF graduate fellowship (NS)

NIH Grant NS036449 (HP)

ONR MURI Award N00014-10-1-0072 (HP)

Title: Deep brain stimulation of the subthalamic nucleus alters the cortical profile of response inhibition in the beta frequency band: A scalp EEG study in Parkinson's disease

Authors: *N. C. SWANN[1], H. POIZNER[2], M. HOUSER[4], S. GOULD[4], D. PETERSON[2], J. STRUNK[3], A. R. ARON[3];
[1]UC San Diego, SAN DIEGO, CA; [2]Inst. for Neural Computation, [3]Psychology, UC San Diego, La Jolla, CA; [4]Scripps Green Clin., La Jolla, CA

Abstract: The ability to stop initiated responses is an important everyday function that goes awry in several neurological disorders. Stopping apparently depends on a structurally-connected network of brain regions including the right inferior frontal cortex (IFC) and the subthalamic nucleus (STN). Recent studies have begun to elucidate the functioning of this network. A study with electrocorticography revealed a response over the right IFC in the beta frequency range (16Hz) approximately 200 ms following a stop signal (Swann et al. 2009). Another study recorded directly from the STN and observed a stopping-related beta frequency band increase (Kuhn et al. 2004). Here we further probed this putative circuit by recording scalp EEG from patients with Parkinson's disease both On and Off STN-deep brain stimulation while they performed a stop signal task. If the STN is functionally important for stopping, then STN stimulation could improve stop

signal performance and modulate scalp EEG, perhaps in the beta frequency band.
So far we have studied 12 patients (On and Off stimulation) and 13 age-matched healthy controls. On each trial the subject initiated a manual motor response. On a minority of trials a visual stop signal was presented, requiring the subject to try to stop. We derived the speed of stopping as stop signal reaction time (SSRT). During the task, scalp EEG was recorded using a 64-channel Biosemi system. For each patient, a time-frequency analysis was performed using a Gabor wavelet procedure and a map of z-scores was generated for the contrast of On vs. Off stimulation, time-locked to the stop signal. We evaluated this time-frequency map for significance in the beta band at the group level using a paired t-test.
Patients Off stimulation were significantly slower than controls at stopping their responses ($p<.05$), while patients On stimulation were slightly faster than Off ($p\sim.07$, Off SSRT:306 ms; On SSRT:277 ms; Controls:247 ms). Thus, STN-DBS improved response inhibition. Strikingly, the EEG comparison of On vs. Off stimulation revealed the predicted increase in the beta frequency band ($p<0.05$) in frontal electrodes. Moreover the beta difference started approximately 200 ms after the stop signal and peaked around 300 ms, and was thus consistent with the time window of stopping (i.e. within SSRT).
We find that cortical activity is modulated by STN stimulation during the stop-signal task. The timing and frequency range of the modulation lends additional credence to a structurally-connected functional cortical-subcortical circuit for inhibitory control.

Disclosures: N.C. Swann, None; **H. Poizner**, None; **M. Houser**, None; **S. Gould**, None; **D. Peterson**, None; **J. Strunk**, None; **A.R. Aron**, None.

Nanosymposium

734. Attention: EEG and TMS

Location: Room 6F

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 734.4

Topic: F.01. Human Cognition and Behavior

Support: American Psychological Association Grant #5 T32 MH18882 (BV)

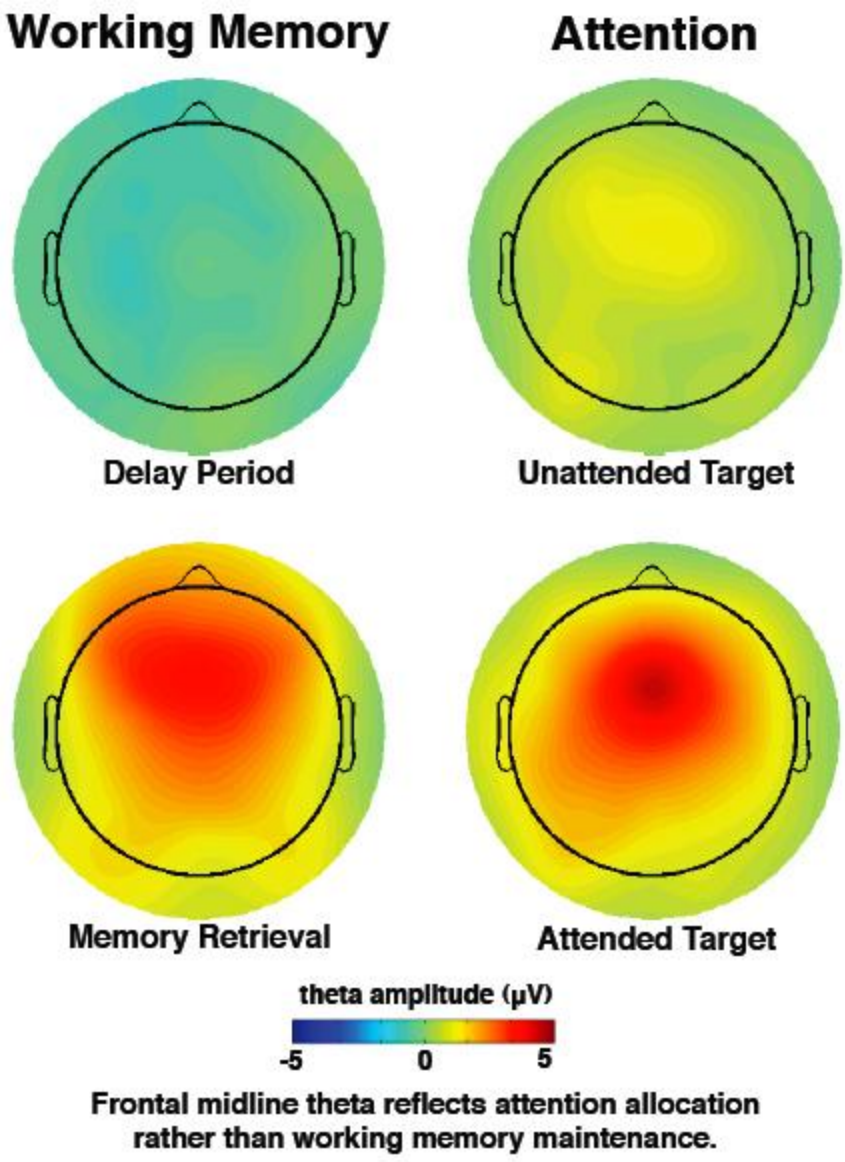
NINDS Grant NS21135-22S1 (BV)

NINDS Grant NS21135 (RTK)

Title: Oscillatory network dynamics in human working memory and attention

Authors: *B. VOYTEK[1], S. LAHUE[1], M. DAVIS[1], L. TSENG[1], R. T. KNIGHT[1,2];
[2]Dept. of Psychology, [1]Univ. California, Berkeley, Berkeley, CA

Abstract: Ongoing low-frequency theta (4-8 Hz) and alpha (8-12 Hz) electrophysiological oscillations are associated with both working memory (WM) and attention processes. Stimulus-related changes in these low-frequency oscillations show stereotyped topographical distributions in scalp EEG. Alpha power is typically associated with WM and attention processes in visual extrastriate cortex whereas theta power is traditionally associated with WM maintenance in frontal cortex. In a set of two experiments with young, healthy controls we employed lateralized visual stimuli to define the roles of these oscillations in attention and WM maintenance and retrieval. During WM alpha power over the visual extrastriate cortex is reduced during the WM maintenance period (900 ms) and during WM retrieval in a load-dependent manner ($F(2,22) = 9.04$, $P = 0.001$). These reductions are stronger in the visual extrastriate cortex contralateral to the hemifield of stimulus presentation ($F(1,11) = 15.84$, $P = 0.002$). A similar pattern is seen in a separate lateralized visual attention task revealing visual extrastriate alpha power reductions for attended targets compared to standards (attention X stimulus interaction, $F(1,15) = 8.86$, $P = 0.009$) with larger reductions in contralateral visual extrastriate ($F(1,15) = 13.21$, $P = 0.002$).
Frontal midline theta power was enhanced during WM retrieval compared to maintenance ($F(1,11) = 37.95$, $P < 0.0005$) independent of load ($F(2,22) = 1.22$, $P = 0.31$). During visual attention frontal midline theta was also enhanced for attended compared to unattended stimuli ($F(1,15) = 14.55$, $P = 0.002$) and is largest for attended targets (attention X stimulus interaction, $F(1,15) = 7.90$, $P = 0.013$). Taken together these results suggest that WM-related changes in alpha and theta power are dependent on attentional allocation and not due to WM maintenance alone. The finding that frontal midline theta power is larger during WM retrieval suggests that frontal theta activity indexes response selection and template-matching processes.



Disclosures: B. Voytek, None; **S. LaHue**, None; **M. Davis**, None; **L. Tseng**, None; **R.T. Knight**, None.

Nanosymposium

734. Attention: EEG and TMS

Location: Room 6F

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 734.5

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01 NS031443

DoD W911NF-09-0001

NSERC

Title: Specialization within the ventrolateral attentional control network for the detection of automatic vs. explicit expectancy violations

Authors: *J. RISTIC[1], B. GIESBRECHT[2];
[1]Psychology, McGill Univ., Montreal, QC, Canada; [2]Psychology, Univ. of California Santa Barbara, Santa Barbara, CA

Abstract: Neuroimaging studies implicate two distinct cortical systems that control visual orienting. Orienting based on internal expectations (i.e., voluntary orienting) is mediated by the bilateral dorsolateral frontoparietal network, which includes the frontal eye fields and the parietal lobe (i.e., the intraparietal sulcus, IPS). Orienting to unexpected events (i.e., reflexive orienting) is mediated by a right lateralized ventrolateral frontoparietal network, which includes the middle frontal gyrus (MFG), ventral frontal cortex (VFC), and the temporal-parietal junction (TPJ). According to a recent proposal, another role of the ventral network is to track relevant environmental contingencies and to initiate the reorienting of attention towards those events (e.g., MFG; Corbetta et al., 2008). We tested this hypothesis by recording EEG while subjects performed a cuing task that manipulated spatial contingencies between a central number cue and a peripheral target. The cues were either spatially uninformative or spatially informative of the target position. In the uninformative condition, the number cue induced a typical left-to-right mental number line, engaging reflexive orienting; in the informative condition, the number cue induced a right-to-left mental number line, engaging volitional orienting. Because the predictive condition explicitly induced a spatial expectancy running in the opposite direction to the automatically generated number line, one could simultaneously track the activity of the ventral network while the dorsal network was engaged by the explicit task. The spatio-temporal dynamics of the two networks were analyzed using a multiple source beamformer analysis of the spectral density in the theta frequency band (4-8 Hz), which has been implicated as important index of attentional control (Green & MacDonald, 2008). The results revealed that the dorsal network was engaged in both conditions. However, the ventral network was engaged concurrently with the dorsal network in the predictive condition indicating that it was tracking automatically created contingencies induced by the typical mental number line. Additionally, we observed a functional dissociation within the ventral network: VFC responded more to violations of the explicit expectancy in the predictive condition whereas TPJ and MFG responded more to violations of expectancy in the nonpredictive condition. These data indicate that subregions of the ventral network may be specialized

for detecting automatically versus explicitly created spatial contingencies.

Disclosures: J. Ristic, None; **B. Giesbrecht**, None.

Nanosymposium

734. Attention: EEG and TMS

Location: Room 6F

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 734.6

Topic: F.01. Human Cognition and Behavior

Support: 2 R01 EY016984-35

KIBM Innovative Research Award

Title: Visual processing of seen and unseen patterns during inattentional blindness

Authors: *M. A. PITTS, A. MARTINEZ, S. A. HILLYARD;
Neurosciences, UCSD, La Jolla, CA

Abstract: Previous studies have reported early differences in cortical visual processing for various types of objects versus non-objects (e.g. intact vs. scrambled, textured vs. homogenous, shapes vs. control stimuli). The event-related potential (ERP) most commonly associated with object perception is an occipital-parietal negativity at ~150-300ms post-stimulus onset, most likely generated in the lateral occipital complex (LOC). While this early ERP component is assumed to reflect automatic sensory encoding of basic object form, its dependence on attention and awareness has not yet been tested. Here, we adapted an inattentional blindness paradigm to examine ERPs associated with conscious and non-conscious visual processing of basic shapes. In the first phase of the experiment, subjects viewed an array of randomly oriented line segments presented in central vision while performing a separate, attention-demanding task in the visual periphery. Once every second, the line segments rotated to form a random configuration (50%), a salient square pattern (40%), or a diamond-shaped pattern (10%). After the square was presented more than 200 times, subjects answered a series of questions that assessed their awareness of these task irrelevant patterns. More than half of all subjects failed to notice the squares and were thus considered inattentionally blind. In the second phase of the experiment the task and stimuli were identical, but all of the subjects now reported seeing the square pattern, presumably because the prior questioning caused them to partially attend to the central array of line segments. In a third and final phase, subjects

focused their attention directly on the line segments in order to detect the diamond patterns.
When subjects attended to the patterns directly (third phase) a prominent object-related negativity was evident in the ERP from ~200-300ms over occipital-parietal regions. When subjects performed the separate attention-demanding task (first and second phases), this same negativity was reduced in amplitude but was nevertheless still present regardless of whether subjects were aware of the patterns or not. Analyses of subsequent components showed differences based on subjective awareness of the shapes. These results will be discussed within the context of current theories of object perception, attentional selection, and visual awareness.

Disclosures: M.A. Pitts, None; **A. Martinez**, None; **S.A. Hillyard**, None.

Nanosymposium

734. Attention: EEG and TMS

Location: Room 6F

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 734.7

Topic: F.01. Human Cognition and Behavior

Support: Netherlands Organization for Scientific Research Veni grant to M.V.

Title: Effects of prefrontal stimulation on stop-signal response inhibition: A combined fMRI/TMS study

Authors: *M. BLOEMENDAAL, B. B. ZANDBELT, R. S. KAHN, M. VINK; Psychiatry, UMC Utrecht, Utrecht, Netherlands

Abstract: The ability to stop a manual response from being executed requires suppression of corticospinal neurons in the primary motor cortex (M1). Converging lines of evidence suggest that inhibitory control over M1 relies on a fronto-basal ganglia network, in which the right inferior frontal cortex (rIFC) and the supplementary motor complex (SMC) seem to play important roles. M1 is modulated both before (proactive inhibitory control) and after (reactive inhibitory control) occurrence of a stop signal. However, the specific contribution of rIFC and SMC to proactive and reactive inhibitory control and how they interact with other areas in the stopping network remains unclear. Here, we combine repetitive transcranial magnetic stimulation (rTMS) with functional magnetic resonance imaging (fMRI) to investigate how perturbation of neural activity in the rIFC and SMC affects behavioral measures of reactive and proactive inhibitory

control as well as activation in the stopping network.
In three separate sessions, 24 healthy volunteers received rTMS over rIFC, SMC and sham rTMS in counterbalanced order. Immediately after rTMS they underwent fMRI while performing a stop-signal task in which anticipation of stopping was manipulated using a visual cue indicating stop-signal probability (range: 0% - 33%). The rTMS protocol consisted of a priming phase (20 trains of 30 6-Hz pulses at 90% resting motor threshold (RMT), separated by 25 s), followed by 600 1-Hz pulses at 110% RMT. This protocol has been shown to produce sufficiently long-lasting (> 30 min) effects on M1 corticospinal excitability and fMRI resting-state connectivity.
TMS over rIFC and SMC both affected reactive inhibitory control, as stop-signal reaction times were shorter after TMS over rIFC and SMC than after sham TMS. In contrast, TMS had no effect on proactive control, given that response slowing as a function of stop-signal probability did not differ between stimulation conditions. There were also no effects of TMS on baseline Go response times. As TMS influenced reactive inhibitory control only, fMRI analyses focused on TMS effects on stopping-related activation. TMS over the rIFC resulted in stronger deactivation of the left primary motor cortex during successful stopping, as compared to TMS over the SMC. TMS over the SMC resulted in stronger activation of the left putamen during successful stopping, as compared to TMS over the rIFC and sham TMS.
These preliminary results confirm the right inferior frontal cortex and supplementary motor complex as important nodes in the network subserving reactive inhibitory control. However, their contribution to successful stopping may be mediated through different pathways.

Disclosures: M. Bloemendaal, None; **B.B. Zandbelt**, None; **R.S. Kahn**, None; **M. Vink**, None.

Nanosymposium

734. Attention: EEG and TMS

Location: Room 6F

Time: Wednesday, November 17, 2010, 8:00 am - 10:00 am

Program Number: 734.8

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH68004

Title: Fast-action gaming affects the neural strategies underlying selective attention

Authors: *L. KRISHNAN, A. KANG, G. SPERLING, R. SRINIVASAN;

Univ. of California, Irvine, Irvine, CA

Abstract: Fast-action video game players are better at visuo-spatial tasks than non-video game players (Green and Bevalier, 2007). The enhanced performance of fast-action players has been attributed to their increased ability to select, process and respond to visual information because of their prior fast-action gaming experience. We investigated the neural basis of the difference between fast-action video game players (first-person shooter game players, FPS) and non-action video game players (role-playing game players, RPG) by examining both behavioral and neurophysiological measures in a visual search task. Task difficulty was manipulated by varying the number of regions to be attended and ignored from 1 to 4, and by using three data frame rates: 3 Hz, 8.6 Hz and 20 Hz. Subjects were chosen based on whether they were exclusively FPS players or exclusively RPG players, equating total game-playing experience between the two groups.
At all the temporal frequencies, the hit rates for FPS players and RPG players showed the largest differences when 4 regions had to be simultaneously attended. We measured steady state visually evoked potentials (SSVEPs) to the stimuli flickering at either 3 Hz, 8.6 Hz or 20 Hz. The spatial distribution of the SSVEP S/N ratio depended on the temporal frequency of the stimulus. In both groups of gamers, the 3 Hz and 8.6 Hz stimuli engaged global cortical networks over frontal, parietal, medial and occipital cortices while the 20 Hz stimulus engaged a local network over occipital and parietal areas. By fitting a partial least squares (PLS) regression model to the SSVEP S/N ratio and the hit rate, we found that the strength of the S/N ratio that predicted the hit rates at each temporal frequency, was modulated by the increasing number of attended and ignored regions. In both gamer groups the S/N ratio at 3 Hz increased over the frontal areas with increasing number of attended locations, while at 20 Hz the S/N ratio decreased over occipital and parietal areas. Additionally in FPS players at 3 Hz, the S/N ratio when more than 1 region was attended, was larger over the frontal cortex when the flickering stimulus was ignored versus when it was attended. At 8.6 Hz, the direction of change in the strength of the S/N ratio with the number of attended locations, distinguished the two groups of gamers. In the FPS players, the 8.6 Hz responses over the medial and frontal cortices decreased with the number of attended locations, while in the RPG players these responses increased. These results suggest that the performance differences between FPS players and RPG players might be influenced by the differential neural strategies adopted to deploy selective attention.

Disclosures: L. Krishnan, None; **G. Sperling**, None; **R. Srinivasan**, None; **A. Kang**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.1

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH58405

NIH Grant AG19645

Title: Perirhinal cortex, entorhinal cortex, and trace fear conditioning

Authors: *T. H. BROWN, S. BANG, A. PARSANA;
Yale Univ., New Haven, CT

Abstract: In a trace conditioning paradigm, unlike a delay paradigm, a temporal gap separates the offset of the conditional stimulus (CS) and the onset of the unconditional stimulus (US). This "trace interval" can be tens of seconds or longer. Since the two stimuli are not temporally contiguous, any contiguity-based encoding mechanism requires that a transient representation of the conditional stimulus (CS) persist during the trace interval. The hippocampus (HC) is well-known for its essential role in trace conditioning. Because of the massive recurrent network in HC, one possible mechanism for a persistent representation could entail transient synaptic plasticity within re-entrant circuitry. Relatively little research has been done on the role of two adjacent structures_the perirhinal cortex (PR) and the entorhinal cortex (EC). These structures have reciprocal connections with each other and with HC. Recently-published lesion studies discovered that both PR and EC are critically-involved in trace fear conditioning. A conventional interpretation might be that PR and EC lesions impair trace conditioning because these two structures furnish a key part of the input pathway to HC as well as part of the output pathway from HC. Recently, a radically-different hypothesis was advanced. This hypothesis asserts that the reason PR and EC are involved in trace fear conditioning is that both structures help maintain a transient representation of the CS through the continuing activity of so-called "persistent-firing neurons". In the presence of a muscarinic acetylcholine receptor (mAChR) agonist, these neurons continue to discharge long after the termination of the original excitatory stimulus. The persistent-firing duration can last seconds to minutes. In two different laboratories, one studying PR and the other studying EC, an initial test of this hypothesis has now been performed. If mAChR-enabled persistent-firing plays an essential role in maintaining the CS representation, then infusing these structures with a mAChR antagonist should impair trace but not delay fear conditioning. This is exactly what was observed in both PR and EC. We suggest that persistent-firing neurons in these and nearby structures serve to support a regional memory buffer system. This concept begs the question of what controls acetylcholine release from the cholinergic neurons in the basal forebrain that project to PR and EC. One possibility is that release is controlled through known neuroanatomical projections from the prefrontal cortex (PFC) to the basal forebrain. In

this way, PFC could gate the eligibility period for persistent firing in PR and EC.
Supported by MH58405 and AG19645 to THB.

Disclosures: T.H. Brown, None; **S. Bang**, None; **A. Parsana**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.2

Topic: F.02. Animal Cognition and Behavior

Support: MH069558

MH090426

MH083422

Title: Involvement of the ventral hippocampus to medial prefrontal cortex pathway in trace fear conditioning

Authors: *M. R. GILMARTIN, F. J. HELMSTETTER;
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Abstract: Trace fear conditioning (TFC) is a learning task that requires rats to associate an auditory conditional stimulus (CS) and a fear-producing shock unconditional stimulus (UCS) that are separated by an empty trace interval. Unlike standard delay fear conditioning (DFC), trace fear conditioning is dependent upon an intact hippocampus (McEchron et al., 1998, 2000; Quinn et al., 2002). We have recently shown that the prelimbic area of the medial prefrontal cortex (mPFC) is also necessary for the acquisition of trace, but not delay, fear memories (Gilmartin & Helmstetter, 2010). Specifically, inactivating prelimbic mPFC with the $GABA_A$ agonist muscimol or blocking NMDA receptor-mediated neurotransmission with APV impairs the formation of trace and contextual fear responses (Gilmartin & Helmstetter, 2010). Previous work using single neuron recording in rats and fMRI BOLD in humans suggest that hippocampal and prefrontal regions may serve distinct but complementary roles in TFC (Gilmartin & McEchron, 2005a,b; Knight et al., 2004). We hypothesize that learning the CS-UCS association across a trace interval in TFC requires a functional interaction between the ventral hippocampus and prelimbic mPFC. This study tests this hypothesis

using targeted unilateral inactivation of ventral hippocampus and the contralateral or ipsilateral prelimbic mPFC prior to TFC, using infusions of the $GABA_A$ agonist muscimol. We found that simultaneous unilateral inactivation of the ventral hippocampus and mPFC impaired the formation of memory for the CS and context measured 24 hours later, compared with saline-infused control rats. Initial results support a requirement for cooperative participation of the ventral hippocampus and prelimbic mPFC in the formation of trace and contextual fear memory.

Disclosures: M.R. Gilmartin, None; **F.J. Helmstetter**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.3

Topic: F.02. Animal Cognition and Behavior

Support: Research Growth Initiative from UW-Milwaukee

Quincy Bioscience, LLC

Title: Trace fear conditioning enhances synaptic and intrinsic plasticity in rat hippocampus

Authors: *C. SONG[1], J. A. DETERT[1], M. SEHGAL[1], J. R. MOYER, Jr.[1,2];
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Abstract: Experience-dependent synaptic (LTP, LTD) and intrinsic (membrane excitability) plasticity are thought to be important substrates for learning-related changes in behavior. In order to understand how these forms of plasticity interact as a function of associative learning, we used trace fear conditioning as a model system for hippocampus-dependent learning and studied hippocampal plasticity after conditioning. Adult rats received one 10-trial session of trace fear conditioning (15 s white noise CS, 30 s trace interval, 1 s footshock US; 5.2 min ITI) or they were pseudoconditioned. The next day, rats were placed into a novel context where they received 2 CS-alone test trials. Brain slices were prepared within 1 hr following the CS test. For synaptic plasticity studies, dendritic field recordings were obtained from the *stratum radiatum* of CA1 using pipettes filled with aCSF. Concentric bipolar stimulating electrodes were positioned in the *stratum radiatum* on either side of the field electrode to elicit field EPSPs from both

control and test pathways. After establishing a stable baseline, LTP was induced in the test pathway by delivering a single 1 s train at 100 Hz. Both pathways were monitored for at least 30 min. For intrinsic plasticity studies, somatic intracellular recordings were obtained from CA1 neurons using 3M potassium acetate-filled sharp microelectrodes. Intrinsic excitability was evaluated by studying the current-voltage relation, the post-burst AHP following a burst of four action potentials, and spike frequency adaptation at rest and at -65 mV. Behavioral performance was positively correlated with the amount of LTP in trace fear conditioned but not the pseudoconditioned animals. Moreover, LTP was significantly greater in animals that were classified as "good learners" compared with "poor learners", naïve, pseudoconditioned, and chamber exposed controls. The enhanced LTP was also input specific. Likewise, trace fear conditioning resulted in a learning-specific enhancement of intrinsic excitability in CA1 pyramidal neurons. Animals classified as "good learners" exhibited a significantly smaller post-burst AHP and less spike frequency adaptation than "poor learners" or the other control groups. No differences in resting membrane potential, action potential characteristics or input resistance were observed. These data suggest that acquisition of trace fear conditioning enhances both synaptic and intrinsic plasticity in hippocampal CA1 neurons. Thus, an interaction between intrinsic and synaptic plasticity may underlie the acquisition of trace fear conditioning.

Disclosures: C. Song, None; **J.A. Detert**, None; **M. Sehgal**, None; **J.R. Moyer**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.4

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant 0191959

Title: The Arc of fear conditioning: NMDA-receptor mediated expression of the ieg Arc in both dorsal and ventral hippocampus contributes to the acquisition of contextual and trace fear conditioning

Authors: *J. CZERNIAWSKI, F. REE, T. OTTO;
Dept of Psychology, Rutgers Univ., Piscataway, NJ

Abstract: The dorsal and ventral subregions of the hippocampus are differentially

involved in several of types of learning, including fear conditioning. For example, we have previously demonstrated that the integrity of ventral, but not dorsal, hippocampus is necessary for the acquisition and expression of trace fear conditioning (Czerniawski, Yoon & Otto, 2009), while dorsal, but not ventral, hippocampus is critically involved in spatially-guided reinforced alternation. In contrast to the partially dissociable effects of either lesions or inactivation, however, several lines of research suggest that, in intact subjects, both subregions are normally involved in the acquisition of many hippocampal-dependent tasks. We have recently begun a series of studies investigating the molecular basis of these forms of learning by determining whether NMDA-receptor-mediated immediate early gene expression in dorsal vs. ventral hippocampus contributes to the acquisition and/or retention of trace and contextual fear conditioning. Preliminary data indicate that both NMDA-receptor antagonism and the infusion of antisense oligodeoxynucleotides for the immediate early gene Arc (activity-regulated cytoskeletal protein) into dorsal or ventral hippocampus impair the acquisition of contextual and trace fear conditioning. Together these studies support the notion that NMDA-receptor mediated expression of the immediate early gene Arc in both dorsal and ventral hippocampus may underlie the acquisition of a variety of forms of hippocampal dependent learning.

Disclosures: J. Czerniawski, None; **T. Otto**, None; **F. Ree**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.5

Topic: F.02. Animal Cognition and Behavior

Support: MH077111

T32 DA007262

Title: The amygdala and trace fear conditioning in C57Bl6 mice?!

Authors: *J. D. RAYBUCK, K. M. LATTAL;
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Abstract: The classic neurobiology of fear conditioning demonstrates that associative fear learning is dependent on the amygdala. Additionally, studies of contextual fear

conditioning show that this form of learning depends both on the amygdala and on the dorsal hippocampus, suggesting that multiple functions occur during acquisition of fear learning,(a) the formation of a conjunctive contextual representation by the hippocampus and (b) the association of this representation with an aversive US by the amygdala. More recently the substrates of trace fear conditioning have been investigated. In trace fear conditioning the CS is separated from the US by a temporal interval, forcing the subject to maintain a representation of the CS in order to bridge the interval and associate the two stimuli. Here it has been demonstrated that both hippocampus and prefrontal cortex are critically involved in acquisition of the CS-US association. However, the role of the amygdala in trace conditioning has yet to be examined. It is not clear whether the recruitment of cortical structures in trace fear conditioning alters the role of the amygdala in this task, as compared to delay fear conditioning. Thus, to examine the role of the amygdala in trace fear conditioning we deactivated the amygdala complex of C57Bl6 mice by infusing the GABA A agonist muscimol (0.25, 0.5, ug/side, in 0.5 ul PBS) into the amygdala through surgically implanted intra-cranial cannula (A/P -1.46, M/L 3.1, D/V -8.0; relative to bregma) prior to 2 CS-US pairing trace (30s interval) or delay fear conditioning (30s, 85 dB CS, 2s 0.35 mA footshock US). Muscimol infusion produced deficits in contextual learning, regardless of training protocol. However, while muscimol infusion produced robust deficits in delay fear conditioning, it had no effect on trace fear conditioning. These data suggest that trace fear conditioning, in addition to involving forebrain areas not necessarily involved in delay fear conditioning, may occur independent of amygdala activity. Thus, it is possible that involvement of the medial prefrontal cortex and hippocampus in this task allows rescue of function that may normally necessitate the amygdala, or that certain training protocols can produce amygdala independent fear learning. These findings have general implications for rodent models of aversively motivated learning and memory, as well as for anxiety treatment models based around amygdala dependent theories of fear learning.
Support: Supported by MH077111, JDR supported by T32 DA007262.

Disclosures: J.D. Raybuck, None; **K.M. Lattal**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.6

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH74006

NIH Grant MH46904

Title: Dissociation of upstream and downstream learning signals in the mPFC during associative learning

Authors: *J. J. SIEGEL[1], M. D. MAUK[1,2];
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Abstract: The ability to predict events and take the appropriate anticipatory action is essential to survival. There is often a temporal delay between associated events, and it is of substantial interest to understand how the brain is able to learn and take action under such conditions. To study the neural basis of this kind of associative learning we use a classical conditioning paradigm in which a tone (500 ms) predicts a stimulation induced eye closure (US) but which are separated by a stimulus-free temporal gap (500 ms), known as trace eyelid conditioning. After many pairings animals learn to close the eyelid in anticipation of the US (conditioned responses, CRs). The cerebellum has been shown to mediate eyelid conditioning when the tone and US overlap in time, but for trace conditioning requires an input that bridges the temporal gap between the tone and US. Single-unit recordings from the rabbit mPFC during trace conditioning revealed two patterns of activity that are evoked by the tone and persist to overlap with the US. The first pattern has an early onset, typically within 200 ms after tone presentation. The second pattern has a delayed onset, and sometimes appears correlated with behavioral responses (CRs) during the stimulus-free trace interval (Siegel et al., Prefrontal cortex and lateral pontine neurons display tone-evoked persistent activity during trace eyelid conditioning. Program No. 384.9. Chicago, IL: SFN, 2009. Online.). To determine whether these response patterns are upstream of the cerebellum (inputs) or downstream, reflecting behavioral feedback to the mPFC, we blocked the expression of CRs in trained animals (n=2 rabbits) with temporary inactivation of the deep cerebellar nucleus by infusion of muscimol. Delayed onset trace interval associated activity (identified in pre-infusion trials) was not observed when CR expression was blocked (11/11 cells, 6 sessions). Early onset persistent activity and phasic tone-evoked responses were not significantly affected (0/5 cells, 3 sessions; 4/13 cells, 6 sessions). The data suggest that trace interval associated changes in activity reflect behavioral feedback to the mPFC network regarding the learned response, while tone evoked persistent activity is upstream of cerebellar output, putatively providing an input to the cerebellum that bridges the temporal gap between two events to allow for associative learning.

Disclosures: J.J. Siegel, None; **M.D. Mauk**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.7

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH59970

NIH Grant ARRA3R01MH059970-10S1

NSF Grant IOB0444364

NSF Grant IOS0914386

Title: The effects of learning on neurogenesis: Cell production versus cell survival

Authors: *M. L. ANDERSON[1,2], T. J. SHORS[3];
[1]Monmouth Junction, NJ; [2]Neurosci. and Cell Biol., [3]Behavioral Neurosci., Rutgers Univ., Piscataway, NJ

Abstract: Thousands of new neurons are generated in the adult hippocampus each day (Cameron et al., 1993). Most of these new cells die unless they are exposed to a learning experience. Specifically, cells that are about one week of age at the time of learning a trace conditioning task tend to survive and remain in the hippocampus for months at least (Gould et al., 1999; Leuner et al., 2004, Shors, 2009). Training on a spatial maze task also enhances the likelihood that the new neurons will survive (Sisti et al., 2007; Drapeau et al., 2007). Recently, Epp et al. (2007) reported that learning increased survival when the cells were about 6-10 days of age but not younger or older than that. However, other studies report that spatial learning can increases proliferation of cells that only a few days old (Döbrössy et al., 2003). Here we assessed the effects of trace conditioning on the production versus survival of newly generated cells in the DG. To do this, rats were injected with one dose of BrdU to label one population of dividing cells. Rats began training with trace eyeblink conditioning 30 min, 1 week or 3 weeks after the BrdU injection. Trace conditioning is a hippocampal-dependent task in which a conditioned stimulus (CS; white noise) is paired with an unconditioned stimulus (US; eyelid stimulation) but they are separated in time by a trace interval of 500 ms. The rats received 150 trials a day for 4 days. The rats were euthanized 24hr after the end of training to assess training's effect on proliferation or 28 days after the BrdU injection to assess the effect of training on survival. Our results indicate that the number of cells that were still mitotic at the time of the training experience did not increase in number as a result of training. Neither exposure to a new context and novel stimuli nor learning increased the number of new cells produced. The present experiment confirms previous data showing that neural precursors cells can be rescued from death if they are about 1 week old at the

onset of this training regime but not if they are younger or older (Dalla et al., 2007; Waddell & Shors, 2008). The number of BrdU+ cells was not increased when rats were trained as cells were still being produced or when they were already quite mature. Interestingly, training on the trace conditioning task 30 minutes after injection decreased the number of BrdU-labeled cells that survived 28 day later. These data support our previous studies indicating that learning does not enhance proliferation of new neurons in the hippocampus but rather enhances the survival of cells that are already present at the time of training. Thus, there is a critical period during which new neurons are responsive to learning in the adult brain.

Disclosures: M.L. Anderson, None; **T.J. Shors**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.8

Topic: F.02. Animal Cognition and Behavior

Support: Academy of Finland (114258 and 130013) to JW

Emil Aaltonen Foundation Young Researcher Grant to MSN

Title: Hippocampal ripple -contingent training accelerates trace eyeblink conditioning and retards extinction in rabbits

Authors: *M. S. NOKIA[1,2], M. PENTTONEN[1], J. WIKGREN[1];
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Abstract: There are at least two distinct oscillatory states of the hippocampus that are related to distinct behavioral patterns. Theta (~6 Hz) oscillation, seen during active exploration as well as during immobile alertness, has been suggested to indicate selective attention when the subject concentrates on some features of the environment while suppressing reactivity to others. In contrast, sharp-wave ripples (~200 Hz) reflect a state in which the hippocampus is most responsive to any kind of afferent stimulation. In addition, external stimulation tends to evoke and reset theta oscillation, the phase of which has been shown to modulate synaptic plasticity in the hippocampus. In the present study, we used a brain-computer interface to detect hippocampal ripples in rabbits in

order to deliver trace eyeblink conditioning and extinction trials selectively contingent upon them. A yoked control group was trained irrespective of their ongoing neural state. That is, they received a trial whenever the animal in the experimental group showed a ripple and received a trial. Theoretically, training on this or any hippocampus-dependent learning task contingent upon ripples could enhance learning rate due to elevated responsiveness and enhanced phase locking of the theta oscillation in response to the conditioning stimuli. As assumed, ripple-contingent training expedited acquisition of the conditioned response early in training and evoked stronger theta-band phase locking to the conditioned stimulus. Surprisingly, ripple-contingent training also resulted in slower extinction in well-trained animals. We suggest that the ongoing oscillatory activity in the hippocampus determines the extent to which a stimulus can induce a phase reset of the theta oscillation, which in turn is the determining factor of learning rate in trace eyeblink conditioning.

Disclosures: M.S. Nokia, None; **M. Penttonen**, None; **J. Wikgren**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.9

Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 AG021501

Title: Functional MRI of human neural substrates important for CS-US contingency awareness during delay and trace eyeblink conditioning

Authors: *D. T. CHENG[1], A. M. KATZENELSON[2], M. L. FAULKNER[1], J. F. DISTERHOFT[3], J. E. DESMOND[1];
[1]Neurol, [2]Neurosci., Johns Hopkins Univ. Sch. Med., BALTIMORE, MD; [3]Physiol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Eyeblink classical conditioning is a model system used to study the neurobiological mechanisms underlying learning and memory. In this fundamental form of learning a neutral conditioned stimulus (CS; e.g. tone) is paired with a biologically salient, unconditioned stimulus (US; e.g. corneal airpuff). After repeated pairings, the CS alone reliably elicits a conditioned response (CR), indicating that a CS-US association has been formed. In the delay conditioning procedure the CS and US coterminate while

in trace conditioning, a silent "trace interval" separates the CS offset and US onset. Declarative knowledge (awareness) of the CS-US relationship has been shown to affect behavioral conditioning performance but to date, the neural structures underlying this contingency awareness remain largely unexplored. Namely, it is unclear whether additional brain structures are recruited in subjects demonstrating CS-US awareness relative to subjects unaware of the stimulus contingencies. The present study used event-related functional MRI to investigte the neural substrates important for reporting awareness of the CS-US relationship during human eyeblink conditioning. Single-cue delay or trace conditioning trials were presented to both older (60-70 yrs old) and younger (20-30 yrs old) adults. Delay (1350 ms) and trace (250 ms, 1000 ms trace interval) CSs were 1000 Hz tones and the US was a 100 ms corneal airpuff (5 psi). All subjects watched a silent movie (The Gold Rush) while receiving tone-airpuff presentations. They were informed that the study was designed to investigate how distracting tones and airpuffs affected their ability to remember details about the movie. Awareness of the CS-US relationship was assessed with a post-experimental questionnaire. Subjects correctly answering 6 or more of the 7 questions were classified as aware and subjects correctly answering 5 or fewer were classified as unaware. Behavioral findings showed that younger subjects were significantly more aware than older subjects. Younger subjects also showed more conditioned responses than older subjects in early phases of trace conditioning. Neuroimaging data indicated that trace conditioning, a form of learning thought to require awareness, elicited greater activity in the left inferior frontal/opercular cortex compared to delay conditioning. Furthermore, aware subjects demonstrated greater activity in the right inferior parietal cortex and parahippocampal gyrus relative to unaware subjects. These findings suggest that unique brain regions may be engaged as a function of awareness of the CS-US relationship.

Disclosures: D.T. Cheng, None; **A.M. Katzenelson**, None; **M.L. Faulkner**, None; **J.F. Disterhoft**, None; **J.E. Desmond**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.10

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG008796

NIH Grant AG017139

NIH Grant MH047340

Title: Successful modulation of the postburst afterhyperpolarization is essential for learning trace conditioning tasks

Authors: *M. M. OH, F. A. OLIVEIRA, F. L. NÚÑEZ-SANTANA, J. F. DISTERHOFT;
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Abstract: In this nanosymposium session, I will discuss our working hypothesis that the postburst afterhyperpolarization (AHP) is an essential intrinsic cellular mechanism that needs to be modulated for successfully learning hippocampus dependent tasks. The postburst AHP is a calcium-dependent potassium current that has been demonstrated to be significantly reduced in hippocampal pyramidal neurons following successful learning the trace eyeblink, trace fear and spatial water maze tasks. The postburst AHP has also been shown to be significantly enlarged in CA1 pyramidal neurons from aging animals, and thus, suggested to be an underlying cause of the aging-related learning impairments observed in hippocampus dependent tasks, such as trace eyeblink conditioning. The aging-related learning deficit can be ameliorated by pharmacological compounds that reduce the postburst AHP. I will also present data from recent unpublished work that demonstrates a functional linkage between calcium and the postburst AHP following successful learning and with normal aging: a learning-related reduction in the L-type voltage-gated calcium channels; and an altered calcium homeostatic mechanism with aging. Lastly, I will discuss our recent work using calcium imaging techniques that examine a functional correlation between the postburst AHP and the calcium transients evoked with a burst of action potentials in CA1 pyramidal neurons. These recent data further provide evidence that the postburst AHP is a cellular biomarker that needs to be successfully modulated for learning a hippocampus-dependent task, such as trace eyeblink conditioning.

Disclosures: M.M. Oh, None; **F.A. Oliveira**, None; **F.L. Núñez-Santana**, None; **J.F. Disterhoft**, None.

Nanosymposium

735. Associative Learning and Fear Conditioning

Location: Room 7B

Time: Wednesday, November 17, 2010, 8:00 am - 10:45 am

Program Number: 735.11

Topic: F.02. Animal Cognition and Behavior

Support: Howard Hughes Medical Institute

Title: Imaging the cortical ensemble in mice during learning

Authors: T. KOMIYAMA[1,2], *T. SATO[1], D. H. O'CONNOR[1], Y.-X. ZHANG[1,3], D. HUBER[1], B. M. HOOKS[1], M. GABITTO[4], K. SVOBODA[1];
[1]Janelia Farm Res. Campus, ASHBURN, VA; [2]UCSD, San Diego, CA; [3]Johns Hopkins Univ., Baltimore, MD; [4]Columbia Univ., New York, NY

Abstract: Cortical neurons form specific circuits, but the functional structure of this microarchitecture and its relation to behaviour are poorly understood. Two-photon calcium imaging can monitor activity of spatially defined neuronal ensembles in the mammalian cortex. Here we applied this technique to the motor cortex of mice performing a choice behaviour. Head-fixed mice were trained to lick in response to one of two odours, and to withhold licking for the other odour. Mice routinely showed significant learning within the first behavioural session and across sessions. Microstimulation and trans-synaptic tracing identified two non-overlapping candidate tongue motor cortical areas. Inactivating either area impaired voluntary licking. Imaging in layer 2/3 showed neurons with diverse response types in both areas. Activity in approximately half of the imaged neurons distinguished trial types associated with different actions. Many neurons showed modulation coinciding with or preceding the action, consistent with their involvement in motor control. Neurons with different response types were spatially intermingled. Nearby neurons (within ~150 micrometers) showed pronounced coincident activity. These temporal correlations increased with learning within and across behavioural sessions, specifically for neuron pairswith similar response types.Wepropose that correlated activity in specific ensembles of functionally related neurons is a signature of learning-related circuit plasticity. Our findings reveal a fine-scale and dynamic organization of the frontal cortex that probably underlies flexible behaviour.
Reference: Komiyama, et al. Nature 2010, 464(22), 1182-6

Disclosures: T. Komiyama, None; **T. Sato**, None; **D.H. O'Connor**, None; **Y. Zhang**, None; **D. Huber**, None; **B.M. Hooks**, None; **K. Svoboda**, None; **M. Gabitto**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.1

Topic: B.11. Glia-Neuron Interactions

Support: BFU2007-63031/BFI

Title: ATP stimulates CREB-dependent transcription in astrocytes via ERK1/2 and TORCs

Authors: P. CARRIBA, R. MASGRAU, M. LICHTENSTEIN, L. PARDO, *E. GALEA;
Univ. Autonoma De Barcelona, Bellaterra, Barcelona, Spain

Abstract: Although much evidence implicates CREB in synaptic plasticity and learning, most of the studies have focused exclusively on neurons with little consideration for astrocytes, despite evidence that these cells are key regulators of synaptic plasticity. Here we sought to dissect the signaling pathways leading to CREB activation by ATP. We report that ATP (100 microM) stimulated CREB dependent transcription (CREB-dt) in primary cortical astrocyte cultures, as judged by CRE-luciferase assays (Promega). Pharmacological analyses revealed that the effect of ATP was mostly mediated by P2X receptors, with a partial contribution of purinergic P1 receptors, indicating ATP degradation into adenosine.The canonical view holds that CREB can be regulated by the coactivator CBP that binds to phosphorylated CREB (pCREB), the family of calcium/calcineurin-dependent coactivators TORCs, also known as CRTCs, or the transcriptional repressor DREAM, that is deactivated by calcium. ATP induced a transient increase of pCREB, measured by western blots. The increase was not inhibited by Rp-cAMP, suggesting that protein kinase A (PKA) was not involved. Rather, CREB phosphorylation appeared mediated by ERK1/2 because the ERK1/2 inhibitor U0126 inhibited both the pCREB increases (western blot analysis), and the CREB activation (luciferase assays) caused by ATP. Accordingly, ATP increased ERK1/2 phosphorylation. CREB activation was completely blocked by BAPTA, a chelator of intracellular calcium, but not by two selective inhibitors of PKA, the myristoylated 14-22 Amide (Calbiochem) or Rp-cAMP. This suggested that calcium-, but not cyclicAMP/PKA-dependent signaling mediate CREB activation by ATP. However, the role of calcium has to be put on hold for now because the pGL4 vectors used in the luciferase assays appear to be strongly calcium-dependent, thus casting doubts on any evidence obtained with these vectors about the calcium-dependency of a given transcription factor. Analyses with other reporters are in progress. Over-expression of DN-TORC1 and DN-TORC2 inhibited the ATP-elicited CREB-dt by approximately 50%, thus implicating TORCs in the effect of ATP. TORC activation was confirmed by immunocytochemical detection of its nuclear translocation upon ATP exposure. Unexpectedly, the calcineurin inhibitors CsA and FSK506 did not inhibit CREB-dt, revealing that TORCs can be activated in astrocytes by a mechanism other than calcineurin. Ongoing work is aimed at investigating, aside from the role of calcium, the implication of DREAM, and the upstream signaling pathways leading to TORC activation.

Disclosures: P. Carriba, None; **L. Pardo**, None; **E. Galea**, None; **M. Lichtenstein**, None; **R. Masgrau**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.2

Topic: B.11. Glia-Neuron Interactions

Support: BBSRC UK Grant BB/F0221445

Title: Modulation of GABA receptors by astrocyte-driven ATP: Implication to glia-neuron communication in the neocortex

Authors: *Y. PANKRATOV[1], O. PALYGIN[1], J. ANDREW[1], U. LALO[2];
[1]Univ. of Warwick, Coventry, United Kingdom; [2]Univ. of Leicester, Leicester, United Kingdom

Abstract: Communication between neuronal and glial cells is thought to be very important for many brain functions. The importance of neuron-glia interaction for brain function is embedded in the concept of "tri-partite synapse". Astroglial cells possess the SNARE-like Ca^{2+}-dependent machinery for vesicular release of "gliotransmitters" like D-serine, glutamate, and ATP. Acting via release of gliotransmitters, astrocytes can modulate synaptic strength.
One of the important pathways for ATP action as gliotransmitter can be modulation of Ca-dependent phosphorylation of postsynaptic receptors. We observed ATP-induced down-regulation of postsynaptic signalling in the individual inhibitory neocortical synapses. This effect was mediated by PKA-dependent phosphorylation of GABA receptors. The regulatory cascades were triggered by Ca^{2+}-entry through the P2 purinoreceptors, which are functionally expressed in the central neurons.
We investigated release of ATP from acutely dissociated cortical astrocytes using "sniff"-cells (HEK293-cells transfected with P2X2 purinoreceptors) and demonstrated that vesicular release of gliotransmitters from astrocytes can be activated via various pathways including Ca^{2+}-permeable ionotropic astroglial receptors, IP3-pathway, or direct UV- uncaging of intracellular Ca^{2+} in the cells loaded with photolabile calcium chelator NP-EGTA. We did not observed release of ATP from astrocytes of dn-SNARE transgenic mice in which the SNARE-dependant release of gliotransmitters was

selectively impaired.
Our experiments *in situ* showed that activation of Ca-signalling in astrocytes either by electrical stimulation or UV-uncaging or by application of specific agonists triggered release of ATP followed by considerable decrease in the amplitude of inhibitory postsynaptic currents in the cortical pyramidal neurons. Furthermore, modulation of postsynaptic GABA by astrocyte-driven ATP affected the induction of long-term potentiation in the neocortex. Both release of ATP from astrocytes and its modulatory effects on synaptic transmission were eliminated in dnSNARE mice.
These findings demonstrate an important role for SNARE complex-dependent release of gliotransmitters for glia-neuron interaction in the neocortex. Our results might help to resolve some recently highlighted controversies about mechanism of exocytosis in astrocytes. Our results also show a novel pathway of modulation of signalling in the tripartite synapse involving vesicular release of ATP from astrocytes and interaction between P2X and other postsynaptic receptors.

Disclosures: Y. Pankratov, None; **O. Palygin**, None; **J. Andrew**, None; **U. Lalo**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.3

Topic: B.11. Glia-Neuron Interactions

Support: NIH R01 HL 84464 (to FFB)

NIH R01 NS 50350 (to MN)

NIH R01 NS 55363 (to MVLB)

Title: FGF-1, ATP, P2X receptors, and gap junction hemichannels in astrocytes mediate spinal inflammation and secondary degeneration after traumatic injury

Authors: J. M. GARRE[1], A. TORRES[3], W. PENG[3], F. F. BUKAUSKAS[1], M. NEDERGAARD[3], *M. V. L. BENNETT[4,2];
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Abstract: Neurodegeneration secondary to traumatic injury of the spinal cord is preceded by ATP release, and significant protection results from blocking P2X7Rs, implicating them in cell death (Wang et al, Nat Neurosci, 2004, 10: 821-7; Peng et al, PNAS, 2009, 106: 12489-93). FGFRs are expressed in the dorsal and ventral spinal cord, and levels of FGF-1 and its receptors may be increased by spinal cord trauma (Koshionaga et al., Exp Neurol, 1993, 120: 32-48; Cassina et al., J Neurochem, 2005, 93: 28-46). FGF-1 application (200 ng/ml) to the mouse spinal cord induces release of ATP. The ATP level reaches a maximum value at 15-45 min and returns to control by ~120 min. This ATP release is blocked by topical PD173074, an inhibitor of FGF receptors [FGFR 1, FGFR 3, VEGFR 3]. Moreover, PD173074 decreases the injury-induced release of ATP. We are investigating whether PD173074 treatment is neuroprotective, as are blockers of P2X7Rs. Studies of spinal astrocytes in pure culture suggest mechanisms for ATP release in vivo. Treatment of cultures with FGF-1/heparin causes secretion of ATP and increases membrane permeability by opening of pannexin1 hemichannels (Px1 HCs) measured at 2 h but starting within minutes. At 7 h treatment, connexin 43 hemichannels (Cx43 HCs) contribute to the increased permeability. Botulinum neurotoxin A, which can block vesicular release, prevents permeabilization when given prior to FGF-1 application, but has little effect given at the time of FGF-1 application or 1 h later. In the absence of FGF-1, ATP and BzATP each cause permeabilization that is prevented by purinergic antagonists. Block of FGF-1-induced membrane permeabilization by apyrase, a soluble ATPase, or blockers of purinergic receptors, pannexin hemichannels or connexin hemichannels suggests that, although initially ATP is released from vesicles, its continued release is mediated by action on purinergic receptors and opening of hemichannels. Single channel recordings from cell attached patches after FGF-1 treatment reveal relatively small channels (~25-50 pS) attributed to Px1 HCs and large channels (~220 pS) attributed to Cx43 HCs. Px1 and Cx43 HCs were distinguished by pharmacological criteria and use of Px1 siRNA and Cx43 KOs. Together these data suggest that ATP is released by astrocytes activated by FGF-1 in vivo, driving the inflammatory response and impacting neuronal survival. Analysis of ATP release in culture suggests early FGF-1 stimulation of vesicular secretion and then opening of Px1 and Cx43 HCs, which mediate continued ATP release.

Disclosures: J.M. Garre, None; **A. Torres**, None; **W. Peng**, None; **F.F. Bukauskas**, None; **M. Nedergaard**, None; **M.V.L. Bennett**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.4

Topic: B.11. Glia-Neuron Interactions

Title: Altered synaptic plasticity and behavior in mice deficient in the glial water channel aquaporin-4

Authors: V. A. SKUCAS[1], I. B. MATHEWS[1], A. K. TREISTER[2], Q. CHENG[1], M. J. SCHANER[1], A. S. VERKMAN[3], M. A. WOOD[2], *D. K. BINDER[4], H. E. SCHARFMAN[1];
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Abstract: Aquaporin-4 (AQP4) is the major water channel expressed in the central nervous system (CNS) and is expressed in glial cells. Little is known about the potential for AQP4 to influence synaptic plasticity or behavior, although many studies have demonstrated it regulates the response of the CNS to insults or injury. Therefore, we asked whether AQP4 knockout mice would demonstrate defects in long-term potentiation (LTP) and long-term depression (LTD), and impaired performance when tested using tasks that are commonly used to evaluate learning and memory. The results showed no detectable difference in LTP of the Schaffer collateral pathway in area CA1, using a high-frequency train (HFS-LTP), but there was a defect in LTP using an LTP induction protocol that mimics theta rhythm (theta-burst stimulation; TBS-LTP). There was also a defect in LTD, induced by a 3 Hz train for 5 min. Basal synaptic transmission was similar between wild-type and AQP4 knockout mice, evaluated using field potentials and patch clamp recordings, and there were no significant differences in short term plasticity (paired pulse facilitation, post-tetanic potentiation, or short-tetanic depression). When mice were tested behaviorally, there was no detectable influence of genotype on performance using the Morris water maze or contextual fear conditioning, but AQP4 knockout mice performed significantly worse using a task that requires memory for the location of objects. Our results suggest an unanticipated role of AQP4 channels in select forms of synaptic plasticity and spatial memory, and underscore the growing appreciation of the role of glial cells in functions typically attributed to neurons.

Disclosures: V.A. Skucas, None; **I.B. Mathews**, None; **A.K. Treister**, None; **Q. Cheng**, None; **M.J. Schaner**, None; **A.S. Verkman**, None; **M.A. Wood**, None; **D.K. Binder**, None; **H.E. Scharfman**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.5

Topic: B.11. Glia-Neuron Interactions

Support: The Whitehall Foundation

NIH NINDS (NS060677)

Title: Calcium permeable channels underlie localized calcium transients in astrocytes

Authors: *E. SHIGETOMI[1], B. S. KHAKH[1,2];
[1]Dept Physiol, [2]Dept Neurobiol, Univ. California-UCLA, Los Angeles, CA

Abstract: Astrocytes are known to provide passive supportive roles to neurons. Growing evidence also indicates that astrocytes may actively participate in brain function through interactions with neurons. It has been proposed that astrocyte calcium elevations can trigger the release of neuroactive substances from astrocytes. The intracellular calcium level at the plasma membrane may be an important factor for regulating or even triggering this process. We analyzed real time calcium dynamics within ~100 nm of the plasma membrane using total internal reflection fluorescence microscopy with moderately fast acquisition (10-40 Hz) in rat hippocampal astrocytes. We found that highly-localized (half width 5.5 ± 0.4 μm) calcium transients occurred spontaneously (2.0 ± 0.5 events/min). Localized calcium signals were also observed by newly developed membrane-targeted optical sensors[1]. The pharmacological profiles of the calcium signals were distinct from GPCR-mediated calcium signals and suggested that TRP-like calcium permeable plasma membrane channels mediate localized calcium elevations at the membrane. We will present their characterization data on the molecular identity of the channels in astrocytes, but, overall our results suggest that TRP-like calcium permeable plasma membrane channels mediate compartmentalized calcium signals in astrocytes. The calcium permeable plasma membrane channels underlying localized calcium signals in astrocytes may be good candidates to mediate physiological calcium signals in astrocytes *in vivo* at resting membrane potential levels, near the potassium equilibrium potential, when the electrochemical gradient for calcium entry is high.

Reference

1.
Shigetomi E et al. (2010) A genetically targeted optical sensor to monitor calcium signals in astrocyte processes. *Nat Neurosci*, in press.

Disclosures: E. Shigetomi, None; **B.S. Khakh**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.6

Topic: B.11. Glia-Neuron Interactions

Support: NIH grant NS061953

Title: Cellular swelling strongly modifies glutamate-glutamine cycle in the rat brain in vivo and in astrocyte cultures in vitro

Authors: M. C. HYZINSKI[1], M. Y. VINCENT[1], P. DOHARE[1], R. E. HASKEW-LAYTON[3], A. RUDKOUSKAYA[4], R. W. KELLER, Jr.[1], *A. A. MONGIN[2]; [1]Ctr. for Neuropharm. and Neurosci., [2]Albany Med. Col., ALBANY, NY; [3]Burke/Cornell Med. Res. Inst., White Plains, NY; [4]Dept. of Physiol. and Pharmacol., Univ. of Western Ontario, London, ON, Canada

Abstract: Cellular swelling in the brain occurs in numerous pathologies, including ischemia, traumatic brain injury, and hyponatremia. Cell swelling causes opening of volume-regulated anion channels that are permeable to the excitatory amino acids, glutamate and aspartate. In the present study, we explored the effects of cell swelling on extracellular amino acid levels by delivering hypoosmotic medium via a microdialysis probe into rat cortex *in vivo*. Amino acids were sampled via the same probe and their concentrations were quantified using an HPLC analysis. Predictably, hypoosmotic medium (-90 mM NaCl) caused strong increases in the cortical levels of glutamate and aspartate. However, we also, unexpectedly, found a dramatic decrease in the extracellular levels of glutamine that within 1 hr fell to 25-30% of the controls values. This decrease was reversible and was not observed when NaCl was replaced with mannitol, suggesting that changes in glutamine levels were related to cell swelling. To determine the mechanisms responsible for the dramatic change in glutamine levels *in vivo*, we further tested the effects of hypoosmotic and low NaCl media on glutamine transport and metabolism in primary cultures of rat cortical astrocytes_the cell type that is responsible for brain glutamine synthesis and export. HPLC analysis detected ~30% decrease in intracellular glutamate and ~20% decrease in intracellular glutamine levels after 30-min exposure to hypoosmotic medium. Using D-[^{3}H]aspartate as radiotracer, we linked decreases in the intracellular glutamate levels to enhanced amino acid release. On the contrary, no substantial changes were observed for the release or uptake of L-[^{3}H]glutamine. We then measured activities of glutamine synthetase and glutaminase, key enzymes of intracellular glutamate and glutamine metabolism. Glutaminase activity, measured in cell cultures via enzymatic conversion of L-[^{3}H]glutamine, was not affected by hypoosmotic cell swelling. In contrast, activity of glutamine synthetase, that was

measured via enzymatic conversion of L-[^{3}H]glutamate, was reduced by 30%. The latter effect is likely responsible for strong changes in the extracellular and whole-tissue glutamine levels found by us and others in animal models of hyponatremia.

Disclosures: M.C. Hyzinski, None; **M.Y. Vincent**, None; **P. Dohare**, None; **A.A. Mongin**, None; **R.E. Haskew-Layton**, None; **A. Rudkouskaya**, None; **R.W. Keller**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.7

Topic: B.11. Glia-Neuron Interactions

Support: NIH

Title: Role of astrocytic Gq GPCR signaling in sensory activated synaptic transmission

Authors: *C. AGULHON, T. RIDAY, B. ROTH, B. PHILPOT, K. B. CASPER, K. D. MCCARTHY;
Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstract: The concept that astrocytes release neuroactive molecules (gliotransmitters) in a Gq GPCR Ca2+ dependent manner to affect synaptic transmission is one of the most important paradigm shifts in neuroscience over the past decade. Using two mouse lines to either selectively increase or obliterate astrocytic Gq GPCR Ca2+ signaling, we recently reported that neither increasing nor obliterating astrocytic Ca2+ fluxes affects miniature, spontaneous and evoked excitatory synaptic transmission or synaptic plasticity in acute hippocampal slices (Agulhon et al., Science, 2010; Petravicz et al., J Neurosci, 2008; Fiacco et al., Neuron, 2007). These findings call into question the developing consensus that Gq GPCR Ca2+-dependent release of gliotransmitters is both necessary and sufficient to directly affect neuronal transmission and plasticity in situ.
In an attempt to circumvent the caveats associated with in situ experimental approaches, here we test the possibility that astrocytic Gq GPCR signaling is involved in the modulation of physiological sensory activated synaptic transmission in vivo. We have developed and characterized a new genetically modified mouse line that enables selective activation of astrocytic Gq GPCR Ca2+ signaling in awake mice. This mouse line expresses an engineered Gq GPCR (Gq-DREADD receptor) selectively in astrocytes.

The Gq-DREADD is not activated by any known endogenous ligand, but is potently activated by a synthetic drug-like compound (CNO) that penetrates the CNS and does not not activate any known GPCR (Armbruster et al., PNAS, 2007; Alexander et al, Neuron 2009). We demonstrate that selectively activating astrocytic Gq GPCR signaling cascades depresses sensory-driven synaptic transmission in the visual cortex 20-25 min after peripheral CNO administration. The magnitude of this effect is dose-dependent and blocked by an antagonist of Gq-DREADD receptors. The mechanisms underlying this observation are currently under investigation, in particular whether Ca2+, gliotransmission or other cellular mechanism(s) are involved in this phenomenon.

Disclosures: C. Agulhon, None; **T. Riday**, None; **B. Roth**, None; **B. Philpot**, None; **K.B. Casper**, None; **K.D. McCarthy**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.8

Topic: B.11. Glia-Neuron Interactions

Support: NIH Grant NS050705-01

Title: Connexin expression profiles in sciatic nerves from injured and aging WT and Cx32-null mice

Authors: *M. M. FREIDIN, S. ASCHE-GODIN, C. K. ABRAMS;
Dept Neurol, SUNY Downstate Med. Ctr., BROOKLYN, NY

Abstract: Mutations in Cx32 alter channel function and are clearly associated with X-linked Charcot-Marie-Tooth Disease (CMTX). Data from our laboratory and others suggests: 1) mice lacking Cx32 show reduced capacity for regeneration associated myelination; 2) Cx32 is expressed and regulated in cultures of primary SCs; 3) SCs expressing two different CMTX mutant forms of Cx32 have strikingly different effects on regeneration in a xenograft model; and 4) SCs from Cx32 knockout (32KO) mice show decreased proliferative responses to stimulation by GGF2 (Glial Growth Factor 2), a member of the neuregulin family of growth factors. These findings suggest that that Cx32, acting in junctional and non-junctional capacities, is required for normal function of myelinating and non-myelinating SC populations. In additional studies, the expression profiles of 20 mouse connexin genes examined whether GGF2 regulates other connexins

neonatal and adult SCs from neonatal and adult mice. Real time PCR studies revealed GGF2 treatment results in differential regulation of Cx45, Cx47, Cx50, and Cx29 with respect to genotype and age. To determine whether there is a corresponding change in connexin expression in 32KO peripheral nerves in vivo nerve during periods of SC proliferation, myelination, and repair, connexin gene expression profiles were obtained from sciatic nerves of WT and 32KO mice taken at increasing post-natal ages of 1 week, 2 weeks, 1 month, 3, months, 6 months, and 12 months old. In addition, to more directly probe changes in connexin expression in proliferating populations of adult SCs, real time PCR studies compared samples from WT and 32KO transected nerves at 4 and 6 days post-injury (a period of SC de-differentiation and proliferation). As predicted by in vitro studies, aging and injury revealed significant differences between WT and 32KO in several SC connexins, including Cx45 and Cx47. These and related studies will ultimately determine the regulatory features of Cx32 in aging and regenerating peripheral nerves from WT and 32KO mice; identifying and confirming pathways associated with SC cell cycle control, survival, and myelination and expression of Cx32.

Disclosures: M.M. Freidin, None; **S. Asche-Godin**, None; **C.K. Abrams**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.9

Topic: B.11. Glia-Neuron Interactions

Title: Neurites-growth inhibitory astrocytes show high mobility after mechanical injury in vitro

Authors: *H. XU[1,2], L. GOU[1], H. DONG[1];
[1]Neurol., UCLA, Los Angeles, CA; [2]Basic science institute, the fourth military medical university, Xi'an, Shaanxi Province, China

Abstract: Astrocytes are the most predominant and heterogeneous glial cell types in central nervous system. Little is known about the functional significance of this heterogeneity. After CNS injury, activated astrocyes migrated into the lesion site and formed glial scar which contributed to the failure of axon regeneration. Understanding astrocyte migration is fundamental to understanding the formation of the glial scar.
In previous experiments, we have shown that subpopulation of cortical astrocytes (referred as astrocytesi) exerted strong neurite-growth inhibition effects on DRG neurons

in vitro. In present study, we investigate the migration behavior of these astrocytei using the classical scratch wound healing model in vitro. Confluent monolayer primary cortical glial cells were obtained from neonatal rat pups (P5-P10), astrocytei and neurite-growth supportive astrocyte(referred as astrocytess) were identified by the neurite-growth inhibitory substructures they formed. After injury, astrocytes migration were recorded and analyzed at different time points. Our results shown that compare to neighboring astrocytess, astrocytesi were different in several aspects: 1)small size, satellite or spindle morphologies; 2) earlier migration initiation; 3) high migration rate; 4) single cell independent migration; 5) no significant proliferation increase; 6) regular arrangement lost after wound heal ; 7) no significant hypertrophy changes during migration; 8) limited lateral(parallel to the injury scratch) migration.
Our results suggested astrcotyesi subpopulation showed high mobility after injury. Considering their neurite-growth inhibition property, astrocytesi may play an import role in glial scarring and axon regeneration failure. To our knowledge, this is the first time to show that heterogeneous astrocytes showed different migration behavior after injury. The underlying molecular mechanism of their high motility will help us to in controlling the glial scar formation and seeking potential therapeutic targets for axon regeneration.

Disclosures: H. Xu, None; **L. Gou**, None; **H. Dong**, None.

Nanosymposium

827. Neuron-Glia Interactions and Astrocyte Activity II

Location: Room 24A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:30 pm

Program Number: 827.10

Topic: B.11. Glia-Neuron Interactions

Support: Sällskapet Barnavård

Jeanssons stiftelse

Stiftelsen Frimurare Barnhuset Stockholm

HKH Kronprinsessan Lovisas/Tielmans Minnesfond

Title: Role of extracellular potassium in the regulation of astrocyte water permeability

Authors: Y. SONG, *E. GUNNARSON;
Karolinska Institutet, Stockholm, Sweden

Abstract: Astrocytes are essential for brain water and ion homeostasis. Fundamental properties include high selective permeability to K^+ ions to support regulation of extracellular potassium concentrations ($[K^+]_o$). During synaptic activity $[K^+]_o$ increases from 2.5mM to 10-12mM. When these systems are overwhelmed, $[K^+]_o$ can reach values as high as 80mM. Astrocytes express the water channel aquaporin 4 (AQP4), which is regulated by phosphorylation and suggested to be important for efficient removal extracellular K^+. AQP4 is coexpressed with the inward rectifying K-(Kir) channel Kir4.1, however there is controversy as to whether a functional relationship exists. We investigated whether high $[K^+]_o$ can regulate astrocyte AQP4 and the mechanisms of such an effect. Studies were performed on rat astrocytes expressing AQP4; primary cultures and a transfected cell line. When exposed to 10mM $[K^+]_o$ or 35mM $[K^+]_o$ for 1 min, astrocyte water permeability significantly increased by 46%, attributable to an effect on AQP4. High $[K^+]_o$ has been suggested to activate soluble Adenylyl Cyclase and increase cAMP production, the prime activator of PKA. Both 10 mM and 35mM $[K^+]_o$ increased cAMP production by 40%. We found that PKA phosphorylated AQP4 Serine 111 *in vitro*. In support of this 10 mM $[K^+]_o$ did not increase water permeability in cells preincubated with the PKA inhibitor KT5720 or in cells expressing mutant AQP4 S111A. After 5 min of 10mM $[K^+]_o$ the increase in water permeability was sustained, but not after 5 min of 35mM $[K^+]_o$. To explore the role of Kir-channels we used 100µM Barium, which is known to specifically inhibit Kir-channels. After Barium, 10mM $[K^+]_o$ failed to increase water permeability, supporting a functional link between Kir-channels and regulation of astrocyte water permeability. We then explored mechanisms behind the observed absence of water permeability increase. 35mM $[K^+]_o$, as opposed to 10mM $[K^+]_o$, triggered a global calcium increase in the cells. When Kir-channels were inhibited, 10mM $[K^+]_o$ also induced a calcium increase in the cells. We hypothesized that a calcium-dependent dephosphorylation of AQP4 prevented water permeability increase and found that inhibition of the protein phosphatase calcineurin resulted in sustained water permeability increase following 35mM $[K^+]_o$. Taken together our data indicate that increases of $[K^+]_o$ within the physiological range will increase astrocyte water permeability via PKA-dependent AQP4 phosphorylation and that this effect can be modulated by the activity of Kir-channels and calcium signaling. Our results support the concept of coupling between water transport via AQP4 and potassium handling in astrocytes.

Disclosures: Y. Song, None; **E. Gunnarson**, None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.1

Topic: C.01. Translational Mechanisms

Support: RO1 MH075916

Title: Streamlining quantitative proteomics in human brain tissue

Authors: E. CICCIMARO[1], M. L. MACDONALD[2], S. PETERMAN[1], *C.-G. HAHN[3], A. PRAKASH[1], I. A. BLAIR[2], M. SANDERS[1];
[1]Thermo Fisher Scientific, Somerset, NJ; [2]Dept Psychiat, Univ. Pennsylvania Sch. Med., Philadelphia, PA; [3]Dept Psychiat, Univ. Pennsylvania Sch. Med., PHILADELPHIA, PA

Abstract: Neuronal protein trafficking and sub-cellular composition is implicated in a number of neuropsychiatric illnesses including Alzheimer's disease, schizophrenia and drug addiction. To date, several groups have reported qualitative and quantitative mass spectrometric based investigations of post-synaptic density fractions isolated from human brain tissue. Future proteomics studies will ideally be capable of assaying a broader number of proteins and, alternatively, target specific families across multiple sub-cellular factions. Toward this end we are attempting to gain both greater proteomic coverage of human brain tissue as well as develop a bioinformatics platform capable of the streamlined quantitative targeting of specific protein groups.
A particularly beguiling challenge associated with experimentation using human brain tissue, as with any primary tissue sample, is the great variability introduced by sample harvesting, storage, and processing. In an attempt to control for these variables, a whole proteome isotope-labeled standard representing hundreds of key human brain proteins was developed and validated. In this work we demonstrate how this labeled proteome was created from olfactory epithelial cultures and utilized in a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify targeted protein families in multiple fractions isolated from human postmortem brain tissue including synaptosomal, synaptic membrane, vesicular, ER/golgi, pre-synaptic and post-synaptic fractions. Additionally we report on an emerging bioinformatics platform for streamlining liquid chromatography-selected reaction monitoring (LC-SRM) assay design, quantitative validation and data analysis. In this paradigm, data dependant LC-MS/MS was first used to characterize the labeled proteome. Following this discovery phase, high resolution full scan MS was performed on a dilution series of the unlabeled to labeled whole proteome standard. This proteome wide stable-isotope dilution series, in conjunction with the discovery data, provided both protein identification, as well as detailed information on how specific tryptic peptides performed analytically in terms of linear response and limits of quantification. Ultimately, protein groups were targeted using software to identify tryptic peptides with excellent analytical characteristics and automatically assign SRM transitions predicted to give optimal selectivity and sensitivity. The resulting transition list was then used to quantify key proteins in cellular-fractions from schizophrenic subjects using LC-SRM. These experiments are currently ongoing.

Disclosures: **E. Ciccimaro:** Employment; Thermo Fisher Scientific. **M.L. MacDonald:** None. **S. Peterman:** Thermo Fisher Scientific. **C. Hahn:** None. **A. Prakash:** Thermo Fisher Scientific. **I.A. Blair:** None. **M. Sanders:** Thermo Fisher Scientific.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.2

Topic: C.01. Translational Mechanisms

Support: NIH Grant MH074313

Stanley Medical Research Institute

Title: Membrane proteomics of superior temporal gyrus in schizophrenia

Authors: *N. S. TANNU[1], S. SUN[3], R. PINTAL[3], S. E. ARNOLD[4], S. E. HEMBY[2]; [1]Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Wake Forest Univ. Sch. of Med., Winston salem, NC; [3]Applied Biosystems, Framingham, MA; [4]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: The superior temporal gyrus (STG) consists of primary and secondary (association) auditory cortex providing initial analysis of auditory signals and language. Several studies have demonstrated the involvement of STG in the pathophysiology of schizophrenia (SCZ), including an inverse correlation between STG activity and severity of hallucinations and thought disorder as well as a number of molecular alterations in post-mortem SCZ brain. In order to further explore STG related biochemical correlates of SCZ, we performed a comparative membrane proteomic analysis between SCZ (M/F: 6/5, Age : 76.2 ± 1.6 yrs; n=11) and age/gender matched non-psychiatric controls (M/F: 6/5, Age : 75.3 ± 2.0 yrs; n=11) in tissue obtained from the University of Pennsylvania Neuropathology Brain Bank. Peptides generated from trypsin digestion of membrane fractions were labeled using isobaric mass tag labels (8plex iTRAQ). Samples were combined and separated by strong cation exchange followed by reverse phase liquid chromatography and spotted onto the MALDI plates for analysis by MALDI ToF/ToF mass spectrometry. Thirty two hundred distinct peptides were successfully annotated with high score and confidence (> 95%). Mass spectra were analyzed by ProteinPilot™ against the NCBI non-redundant database followed by Principal Components Analysis to determine relationships between variables by reducing the dimensionality of the dataset,

identify underlying variables, and detecting the presence of clusters within this multivariate dataset. Proteins that were differentially expressed between the two groups included positive regulators of long-term synaptic plasticity, neurite extension, axonal guidance and synaptogenesis, as well as neuron-glial cell interactions. The identified proteins are being analyzed/correlated with established risk factors to establish a hierarchical model of SCZ neuropathogenesis. Additionally, results are being compared to results from a similar study conducted in rhesus monkeys following chronic clozapine and haloperidol administration to enable better differentiation of medication versus disease related protein expression changes.

Disclosures: N.S. Tannu, None; **S. Sun**, None; **R. Pintal**, None; **S.E. Arnold**, None; **S.E. Hemby**, None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.3

Topic: C.01. Translational Mechanisms

Support: NIH Grant F32AG032848-02

Title: Proteomic and phosphoproteomic characterization of frontotemporal lobar degeneration

Authors: *N. T. SEYFRIED[1], J. H. HERSKOWITZ[2], Y. M. GOZAL[2], E. B. DAMMER[1], Q. XIA[1], D. M. DUONG[1], H. D. REES[2], D. S. COOPER[2], M. GEARING[2], J. J. LAH[2], A. I. LEVEY[2], J. PENG[1];
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Abstract: Frontotemporal lobar degeneration (FTLD) is a progressive neurodegenerative disease characterized by prominent behavioral abnormalities, personality changes, language dysfunction, and can often co-occur with motor neuron disease. The major pathology in FTLD is characterized by intracellular aggregation of ubiquitinated and phosphorylated TAR DNA binding protein-43 (TDP-43), suggesting that dysregulation in phosphorylation events may contribute disease progression. However, to date systematic proteomic or phosphoproteomic analysis in FTLD brains has not been reported. In this study we employed complementary quantitative proteomic approaches (label-free and isotope-labeling) to characterize the insoluble proteome from FTLD and age-matched

control postmortem brain tissue (frontal cortex). The altered proteins in FTLD include among others, regulators of cytoskeletal function and oxidative stress. Western blotting was used to confirm changes in protein abundance. For phosphopeptide analysis immobilized metal affinity chromatography (IMAC) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed. Using this approach we identified approximately 1000 phosphopeptides in human brain (control and FTLD) with significant changes in the FTLD phosphoproteome. These data highlight the utility of combining proteomic and phosphoproteomic strategies to characterize postmortem human brain tissue, providing new insight into FTLD pathogenesis.

Disclosures: N.T. Seyfried, None; **J.H. Herskowitz**, None; **Y.M. Gozal**, None; **E.B. Dammer**, None; **Q. Xia**, None; **D.M. Duong**, None; **H.D. Rees**, None; **D.S. Cooper**, None; **M. Gearing**, None; **J.J. Lah**, None; **A.I. Levey**, None; **J. Peng**, None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.4

Topic: C.01. Translational Mechanisms

Support: NIH 1ZIAMH000279-27

Title: Postsynaptic density proteome composition reflects chronic valproate and lithium treatment in rats

Authors: *S. P. MARKEY[1], D. NANAVATI[1,2], G. CHEN[3], D. AUSTIN[3], L. A. CATAPANO[3], A. DOSEMECI[4], H. MANJI[3];
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Abstract: Bipolar disorder is marked by significant changes in the structure and cell type distribution of several prominent brain regions, including the hippocampus. There is also growing evidence that the disease is correlated with disruptions in synaptic plasticity cascades involved in cognition and mood regulation. The postsynaptic complex is a vast network of cytoskeletal scaffolding and signaling proteins that facilitate plasticity by mediating the movement of receptor and signaling complexes within the postsynaptic active zone. Alleviating the symptoms of bipolar disorder involves chronic treatment with

mood stabilizers like lithium or valproate. These two structurally dissimilar drugs are known to alter prominent signaling cascades in the hippocampus, but their effects on the postsynaptic density complex remain undefined.
We utilized mass spectrometry to investigate the effects of chronic mood stabilizer treatment on the hippocampal postsynaptic proteome isolated from groups of 6 rats treated for 5 weeks. Sucrose density-separated PSDs were tryptically digested, and the peptides separated and analyzed in triplicate using ion exchange chromatography followed by liquid chromatography tandem mass spectrometry. We identified and quantified 605 proteins (>2 peptides) in the 288 LC/MS/MS analyses, of which 99% were found in all treatment conditions. The majority of proteins were functionally classified as signaling molecules (23%), cytoskeletal and cell adhesion proteins (20%), or synaptic vesicle/transport proteins (13%). 300 proteins (~50%) have been previously implicated in psychiatric or neurological disorders. Lithium and valproate significantly altered the concentrations of 20 and 37 proteins, respectively, based on ion current quantification. Seven proteins were affected similarly by both lithium and valproate: Ank3, Grm3, Dyhc1, and four isoforms of 14-3-3 structural proteins. Immunoblotting the same samples confirmed the changes in Ank3 and Grm3 expression. Our findings support the hypothesis that mood stabilizers modulate signaling in the hippocampal PSD proteome. The data also support targeting Ank3 and Grm3 to develop biomarkers and novel therapeutics for bipolar disorder.

Disclosures: S.P. Markey, None; **D. Nanavati**, None; **G. Chen**, None; **D. Austin**, None; **L.A. Catapano**, None; **A. Dosemeci**, None; **H. Manji**, None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.5

Topic: C.01. Translational Mechanisms

Support: MH086257

MH53327

MH064673

MH066392

MH074016

Doris Duke Clinical Scientist Award

Title: Isolation of endosomes and characterization of endosomal AMPA receptor expression in prefrontal cortex in schizophrenia

Authors: *J. C. HAMMOND[1], A. FUNK[1], V. HAROUTUNIAN[2], R. MCCULLUMSMITH[1], J. MEADOR-WOODRUFF[1];
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Abstract: Several lines of evidence point to alterations of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor trafficking in schizophrenia. Multiple proteins, including Synapse Associated Protein 97 (SAP97), Glutamate Receptor Interacting Protein 1 (GRIP1), and N-ethylmaleimide Sensitive Factor (NSF), facilitate the forward trafficking of AMPA receptors toward the synapse. Once localized to the synapse, AMPA receptors are trafficked in a complex endosomal system. We hypothesized that alterations in the expression of these proteins and alterations in the subcellular localization of AMPA receptors in endosomes may contribute to the pathophysiology of schizophrenia. Accordingly, we measured protein expression of SAP97, GRIP1, and NSF in the dorsolateral prefrontal cortex and found an increase in the expression of SAP97 and GRIP1 in schizophrenia. To determine the subcellular localization of AMPA receptor subunits, we developed a technique to isolate early endosomes from postmortem tissue. We found increased GluR1 receptor subunit protein in early endosomes in subjects with schizophrenia. Together, these data suggest that there is an alteration of forward trafficking of AMPA receptors as well as changes in the subcellular localization of an AMPA receptor subunit in schizophrenia. We are currently expanding our previous work on the isolation of early endosomes to the isolation of both late and recycling endosomes, by targeting the proteins Rab7 and Rab11, respectively, for immunoisolation. We will present the total expression level of late and recycling endosomes from postmortem brain in schizophrenia and a comparison group, as well as the level of expression of AMPA receptor subunits in each of these endosomal compartments..

Disclosures: J.C. Hammond, None; **A. Funk**, None; **J. Meador-Woodruff**, None; **R. McCullumsmith**, None; **V. Haroutunian**, None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.6

Topic: C.01. Translational Mechanisms

Support: NIH MH075916

Stanley Foundation

Title: Dysregulated trafficking and phosphorylation of NR1 in the post synaptic density of schizophrenia patients

Authors: *A. BANERJEE[1], M. L. MACDONALD[2], K. BORGMANN-WINTER[3], H.-Y. WANG[4], T. E. MCCLOSKEY[5], C.-G. HAHN[5];
[1]Dept. of Psychiatry, Univ. Pennsylvania, PHILADELPHIA, PA; [2]Pharmacol. and Psychiatry, Univ. Pennsylvania, Philadelphia, PA; [3]Psychiatry, Univ. of Pennsylvania and Children Hosp. of Philadelphia, Philadelphia, PA; [4]Pharmacol. and Physiol., CUNY Med. Sch., New york, NY; [5]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Previously, we have assessed the ligand induced activation of NMDA receptors in postmortem brains and found that tyrosine phosphorylation of NR2 subunits and NR1 association with its signaling partners were attenuated in the prefrontal cortex (PFC) of patients with schizophrenia. Subsequently, we observed postmortem PFC tissues of schizophrenia also contain altered protein associations; NR1 association with phospholipase lipase Cγ (PLCγ) and with protein kinase C gamma and with protein kinase Cγ (PKCγ); in their basal states were decreased. Altered protein interactions in NR complexes could be due to dysregulated partitioning of component proteins of NR complexes into the postsynaptic density (PSD), where NR complexes are highly concentrated. Or, it could be due to changes in posttranslational modifications of component proteins that may determine trafficking of these proteins or their affinity for binding partners. To test this, we first isolated PSD fractions from postmortem brains and quantified component proteins in the PSD and in NR complexes derived from PSD fractions. We found that NR1 protein content was much higher in PSD fractions derived from schizophrenia patients, while it was not altered in total brain lysates of schizophrenia patients. This may raise an interesting possibility that NR1 trafficking and/or posttranslational modifications are altered in schizophrenia. Serine phosphorylation of NR1 is associated with its forward membrane targeting. Serine residue 897 in NR1 in the PSD fraction is of particular interest as phosphorylation of this site was found to be decreased in brain lysates of schizophrenia patients (Emamian et al, 2004). In corroboration of these results, we observed that serine-897 phosphorylation was significantly decreased in PSD fractions of schizophrenia patients' PFC tissues. These observations together suggest that the PSD content and serine phosphorylation of NR1 is altered in the PFC of schizophrenia patients, which can contribute to altered protein interactions in NR complexes.

Disclosures: A. Banerjee, None; **M.L. Macdonald**, None; **K. Borgmann-Winter**,

None; **H. Wang**, None; **T.E. Mccloskey**, None; **C. Hahn**, None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.7

Topic: C.01. Translational Mechanisms

Support: Stanley Medical Research Institute

RO1 MH075916

F31 MH087106

Title: NMDA receptor complex dysfunction in schizophrenia

Authors: *M. L. MACDONALD[1], A. BANERJEE[1], E. CICCIMARO[3], C.-G. HAHN[1], I. A. BLAIR[2];
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Abstract: NMDA receptor hypo-function is a leading hypothesis for schizophrenia. Recently, we provided direct evidence of decreased NMDA receptor activity in postmortem brain tissue of schizophrenic subjects. Additionally, ErbB4 - NR1 and ErbB4 - PSD95 interactions were altered, as were interactions between NR1 and several of its signaling partners. The active NMDA receptor interacts with numerous additional proteins in the postsynaptic density (PSD) forming the NMDA receptor complex. Here, extra and intra PSD signals converge non-linearly to regulate NMDA receptor activity and signaling. Altered protein expression, trafficking, degradation and interactions are all potential contributors to pathological NMDA receptor hypo-function. In the present study we aim to assess both the extent of altered protein-protein interactions at the NMDA receptor and the proximal contributors to these changes. To that end we utilized an absolute quantification liquid chromatography-mass spectrometry/multiple reaction monitoring method (LC-MS/MRM) to quantify 40 NMDA receptor complex components across three fractions of human postmortem brain tissue prepared from 12 schizophrenic and matched control subjects: PSD specific NR1 IP complexes, PSD enrichments, and synaptosomal fractions (SF). To date we have observed few changes in SF and PSD fractions while NMDA receptor complex composition is striking different between

schizophrenic and control subjects. These data suggest that altered protein-protein interactions with in the PSD could contribute to pathological NMDA receptor hypo-function in schizophrenia.

Disclosures: M.L. MacDonald: None. **A. Banerjee:** None. **E. Ciccimaro:** Employment; Thermo Fisher Scientific. **C. Hahn:** None. **I.A. Blair:** None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.8

Topic: C.01. Translational Mechanisms

Support: P50DA006634

R21DA027512

Title: Ionotropic glutamate receptor dysregulation in the hippocampus following chronic cocaine self-administration in rhesus monkeys

Authors: N. TANNU[1], S. HEMBY[1], *K. BORGMANN-WINTER[2];
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Abstract: Numerous studies indicate dysregulation in dopaminergic pathways in humans and animal models, yet pharmacotherapies that directly target dopamine signaling have proven only moderately successful. An alternative strategy is to identify medications that target neurotransmitter systems that augment dopaminergic signaling directly and/or indirectly. Identification of key biochemical processes may provide the basis for development and optimization of next generation pharmacotherapies that can better target cocaine abuse as well as further our understanding of the neurobiological basis of cocaine reinforcement. Previous studies from our lab have demonstrated significant dysregulation of ionotropic glutamate receptor subunits in limbic regions of cocaine overdose victims and rhesus monkeys following chronic cocaine intake. The present study will examine targeted proteomic alterations in NMDA and AMPA receptor complexes in the hippocampus following intravenous cocaine self-administration by comparing limited (6 month; n=4) and binge access (6 month limited access + 6 month binge access; n=6) and controls (n=5). The expression of NMDA and AMPA receptor subunits and associated

synaptic proteins (e.g. PSD95, SynGAP, PICK1 and GRIP) will be assessed using Western blot analysis to extend previous findings in our lab on iGluR dysregulation. To further explore NMDA receptor dysregulation, immunoprecipitation procedures will be used to isolate NMDA receptor subunits and associated complexes in membrane fractions for subsequent proteomic analysis using iTRAQ labeling in combination with multidimensional liquid chromatographic separation followed by MALDI-ToF/ToF analysis. Results will be assessed using biochemical pathway analysis and principal component analysis to determine similarities and differences in protein expression as a function of cocaine exposure in primate brain. Results from these studies extend and compliment previous studies of cocaine-induced biochemical alterations in human post-mortem brain tissue using an animal model that closely recapitulates the human condition and provide new insight into the molecular basis of cocaine addiction and potential targets for pharmacotherapeutic intervention.

Disclosures: N. Tannu, None; **S. Hemby**, None; **K. Borgmann-Winter**, None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.9

Topic: C.01. Translational Mechanisms

Support: NIH Grant RR 01614

Wellcome Trust

Biotechnology and Biological Sciences Research Council

Title: Activity dependent changes in synaptic composition resulting from seizure

Authors: *J. C. TRINIDAD[1], A. THALHAMMER[2], R. SCHOEPFER[2], A. BURLINGAME[1];
[1]Pharmaceut. Chem., Univ. of California, San Francisco, San Francisco, CA; [2]Dept. of Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: At the synapse, AMPA and NMDA-type glutamate receptors are associated with a complex protein network known as the postsynaptic density (PSD). The PSD contains proteins of many functional classes including kinases/phosphatases, scaffolding

molecules, and structural molecules. In addition to its primary role of mediating fast synaptic transmission, the PSD is a dynamic structure that alters its composition over longer time periods in response to the prior pattern of synaptic activity. The balance of synaptic signaling is tightly controlled in the central nervous system and imbalances between synaptic excitation and inhibition can lead to seizures and epilepsy. To better understand the molecular changes associated with this neurological disorder, we employed mass spectrometry to quantitatively profile seizure-induced changes in synaptic composition.
We applied a mass spectrometry-based biochemical approach to quantify synaptic protein components. PSD fractions were obtained via sucrose density fractionation. To relatively quantify protein levels, we applied stable isotope labeling using the iTRAQ reagent. To reproducibly quantify individual phosphorylation sites, we developed selective reaction monitoring assays against a range of phosphopeptides.
We have applied these quantitative proteomic techniques to analyze post-synaptic density preparations from mouse cortex in response to pilocarpine-induced seizure. We isolated synapses at times 0, 10, 20, and 60 minutes post-pilocarpine injection. We have quantified over 950 proteins and 2000 sites of phosphorylation from three biological replicates. Using clustering analysis, we observed that within individual protein subcomplexes, the constituent members display strikingly correlated dynamics as a function of activity. In contrast, the different subcomplexes themselves show a range of distinct dynamic responses. At the level of phosphorylation, a small subset of sites appears highly regulated with greater than two-fold increases or decreases in stoichiometry.

Disclosures: J.C. Trinidad, None; **A. Thalhammer**, None; **R. Schoepfer**, None; **A. Burlingame**, None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.10

Topic: C.01. Translational Mechanisms

Support: NIH Grant R21AG031388

Title: Disruption of protein network at postsynaptic density in Alzheimer’s disease

Authors: *Y. GONG[1], C. LIPPA[1], J. ZHU[2], Q. LIN[2], A. ROSSOA[1];

[1]Neurol., Drexel Univ. Col. of Med., Philadelphia, PA; [2]U Albany Proteomics Facility, Ctr. for Functional Genomics, Univ. at Albany, Rensselaer, NY

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder. Synaptic loss is the strongest correlate of the degree of clinical impairment of AD. However, not much is known about the molecular pathological entities at the synapse in AD development. The accumulating evidence from cellular experiments and clinical treatment shows that excitatory synapses are involved in the development of AD. Glutamate receptors are anchored at excitatory synapses through a proteinaceous network in postsynaptic density (PSD) which is associated by toxic oligomers of β-amyloid peptide (Aβ) released from APP. To understand these molecular pathological interactions, we first used iTRAQ proteomic analysis to screen for the pathological level of proteins at PSD and we found the glutamate receptor network and Shank-postsynaptic platform show alteration at PSD of AD. In cell culture, oligomers of β-amyloid may alter the homeostasis of these proteins at PSD. Currently, the spatial-temporal pathological changes at this protein network are obscured at early AD due to the animal AD models' limitations. The accumulation of pathological changes at protein network of PSD beside at pre-synapse could underlie the synaptic homeostasis loss leading to negative synaptic formation contributing to synaptic loss in AD development. These results may help improve the treatment and prevention of homeostatic deregulation of proteins at the selective synapse in Alzheimer's disease.

Disclosures: Y. Gong: Research Grant; R21AG031388. **C. Lippa:** None. **J. Zhu:** None. **Q. Lin:** None. **A. Rossoa:** None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.11

Topic: C.01. Translational Mechanisms

Support: NIH AG 10485

NIH AG 22550

NIH AG 27956

Title: E2 signaling ameliorates soluble aβ-oligomer-induced mitochondrial fragmentation

Authors: *S. N. SARKAR;
UNTHSC, FORT WORTH, TX

Abstract: Excessive mitochondrial fragmentation, triggered by impaired function of the fission-inducing protein Drp1 (dynamin-related protein 1), leads to synaptic plasticity dysfunction and subsequent neuronal loss due to reactive oxygen species and sluggish ATP generation. Uncontrolled fission results in abnormally small mitochondria, as observed in human Alzheimer's disease neurons. Nitrosylation of Drp1 by β-amyloid protein (Aβ) oligomers induces excessive mitochondrial fission and phosphorylation of Drp1 at its protein kinase A (PKA) site, inhibiting fission and promoting fusion. A myriad of studies have suggested that 17β-estradiol (E2) protects neurons against oxidative insults relevant to the pathogenesis of AD, including exposure to Aβ. Furthermore, E2 increases mitochondrial capacity for oxidative phosphorylation while decreasing production of reactive oxygen species. We sought to determine whether E2-induced signaling abrogates Aβ-induced mitochondrial fission. Primary hippocampal neurons from embryonic day eighteen (E18), grown fifteen days invitro (15 DIV), transfected with the live mitochondrial marker mito-DsRed2, were exposed to soluble Aβ ologomers (human $A\beta_{1-42,}$ 50nM for 12 hr.) and morphological changes in mitochondria were monitored by fluorescence confocal microscopy. In control neurons mitochondria normally displayed an elongated (2-3μm average length), as well as fragmented (0.8-1.4μm average length), but exposure to soluble Aβ oligomers induced highly fragmented, smaller mitochondria (0.8-1.4μm average length.). Exposure to E2 (10nM for 12hr.), resulted in elongated filamentous morphology (2.8-4μm average length). In contrast to Aβ, exposure of E2 and Aβ together ameliorated excessive fission. Ameliorating effects of E2 in Aβ-induced fission was abrogated by treatment of a cell permeable protein kinase A (PKA) inhibitor, KT5720. Taken together, these results suggest that E2-induced signaling ameliorates Aβ-induced mitochondrial fragmentation in an activated PKA-dependent manner. Thus, our results provide a possible mechanism by which E2, by inhibiting mitochondrial fission, increases mitochondrial capacity for oxidative phosphorylation, while decreasing production of reactive oxygen species, and abrogating Aβ-mediated synaptic plasticity dysfunction, synapse loss and neuronal death.

Disclosures: S.N. Sarkar: None.

Nanosymposium

828. Functional Consequences of Synaptopathies

Location: Room 32B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:00 pm

Program Number: 828.12

Topic: C.01. Translational Mechanisms

Support: NIH AG13471

NIH RC1 AG0354878

NIH U0128583

NIH AG021975

Title: Cocktail treatment for cognitive deficits from tau pathology

Authors: *G. M. COLE[1], Q. MA[1], F. YANG[2], D. GANT[2], M. ALAVERDYAN[2], S. A. FRAUTSCHY[2];
[1]GRECC (VA) & Med. (UCLA), UCLA, VA Med. Ctr., SEPULVEDA, CA; [2]Med., UCLA VA Med. Ctr., Los Angeles, CA

Abstract: Numerous effective single treatments exist for beta-amyloid pathology and related deficits in transgenic mouse models, but their success in the clinic is problematic. Targeting beta-amyloid may be insufficient to treat patients with Alzheimer Disease or other tauopathies that involve abnormal age-related accumulations of phosphorylated tau associated with both loss of function (LOF) of normal tau roles (microtubule-stabilization) and toxic gains of function (GOF) from tau aggregate species, including tangles. Here, we report that aged (but not middle-aged) tau knockout mice (LOF) develop robust selective hippocampal excitatory synaptic marker loss that responds partially to treatment with the omega-3 fatty acid, DHA, and even better to the combination of 0.6% DHA plus 500 ppm lipoic acid, an intervention that also corrected spatial memory deficits in the Morris water maze. Successful treatment in this tau LOF model was associated with compensation for tau LOF by restoration of function in other microtubule-associated proteins (MAPs) that are inactivated by tau kinase phosphorylation. In contrast, late intervention at 15-16 months with the same combination of DHA plus lipoic acid in aged tangle-bearing wild type human tau transgenic (htau) mice improved biochemical pathology but was insufficient to restore similar pre-existing spatial memory and synaptic deficits despite reducing AT8 and p422S phospho-tau on Westerns. This implies an additional toxic GOF or that intervention was too late. Experiments with earlier intervention are underway to resolve this. Htau mice have extensive tau pathology as well as synaptic and cognitive deficits by 10-12 months of age. Thus, our late intervention at 15-16 months occured post-tangle and synaptic pathology and worked against established cognitive deficits, rather than in a prevention paradigm. However, combined treatment with DHA, alpha lipoate and the polyphenolic antioxidant curcumin was sufficient to treat spatial memory deficits in htau, even with the treatment beginning late. Available data indicate that successful treatments

targeted JNK kinase-mediated inactivation of multiple molecular mediators of synaptic plasticity. Evidence for synergistic biochemical protective mechanisms for the combined treatment with DHA, ALA and curcumin will be presented. Because this cocktail is safe and also effective in beta-amyloid pathology models, this cocktail approach shows promise for clinical trials.

Disclosures: G.M. Cole, None; **Q. Ma**, None; **F. Yang**, None; **D. Gant**, None; **M. Alaverdyan**, None; **S.A. Frautschy**, None.

Nanosymposium

829. APP Actions on Nerve Cells Development and Survival

Location: Room 23A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 829.1

Topic: C.02. Alzheimer's disease and other dementias

Support: R01AG026146 (SWP)

Alzheimer's Association (SWP)

CART-Rotary funds (SWP)

Title: APP intracellular domain (AICD) inhibits neurite outgrowth by regulating microtubule-binding proteins

Authors: *P. BANERJEE, A. SURYANARAYANA, A. NOLL, S. W. PIMPLIKAR; Dept Neurosci, NC-30, Lerner Res. Institute, CCF, CLEVELAND, OH

Abstract: Amyloid precursor protein (APP) plays a pivotal role in the pathogenesis of Alzheimer’s disease (AD). Although its exact function is undetermined, APP has been implicated in neuronal migration and axonal elongation. APP is proteolytically processed to generate multiple fragments including APP intracellular domain (AICD). We previously showed that AICD transgenic mice display aberrant activation of GSK-3b and increased phosphorylation of microtubule binding proteins, tau and CRMP2. Hyperphosphorylation of tau and CRMP2 is observed in AD brains, underscoring the importance of the role of AICD towards Alzheimer’s pathology.
In this study we analyzed the downstream effects of AICD-induced phosphorylation of tau and CRMP2 on neurite outgrowth. We overexpressed AICD and GFP in mouse primary cortical neurons and measured neurite length at various time points. We

observed that expression of AICD with or without Fe65 resulted in shorter neurites. The inhibitory effect of AICD on neurite length was observed in neurons at 2, 4 or 8 days-in-vitro (DIV) suggesting that AICD inhibits the neurite growth both before and after establishment of axon-dendrite polarity. Mutational analysis of AICD indicated that the YENPTY motif is important for inhibiting neurite growth. Interestingly, co-expression of CRMP2 with AICD resulted in further reduction in neurite growth and this effect was lost when phosphorylation sites in CRMP2 were mutated suggesting that the inhibitory effect of AICD on neurite growth was at least partially mediated by CRMP2 phosphorylation. Similarly, co-expression of tau with AICD also resulted in dramatic reduction in neurite outgrowth. Together, these observations indicate that increased levels of AICD negatively regulate neurite extension by modulating phosphorylation of microtubule binding proteins. We propose that these harmful effects of AICD may be partially responsible for the pathologies observed in vivo in AICD transgenic mice.

Disclosures: P. Banerjee, None; **A. Suryanarayana**, None; **S.W. Pimplikar**, None; **A. Noll**, None.

Nanosymposium

829. APP Actions on Nerve Cells Development and Survival

Location: Room 23A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 829.2

Topic: C.02. Alzheimer's disease and other dementias

Title: The beta-amyloid precursor protein (APP) is a potent tumor growth factor

Authors: *V. VENKATARAMANI, O. WIRTHS, S. SCHWEYER, T. A. BAYER; Univ. of Göttingen, Goettingen, Germany

Abstract: The β-amyloid precursor protein (APP) represents a type I transmembrane glycoprotein that is ubiquitously expressed. In brain it is a key player in the molecular pathogenesis of Alzheimer’s disease. Its physiological function is however less well understood. Previous studies showed that APP is up-regulated in prostate, colon, pancreatic tumor and oral squamous cell carcinoma. In the present study, we show that APP has an essential role in growth control of pancreatic and colon cancer. Abundant APP staining was found in human pancreatic adenocarcinoma and colon cancer tissue. Interestingly, treating pancreatic and colon cancer cells with valproic acid (VPA, 2-propylpentanoic acid), a known histone deacetylase inhibitor (HDACi), leads to up-regulation of GRP78, an ER chaperone immunoglobulin binding protein. GRP78 is

involved in APP maturation, and inhibition of tumor cell growth by down-regulation of APP and secreted sAPPα. Trichostatin A (TSA), a pan-HDAC inhibitor also lowered APP and increased GRP78 levels. In contrast, treating cells with valpromide, a VPA derivative lacking HDAC inhibitory properties had no effect on APP levels. VPA did not modify the level of epidermal
growth factor receptor, another type I transmembrane protein, and APLP2, a member of the APP-family, demonstrating the specificity of the VPA effect on APP. Small interfering RNA (siRNA)-mediated knock-down of APP also resulted in significantly decreased cell growth. Based on these observations, the data suggest that APP down-regulation via HDAC inhibition provides a novel mechanism for pancreatic
and colon cancer therapy.

Disclosures: V. Venkataramani, None; **O. Wirths**, None; **S. Schweyer**, None; **T.A. Bayer**, None.

Nanosymposium

829. APP Actions on Nerve Cells Development and Survival

Location: Room 23A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 829.3

Topic: C.02. Alzheimer's disease and other dementias

Support: CIRM

Title: Somatodendritic and axonal localization of processing and secretion of Amyloid beta from neurons

Authors: *E. DAVIS[1], J. D. FLIPPIN[1], N. JEON[2], L. S. B. GOLDSTEIN[1];
[1]Cell. Mol. Med., UC San Diego, SAN DIEGO, CA; [2]Bioengineering, Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disease characterized by amyloid plaques, neurofibrillary tangles and a massive loss of neurons in affected brains. The major constituent of amyloid plaques is extracellular Amyloid β peptides, which are processed from β-Amyloid Precursor Protein (APP) by γ- and β-secretases. A growing amount of evidence suggests that the localization of APP and its fragments is important for the pathogenesis of AD. To date, the details of regulation and localization of processing remain unclear. We have developed a system using murine hippocampal neurons or neurons derived from human embryonic stem cells (hESCs) in a novel

microfluidic culture device. This device allows us to assess the biochemistry of APP and Aβ in axons independent of the soma in these neurons. Using this system, we have detected significant amounts of amyloid beta secreted from axons. We can also observe the effects of drug inhibition or familial disease mutations on the location of amyloid production and secretion in axons or soma.

Disclosures: E. Davis, None; **J.D. Flippin**, None; **N. Jeon**, None; **L.S.B. Goldstein**, None.

Nanosymposium

829. APP Actions on Nerve Cells Development and Survival

Location: Room 23A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 829.4

Topic: C.02. Alzheimer's disease and other dementias

Support: DFG

NGFNplus

Title: APP and APLP2 provide essential functions at PNS and CNS synapses mediating neuromuscular transmission, spatial learning and synaptic plasticity

Authors: *U. MULLER[1], S. WEYER[1], M. KLEVANSKI[1], K. L. SCHALLER[2], A. DELEKATE[3], V. VOIKAR[4], M. KORTE[3], D. P. WOLFER[4], J. CALDWELL[2]; [1]Inst. Pharm. & Molec Biotech IPMB, Univ. of Heidelberg, Heidelberg, Germany; [2]Physioloy and Biophysics, Univ. of Colorado Denver, Aurora, CO; [3]TU Braunschweig, Braunschweig, Germany; [4]Inst. for Anat., Zurich Univ., Zurich, Switzerland

Abstract: Despite its key role in Alzheimer pathogenesis, the physiological function(s) of the amyloid precursor protein APP and of its proteolytic fragments are still largely unknown. To investigate these functions we have recently generated APPsa knockin (KI) mice expressing solely the secreted APPs-alpha ectodomain from the endogenous APP locus. Comparing APPsa-KI mice with APP-KO mice we previously showed that APPsa was sufficient to rescue the deficits of APP-KO mice and serves a key function for synaptic plasticity, learning and memory (Ring et al., 2007). To test whether APPsa could also rescue the lethal phenotype and neuromuscular deficits of APP/APLP2 double knockout (DKO) mice, we now crossed APPsa -KI mice onto an APLP2-deficient background. Here, we show that the majority of these APPsa-KI/APLP2-KO double

mutant mice (termed APPsa-DM) survive into adulthood. This surprising viability allowed us to assess APP/APLP2-mediated functions in the adult and revealed a complex phenotype characterized by deficits both in the PNS and importantly, also severe dysfunction of adult CNS. Although in APPsa-DM mice defects in neuromuscular transmission were largely ameliorated compared to lethal APP/APLP2 DKO mice, we detected remaining impairments in neurotransmitter release including largely reduced quantal content that resulted in muscular weakness. These data suggest that secreted APPsa modulates neuromuscular transmission to a level sufficient to rescue to a large extent the postnatal lethality of DKO mice. Morphological analysis of adult neuromuscular junctions revealed pre- and postsynaptic alterations and most strikingly a high incidence of immature and fragmented postsynaptic specializations, indicating a novel function of APP/APLP2 for postnatal synaptic maturation and maintenance. Despite normal CNS morphology, APPsa-DM mice showed pronounced deficits in hippocampus-dependent learning and memory that were associated with a strong deficit in long term potentiation LTP, already present in young adults. In contrast to peripheral synapses, neither defects in basal synaptic transmission nor in presynaptic short term plasticity were detectable at glutamatergic CA3/CA1 synapses, suggesting distinct roles of APP family members at peripheral and central excitatory synapses. Collectively, our data show that both APLP2 and APP are synergistically required to mediate neuromuscular function, working memory, spatial learning and synaptic plasticity.

Disclosures: U. Muller, None; **S. Weyer**, None; **M. Klevanski**, None; **A. Delekate**, None; **V. Voikar**, None; **M. Korte**, None; **D.P. Wolfer**, None; **J. Caldwell**, None; **K.L. Schaller**, None.

Nanosymposium

829. APP Actions on Nerve Cells Development and Survival

Location: Room 23A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 829.5

Topic: C.02. Alzheimer's disease and other dementias

Support: Alzheimer's Association Young Investigator Award

Title: Soluble amyloid precursor protein is a proliferation factor for adult neural stem cells

Authors: *M. DEMARS[1], O. LAZAROV[2];
[2]Anat. and Cell Biol., [1]Univ. of Illinois At Chicago, CHICAGO, IL

Abstract: Amyloid Precursor protein (APP) is a ubiquitously expressed, type I transmembrane glycoprotein whose physiological function has yet to be fully elucidated. Mutations in the gene encoding APP are causative of familial forms of Alzheimer's disease. APP is cleaved by several enzymatic activities termed α-, β- and γ secretase to form multiple metabolites of unknown function. In particular, soluble APPα (sAPPα) is a cleavage product of APP by α-secretase. The identity of α-secretase is not fully unraveled. Several members of the disintegrin and metalloproteinase (ADAM) family as well as the β-site APP-cleaving enzyme 2 (Bace 2) exhibit α-secretase activity. In this study, we report that sAPPα regulates the proliferation of a number of stem cell types including adult neural progenitor cells (NPC). We show that inhibition of matrix-metalloproteinase activity reduces the proliferation of NPC and this effect can be reversed by sAPPα administration. Examination of the neurogenic niches of the adult brain uncovered vastly higher levels of sAPP in the subventricular zone (SVZ) when compared with the subgranular layer of the dentate gyrus (SGL), suggesting a potential differential role for sAPP in the different neurogenic niches in the adult brain. Interestingly, levels of mature ADAM10, thought to play a major role as an α-secretase in the mature brain, are significantly higher in the SVZ than in the SGL, as well as in the SVZ of APP wild type mice compared to APP (-/-) mice. Finally, we show that sAPPα is a more potent enhancer of proliferation than the mutant sAPPβSwe, a product of aberrant cleavage in FAD. These results uncover a physiological role for sAPPα and suggest a potential mechanism underlying neurogenic impairments in Alzheimer's disease.

Disclosures: M. Demars, None; **O. Lazarov**, None.

Nanosymposium

829. APP Actions on Nerve Cells Development and Survival

Location: Room 23A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 829.6

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH 5RO1AG014713

NIH 5P01AG15379

Title: Contactin-2 enhances processing of the amyloid precursor protein and the β 2 subunit of the voltage-gated sodium channel

Authors: *V. GAUTAM, D. Y. KIM, C. C. SACHSE, M. T. GERSBACHER, D. M. KOVACS;
Neurobio. of Dis. Laboratory, Genet. and Aging Res. Unit, Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: Accumulation of the β amyloid peptide (Aβ) is associated with the pathogenesis of Alzheimer disease (AD). Aβ derives from a larger precursor, the amyloid precursor protein (APP), cleaved by BACE1 and γ-secretase. Recently, it has been shown that soluble Contactin-2 triggers the generation of the intracellular domain of APP by enhancing APP processing. Contactin-2 is a GPI-anchored protein that belongs to the F3 class of cell-adhesion molecules. Here, we asked whether the membrane-bound form of Contactin also enhances APP processing, given that both proteins colocalize to lipid rafts. To confirm the published report, we first generated stable CHO cells expressing a GPI-anchor deleted form of Contactin-2. These cells secreted high levels of Contactin-2 in the media. On stable co-expression of the secretory form of Contactin-2 with APP, we observed enhanced processing of APP leading to increased C-terminal fragment (CTF) generation. These results nicely agree with the published report. Next, we generated stable CHO cells expressing the membrane-bound form of Contactin-2. Surprisingly, coexpression of this GPI-anchored Contactin-2 along with APP did not significantly alter APP levels or processing. We did not detect any significant changes in APP-CTF levels. Recently, our lab has shown that β_2 subunit of the voltage-gated sodium channel ($Na_v\beta_2$) is also a target for BACE1 and γ-secretase and its processing is enhanced in human brains affected by AD. Therefore, we also asked whether secreted Contactin-2 affects processing of $Na_v\beta_2$. We generated CHO cells stably co-expressing secreted or GPI-anchored forms of Contactin-2 and $Na_v\beta_2$. Similarly to APP, secreted Contactin-2 also increases the processing of $Na_v\beta_2$. However, GPI-anchored Contactin-2 did not have any effect on $Na_v\beta_2$. Furthermore, coimmunoprecipitation experiments revealed that secreted Contactin-2 interacts with both APP and $Na_v\beta_2$ as we successfully pulled down secreted Contactin-2 with APP and $Na_v\beta_2$. The altered processing of both APP and $Na_v\beta_2$ by secreted Contactin-2 was specific to these BACE1 and γ-secretase substrates, as we did not observe any significant increase in the processing of Nectin when we stably coexpressed it along with secreted Contactin-2. Nectin is not cleaved by BACE1, but is a substrate for γ-secretase cleavage. Taken together, our data shows that the secreted, but not the membrane-bound, form of Contactin-2 enhances BACE1-mediated processing of APP and $Na_v\beta_2$, likely by interacting with these proteins as an extracellular ligand. Given that elevated BACE1 activity in a subset of AD patients increases APP and $Na_v\beta_2$ processing, Contactin-2 may play a role in the pathogenesis of AD.

Disclosures: V. Gautam, None; **D.Y. kim**, None; **C.C. Sachse**, None; **M.T. Gersbacher**, None; **D.M. Kovacs**, None.

Nanosymposium

829. APP Actions on Nerve Cells Development and Survival

Location: Room 23A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 829.7

Topic: C.02. Alzheimer's disease and other dementias

Support: AFAR 208065

NIH DP2 OD0006662

Title: Amyloid precursor protein is important for maintaining the integrity of mouse olfactory circuit

Authors: *L. CAO, G. T. RICKENBACHER, S. RODRIGUEZ, E. G. BENZ, M. W. ALBERS;
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Abstract: The amyloid precursor protein (APP) plays a critical role in Alzheimer's disease pathogenesis, but the physiological function of APP remains elusive. Mice with an APP null mutation have altered synaptic transmission in the hippocampus and at neuromuscular junctions. Furthermore, Wang et al., (*J Neurosci*, 29(35):10788-801, 2009) provided evidence that APP promotes synaptogenesis via trans-synaptic interaction at neuromuscular junctions. To investigate the role of APP in the formation and maintenance of the connectivity of a neural circuit, we have examined the integrity of mouse peripheral olfactory neural circuit in an APP null line. The selection and expression of one olfactory receptor (OR) gene by olfactory sensory neurons (OSNs) determines the sites of OSN axonal projection on the surface of the olfactory bulb. In wild type mice, a single locus on the medial and a single locus on the lateral side are the sites of projection of OSNs expressing the same OR. By contrast, OSNs bearing the same OR target several loci on both the medial and the lateral sides of the olfactory bulb in APP null mice. This phenotype was present for several different ORs. APP is cleaved sequentially by beta secretase (BACE1) and gamma secretase to shed the amino-terminus of APP and generate the amyloidogenic peptide Aβ, respectively. A recent study demonstrated that the cleaved amino-terminus of APP binds to death receptor 6 and activates caspase 6 in spinal cord neurons when cultured under limited trophic factor conditions (Nikolaev et al., *Nature,* 457(7232):981-9, 2009). We have examined the precision of OSN targeting in a BACE1 null mouse line. Axons bearing the same OR target to many ectopic loci as well. Accompanying this OSN axon mistargeting phenotype, the architecture of the olfactory bulbs is disturbed in both APP and BACE1 null mice. We also find that markers of cell death and neurogenesis are increased, and the absolute numbers of subpopulations of OSNs expressing specific ORs are reduced in both null lines. Moreover, a marker for odor-evoked activity is reduced in both null lines. Interestingly, we observed these defects in APP+/- but not in BACE+/- mice. Together

these findings suggest that BACE1-dependent cleavage products of APP acting directly to regulate OSN axon targeting, neuronal activity, and cell survival in olfactory system.

Disclosures: L. Cao, None; **G.T. Rickenbacher**, None; **S. Rodriguez**, None; **E.G. Benz**, None; **M.W. Albers**, None.

Nanosymposium

829. APP Actions on Nerve Cells Development and Survival

Location: Room 23A

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 829.8

Topic: C.02. Alzheimer's disease and other dementias

Support: NIH grant GM068596-05S1

Title: Alzheimer’s amyloid-β precursor protein (APP) is a sensor that detects variations in kinesin-1 levels, and signals to the nucleus abnormalities in axonal transport

Authors: *V. MURESAN, Z. MURESAN;
Pharmacol. & Physiol., UMDNJ-New Jersey Med. Sch., NEWARK, NJ

Abstract: The function of APP in neurons, and its metabolism leading to the production of amyloid-β (Aβ), a toxic polypeptide that accumulates in the brain in Alzheimer’s disease (AD), are poorly understood. APP was proposed to modulate axonal transport by serving as linker for the microtubule motor, kinesin-1 to cargo vesicles. We showed that, of the total cellular APP, only the small fraction that is phosphorylated at Thr668 (pAPP) recruits kinesin-1 in vivo. It does so indirectly, via the adaptor protein JNK-interacting protein-1. How the more abundant, non-phosphorylated APP is transported into the neurites remains unknown. Using the CNS-derived neuronal cell line CAD, we show that part of non-phosphorylated APP recruits kinesin-1 via Fe65, a kinesin-1-interacting adaptor protein that has higher affinity for binding to non-phosphorylated APP versus pAPP. Indeed, silencing expression of Fe65 with siRNA decreases the amount of APP that is transported into neurites. Most importantly, we show that, although not involved in the transport of pAPP, Fe65 participates in the phosphorylation of APP by JNK, since the knock down of Fe65 decreases, while moderate overexpression increases the amount of pAPP. Surprisingly, phosphorylation of APP also requires kinesin-1, but not the motor activity of kinesin-1. We show that kinesin-1, with its two light chains (KLCs), acts as divalent scaffold that simultaneously recruits the JNK kinase and the APP:Fe65 complex, thus enabling the phosphorylation of APP. Downregulation of kinesin-1 leads to

diminished phosphorylation of APP, while the moderate expression of KLCs increases the phosphorylation of APP. This phosphorylation selectively modifies the immature APP, suggesting that it targets the fraction of APP present in pre-Golgi compartments, including the endoplasmic reticulum (ER). The phosphorylation of APP decreases its affinity for Fe65, while increasing its susceptibility to cleavage by secretases. As a result, Fe65 is released from pAPP, and translocates into the nucleus; at the same time, Aβ is generated in the ER, a highly oxidizing environment that could trigger the oligomerization of Aβ. These results indicate that the over-accumulation of kinesin-1 in the cell body triggers a cascade of events leading to hyperphosphorylation of APP and signaling by Fe65 to the nucleus. We propose that the APP:Fe65 complex is a sensor in the ER for detecting the increased level of kinesin-1 caused by halted axonal transport, which signals to the nucleus that a halt in transport has occurred, while simultaneously generating an oligomerization-prone pool of Aβ in the ER. Our novel hypothesis could thus explain the pathogenic mechanism in AD.

Disclosures: V. Muresan, None; **Z. Muresan**, None.

Nanosymposium

830. Neuroinflammation

Location: Room 31C

Time: Wednesday, November 17, 2010, 1:00 pm - 2:30 pm

Program Number: 830.1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH AG07684

NIH AG028303

Title: Reproductive aging modulates the neural inflammatory response induced by acute exposure to LPS

Authors: S. BAKE[1], *F. SOHRABJI[2];
[1]Women's Hlth. in Neurosci Program/ Neurosci & Exp Ther, Texas A&M Syst. HSC, College Station, TX; [2]Women's Hlth. in Neurosci Program, Texas A&M Syst. HSC, COLLEGE STA, TX

Abstract: Neural inflammation plays a key role in the onset and progression of neurodegenerative diseases. Increased inflammatory cytokines in systemic circulation increases the risk for neural inflammation. Previous studies from this laboratory have

shown that the blood brain barrier is more permeable in the hippocampus of reproductive senescent females (Bake and Sohrabji, 2004; Bake et al., 2009). We hypothesize that blood brain barrier permeability in the aging brain may increase the probability of neural inflammation resulting from systemic injury or inflammation. To test this hypothesis, senescent and mature adult females were ovariectomized, replaced with estrogen/placebo for 3 weeks . Animals were given saline or LPS (250ug/kg bwt, ip.) and sacrificed 3 h later. Hippocampii were dissected and collected for protein and microvessel extraction. Protein lysates were analyzed for inflammatory cytokines/chemokines using the Milliplex cytokine kit and microvessels were examined for tight junction protein by immunohistochemistry. As expected, LPS increased pro-inflammatory cytokines such as IL1-beta, IL-4, IL-6, and chemokines like MCP-1, GRO-KC, RANTES in both senescent and mature adult hippocampus. However,IL-10, the anti-inflammatory cytokine was increased only in mature adults. Microvessels from the hippocampus were analyzed for the expression of occludin, a tight junction protein. Occludin immunostaining was evaluated for junctional localization of the protein by a rater blind to the treatment conditions. Occludin immunolabel was seen in all groups and was not affected by LPS in most groups, except the estrogen treated mature adults. In this group, junctional occludin was significantly enhanced by LPS, suggesting that estrogen may mobilize junctional proteins to the membrane in an effort to strengthen or preserve barrier function. The present study shows that the mature and aging brain mounts a robust response to systemic LPS and further suggests that compensatory responses such as increased IL-10 and enhanced junctional localization of occuldin, are better maintained in younger animals.

Disclosures: S. Bake: None. **F. Sohrabji:** Consultant/Advisory Board; Advisory Council for Research on Women's Health.

Nanosymposium

830. Neuroinflammation

Location: Room 31C

Time: Wednesday, November 17, 2010, 1:00 pm - 2:30 pm

Program Number: 830.2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DGAPA-IN215408 to S.R-A

Title: Effects of oxidative stress state on inflammation response, insulin growth factor and neurogenesis loss in hippocampus of rats chronically exposed to low doses of ozone

Authors: *S. L. RIVAS-ARANCIBIA, C. R. GALLEGOS-RIOS, O. A. RAMIREZ-

TERAN, D. RODRIGUEZ-ORTIZ, R. GUEVARA-GUZMAN, E. RODRIGUEZ-MARTINEZ;
Facultad De Medicina, UNAM, 04510 Mexico DF, Mexico

Abstract: The objective of this work was to study the effect of oxidative stress on dysregulation of inflammation response, associated with insulin growth factor, insulin growth factor receptor changes, and loss of neurogenesis process in rats chronically exposed to low doses of ozone. Fifty male Wistar rats, with free access to water and food, were randomly divided in 5 groups (n= 10): 1) Control group, exposed to ozone free air stream; 2) 7 days of ozone exposure; 3) 15 days of ozone exposure; 4) 30 days of ozone exposure; and, 5) 60 days of ozone exposure. Ozone exposure was carried out daily for 4 hours at a 0.25 ppm dose. Two hours after the last ozone exposure, the hippocampus of five animals of each group was processed to carry out the western blot technique. Five remaining animals of each group were anesthetised and perfunded to carry out immunohistochemistry technique against tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NFκB), cytochrome C (Cyt C), insulin-like growth factor 1 (IGF-1), insulin-like growth factor 1 receptor (IGF-1R) and doublecortin (DCX). Results show a significantly TNF-α and COX-2 increases during all treatments. We observed that the number of immunoreactive cells to NFkB and IGF-1 increased at 7 and 15 days and decreased at 30 and 60 days. However, the number of immunoreactive cells IGF-1R showed a significantly increases in all treatments compared with control group. Increases to DXC at 7, 15 and 30 day accompanied by a loss of cellular morphology of neuroblasts; and decrease in the number of cells at 60 days. With these results, we can conclude that chronic exposure to low doses of ozone *per se*, causes an oxidative stress state, which induces dysregulation of inflammation response, progressive decreases in IGF-1, up regulation IGF-1R associate to neuroblast death and degenerative changes in neurons.
Supported by DGAPA-IN215408 to S.R-A

Disclosures: S.L. Rivas-Arancibia, None; **C.R. Gallegos-Rios**, None; **O.A. Ramirez-Teran**, None; **D. Rodriguez-Ortiz**, None; **R. Guevara-Guzman**, None; **E. Rodriguez-Martinez**, None.

Nanosymposium

830. Neuroinflammation

Location: Room 31C

Time: Wednesday, November 17, 2010, 1:00 pm - 2:30 pm

Program Number: 830.3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 EY09024

R01 NS046994

P01 ES016738

P30 NS057096

Title: Activation of matrix metalloproteinases (mmps) by s-nitrosylation in hiv-associated neurocognitive disorders

Authors: *R. RUSSO[1,2], Z. GU[2,3], M. SEKI[2], S. BANERJEE[2], S. A. LIPTON[2,4,5]; [1]Dept. of Pharmaco-Biology, Univ. of Calabria, Fac. of Pharmacy, Nutritional and Hlth. Sci., Arcavacata Di Rende, Cosenza, Italy; [2]Ctr. for Neuroscience, Aging, and Stem Cell Research, Sanford-Burnham Med. Res. Inst., La Jolla, CA; [3]Dept. of Pathology & Anatom. Sci., Univ. of Missouri-Columbia Sch. of Med., Columbia, MO; [4]Departments of Neurosci., [5]Departments of Psychiatry, Univ. of California at San Diego, La Jolla, CA

Abstract: Matrix metalloproteinases (MMPs) represent a family of zinc dependent proteolityc enzymes that are physiological mediators of extracellular matrix (ECM) degradation and turnover. A role for MMPs has been suggested in the pathogenesis of several inflammatory and neurodegenerative disorders. S-Nitrosylation, or transfer of an NO group to the thiol of a critical cysteine residue to form a SNO-protein, is a key posttranslational modification that regulates protein function. We have previously demonstrated that S-nitrosylation and subsequent further oxidation activates MMP-9 during cerebral ischemia, thus contributing to neuronal apoptosis (Gu et al., Science 2002;297:1186-1190). In the present study, we extend these studies to HIV-associated neurocognitive disorders (HAND). A significant proportion of HIV-1 infected individuals suffer from neurological complications, characterized by motor dysfunction and cognitive impairment, recently designated HAND. HIV-associated dementia (HAD) is the most severe manifestation of HAND. MMP-2 (gelatinase A) and MMP-9 (gelatinase-B) are both upregulated in the cerebrospinal fluid of patients with HAD and correlate with the degree of cognitive dysfunction (Conant et al., Ann. Neurol. 1999;46: 391-398; Liuzzi et al., J. Neurovirol. 2000;6:156-163; Suryadevara et al., Glia 2003;44:47-56). However, the mechanism(s) of MMP dysregulation in HAD is not well established. Here, we report that MMPs are upregulated in the brains of human patients with HAD, and this proteolytic activity co-localizes with increased immunoreactivity for S-nitrosylated (SNO)-protein. Additionally, we show that the HIV-envelope glycoprotein gp120, which is associated with HAD pathogenesis, activates nitric oxide synthase and MMPs in the retina after intravitreal injection as well as in the brain of HIV/gp120 transgenic mice. We therefore suggest that S-nitrosylation may be a key regulatory mechanism for MMP activation during HIV-associated neurodegeneration.

Disclosures: R. Russo, None; **Z. Gu**, None; **M. Seki**, None; **S. Banerjee**, None; **S.A. Lipton**, None.

Nanosymposium

830. Neuroinflammation

Location: Room 31C

Time: Wednesday, November 17, 2010, 1:00 pm - 2:30 pm

Program Number: 830.4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH AG23708, AG20603

Biogen Idec

Title: Macrophage colony-stimulating factor (M-CSF) receptor is expressed in injured neurons and mediates survival and protection

Authors: *J. LUO[1], F. PICKFORD[1], M. BRITSCHGI[1], R. NARASIMHAN[1], H. ALABSI[1], S. VILLEDA[1], Z. DING[1], H. ZHANG[1], M. WILSON[1], G. WONG[2], J. RELTON[2], J. W. POLLARD[3], T. WYSS-CORAY[1,4];
[1]Neurol., Stanford Univ., PALO ALTO, CA; [2]Biogen Idec, Cambridge, MA; [3]Albert Einstein Col. of Med., Bronx, NY; [4]GRECC, VA Palo Alto Hlth. Care Syst., Palo Alto, CA

Abstract: Macrophage colony-stimulating factor (M-CSF) is a key regulator of the monocyte-macrophage lineage. M-CSF levels are reduced in Alzheimer disease (AD) plasma and systemic administration of M-CSF ameliorates AD-like disease in mouse models, but how it exerts this effect is unclear. Using lineage tracing experiments we discovered that a small number of neurons in the hippocampus and cortex express the M-CSF receptor under physiological conditions, and that excitotoxic injury results in a dramatic increase in neuronal receptor expression. M-CSF strongly reduced excitotoxin-induced neuronal cell loss and gliosis in wildtype mice when administered systemically before or up to 6 h after injury. These effects were accompanied by activation of cAMP responsive element binding protein (CREB) signaling in neurons rather than in microglia. Selective deletion of the M-CSF receptor in neurons exacerbated excitotoxin-induced death and neurodegeneration. We conclude that M-CSF is a powerful neuroprotective factor signaling via neuronal receptors in the injured brain.

Disclosures: J. Luo, None; **F. Pickford**, None; **M. Britschgi**, None; **R. Narasimhan**, None; **H. Alabsi**, None; **S. Villeda**, None; **Z. Ding**, None; **H. Zhang**, None; **M. Wilson**, None; **T. Wyss-Coray**, NIH, Biogen Idec, Research Grant; **G. Wong**, Biogen Idec, Employment; **J. Relton**, Biogen Idec, Employment; **J.W. Pollard**, None.

Nanosymposium

830. Neuroinflammation

Location: Room 31C

Time: Wednesday, November 17, 2010, 1:00 pm - 2:30 pm

Program Number: 830.5

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Chronic exposure of SiO2 nanoparticles activates astrocytes and induces myelin degradation in selective brain areas in the rat

Authors: *H. C. PANT[1], A. SHARMA[2], H. S. SHARMA[2];
[1]LNC NINDS, NIH, BETHESDA, MD; [2]Uppsala Univ., Uppsala, Sweden

Abstract: Silica dust (SiO2) exposure in desert environments is a common manifestation of human ill health. However, effects of SiO2 exposure on central nervous system (CNS) dysfunction are still not well known. Previous reports from our laboratory showed that chronic exposure of SiO2 in rats (20-30 nm or 50-60 nm, in separate groups, 50 mg/kg, i.p.) once daily for 7 days) lead to alterations in sensory motor and cognitive dysfunction in rats. These rats were often more prone to trauma or hyperthermia induced brain pathology than saline treated animals. Thus, a possibility exists that SiO2 exposure could alter the microenvironment of the CNS function. Since glial cells effectively regulate the microenvironment of the CNS, in this study we examined the effects of chronic SiO2 exposure on astrocytic activation in rats. In addition, alterations in axonal function following SiO2 exposure were evaluated using structural changes in the myelin. Male Sprague Dawley rats (250 to 300 g) were administered SiO2 (20-30 nm or 50-60 nm, in separate groups, 50 mg/kg, i.p.) once daily for 7 days. On the 8th day the animals were tested for behavioral functions on Rota Rod, Inclined Plane angle test, Grid walking test as well as gait disturbances. The saline treated rats were used as controls. After these behavioral tests, the animals were anesthetized with Equithesin (3 ml/kg, i.p.) and their brains were perfused transcardially with 4 % buffered neutral formalin proceeded with a brief saline rinse. After perfusion the brains and spinal cord were dissected out and small tissue pieces from different brain regions were embedded in paraffin. About 3-µm thick sections were cut and immunostianed with gilal fibrillary acidic protein (GFAP), myelin basic protein (MBP) and albumin immunoreactivity. In addition, luxol fast blue (LFB) and Nissl staining was done using standard histopathological techniques. Animals that received SiO2 showed abnormal behavioral functions as compared to saline treated rats. This effect was most pronounced in rats that received 20-30 nm SiO2 exposure. A massive upregulation of GFAP was seen in the cerebral cortex followed by hippocampus,

cerebellum, thalamus, hypothalamus, brains tem and spinal cord. In these areas damage to myelin was evident as seen using MBP degradation or loss of LFB staining. Leakage of albumin closely corresponded activation of GFAP and loss of MBP immunostaining. Several distorted neurons were seen in these areas in SiO2 treated rats. These pathological effects were most prominent in rats that received 20-30 nm SiO2 treatment. These observations are the first to show that SiO2 dependent on its size could induce profound neurotoxicity in the CNS.

Disclosures: H.C. Pant, None; **A. Sharma**, None; **H.S. Sharma**, None.

Nanosymposium

830. Neuroinflammation

Location: Room 31C

Time: Wednesday, November 17, 2010, 1:00 pm - 2:30 pm

Program Number: 830.6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 NS41858

R01 NS61642

R21 MH83525

P20RR15635

P01NS43985

Title: IL-1β and/or TNF-α induce neurotoxicity through glutamate production: A potential role for neuronal glutaminase

Authors: L. YE, *C. TIAN, Y. HUANG, J. C. ZHENG;
UNMC, OMAHA, NE

Abstract: Cytokines (such as IL-1β and TNF-α) are primary mediators of neuroinflammation during neurodegenerative diseases. Although the mechanism of inflammation-induced neuronal injury remains unclear, excess glutamate and its related excitotoxicity may contribute to the neurotoxicity. Phosphate-activated glutaminase (PAG), the main enzyme responsible for glutamate production in neurons, may play a vital role in this process. We hypothesize that IL-1β and/or TNF-α stimulation regulates

neuronal glutaminase expression, leading to excess levels of glutamate and excitotoxic neuronal death. Through qRT-PCR analysis and Western blotting, we have found that treatment with IL-1β and/or TNF-α significantly upregulated the kidney-type glutaminase (KGA), a glutaminase isoform, in primary human neurons. The increase of neuronal glutaminase by IL-1β and/or TNF-α was also confirmed by immunocytochemistry. The increase of glutaminase was associated with a reduction of MAP-2 staining, a decrease of neuronal number, and damage of neuronal dendrites. Furthermore, glutamate levels were significantly higher in rat cortical neuron cultures following IL-1β and/or TNF-α treatment, as measured by reversed phase-high performance liquid chromatography. NMDA receptor antagonist, MK-801, pretreatment blocked the cytokine-induced glutamate production and alleviated the neurotoxicity. We conclude that both IL-1β and TNF-α induce neuronal glutaminase, increase glutamate production, and subsequently result in neurotoxicity through the NMDA receptor. The identification of glutaminase as a pathogenic factor during neuroinflammation may provide a novel therapeutic target for the treatment of neurodegenerative diseases.

Disclosures: L. Ye, None; **C. Tian**, None; **J.C. Zheng**, None; **Y. Huang**, None.

Nanosymposium

831. Genetics and Brain Imaging in Psychiatric Illness

Location: Room 4

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 831.1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH grant R01 MH052857 (to TDC)

gift to the UCLA Foundation from the International Mental Health Research Organization (IMHRO)

Title: Genome-wide association study of brain phenotypes in Swedish twin cohort

Authors: *E. S. LUTKENHOFF[1], C. M. HULTMAN[2], P. LICHTENSTEIN[2], T. D. CANNON[1];
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Abstract: This study aims to discover genetic determinants of brain structure variation and susceptibility to bipolar disorder and schizophrenia. We used a genome-wide

association study (GWAS) design to associate one million single nucleotide polymorphisms (SNPs) with neural phenotypes measured by structural magnetic resonance imaging. These phenotypes included gray and white matter volume normalized to intracranial volume. Though data collection is on-going, the sample used in this analysis was composed of over one hundred twin pairs ascertained from the Swedish Twin Registry, including healthy pairs and pairs discordant for schizophrenia or bipolar disorder. Genotyping was done on the Illuminex Human Omni1 array. The genome-wide association analysis was done within the PLINK software package (PLINK v1.07, Shaun Purcell, http://pngu.mgh.harvard.edu/purcell/plink/). The analysis used linear and logistic regression models that allowed for age and sex covariates when testing both quantitative trait and disease trait SNP associations and accounted for genetic similarity among co-twins. In addition, several methods of multiple testing correction were applied to determine valid genome-wide significance. Intracranial and brain volume were both associated with several SNPs on chromosome X, 17, and 8 at a genome-wide significance level across diagnosis groups. Preliminary analyses suggest that several SNPs on chromosome 11 are associated with familial loading for severe psychiatric illness, but no SNPs were associated with either diagnostic category when considered separately in case-control comparisons at a genome-wide significance level. These results suggest several novel genetic loci associated with brain structure. Future analyses will use voxel-wise methods to refine the localization of the genetic signal to specific brain regions and, with expanded sample sizes, will examine the potential relevance of the brain-associated SNPs to susceptibility to schizophrenia and bipolar disorder.

Disclosures: E.S. Lutkenhoff, None; **C.M. Hultman**, None; **P. Lichtenstein**, None; **T.D. Cannon**, None.

Nanosymposium

831. Genetics and Brain Imaging in Psychiatric Illness

Location: Room 4

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 831.2

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant P20, RC1, RO1, R21

Stanley Medical Research Institute

NARSAD

RUSK

Title: DISC1 Leu607Phe rare variant in schizophrenia and altered inflammatory/immune responses against Toxoplasma gondii infection: Possible role of genetic susceptibility in glial function

Authors: *S.-I. KANO[1], C. HODGKINSON[2], L. JONES-BRANDO[3], S. EASTWOOD[4], K. ISHIZUKA[1], M. NIWA[1], F. DUCCI[2], D. CAYCEDO[2], E. HEINZ[2], P. DEROSSE[5], K. BURDICK[5], T. LENCZ[5], E. NEWMAN[2], J. KANE[5], A. ROY[6], A. MALHOTRA[5], D. GOLDMAN[2], P. HARRISON[4], R. YOLKEN[3], A. SAWA[1];
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Abstract: Schizophrenia is thought to involve gene-environment interactions. Many epidemiological studies show that elevated level of serum anti-*Toxoplasma gondii* (*T. gondii*) antibodies is observed in schizophrenia. However, genetic risk variants associated with schizophrenia susceptibility have not been linked to *Toxoplasma*-related inflammatory/immune responses. Here we report that rs6675281 SNP in *Disrupted in schizophrenia 1 (DISC1)* gene that produces Leu607Phe missense mutation is associated with elevated levels of serum anti-*T. gondii* antibodies. Interestingly, the same rs6675281 SNP is also associated with schizophrenia in African Americans. Other schizophrenia-associated SNPs examined were not related to seropositivity for *T. gondii*. We found that DISC1 Phe607 mutant protein had altered nuclear localization. Notably, cells derived from subjects with DISC1 Phe607 rare variant showed differential gene expression profiles in immune- and stress- related genes following *T. gondii* infection *in vitro,* when compared to cells from subjects with DISC1 Leu607 variant. Detailed analysis of cellular response of DISC1 Phe607 mutant cells (human subject-derived cells and rodent glial cells) in *T. gond*ii infection is in progress. These findings suggest an unexpected role for a schizophrenia-associated *DISC1* SNP in host-parasite interactions against *T. gondii* infection.

Disclosures: S. Kano, None; **C. Hodgkinson**, None; **L. Jones-Brando**, None; **S. Eastwood**, None; **K. Ishizuka**, None; **M. Niwa**, None; **F. Ducci**, None; **D. Caycedo**, None; **E. Heinz**, None; **P. DeRosse**, None; **K. Burdick**, None; **T. Lencz**, None; **E. Newman**, None; **J. Kane**, None; **A. Roy**, None; **A. Malhotra**, None; **D. Goldman**, None; **P. Harrison**, None; **R. Yolken**, None; **A. Sawa**, None.

Nanosymposium

831. Genetics and Brain Imaging in Psychiatric Illness

Location: Room 4

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 831.3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant 1RO1MH08373301

NIH Grant F32MH084528

NIH grant 5RO1MH057483

Title: Neurodevelopmental role for miR-132 in schizophrenia

Authors: B. H. MILLER[1], Z. ZEIER[1], L. XI[2], T. A. LANZ[2], S. DENG[2], D. WILLOUGHBY[3], P. J. KENNY[4], J. D. ELSWORTH[5], M. LAWRENCE[6], R. H. ROTH[5], R. J. KLEIMAN[2], *C. R. WAHLESTEDT[1];
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Abstract: Schizophrenia is characterized by a complex combination of affective, cognitive, neuromorphological, and molecular abnormalities that are thought to have a neurodevelopmental origin. MicroRNAs (miRNAs) are small non-protein coding RNA sequences that play an important gene regulatory role in both neurodevelopment and adult neuronal processes by coordinating the activity of multiple genes within biological networks. In the present work, we examined the expression of 854 miRNAs in dorsolateral prefrontal cortex tissue from a large sample of control, schizophrenic, and bipolar patients. The brain-enriched miRNA miR-132 was significantly downregulated in both a discovery cohort and a second case/control population, indicating that miR-132 dysregulation is a common molecular characteristic of schizophrenia. MiR-132 expression is induced by CREB signaling, has a developmental expression pattern that overlaps with critical neurodevelopmental processes during the adolescent period, and regulates NMDA signaling and activity-dependent neurite outgrowth. Our data suggest that miR-132 dysfunction, and abnormal expression of miR-132 protein-coding targets, may underlie a number of the neurodevelopmental and neuromorphological pathologies characteristic of schizophrenia.

Disclosures: B.H. Miller: Research Grant; F32MH084528. **Z. Zeier:** None. **L. Xi:** Employment; Pfizer Global Research. **T.A. Lanz:** Pfizer Global Research. **S. Deng:** Pfizer Global Research. **D. Willoughby:** Ownership Interest; Ocean Ridge Biosciences. **P.J. Kenny:** Research Grant; 1RO1MH08373301. **J.D. Elsworth:** None. **M. Lawrence:** Consultant/Advisory Board; RxGen. **R.H. Roth:** Research Grant; 5RO1MH057483. **R.J.**

Kleiman: Employment; Pfizer Global Research. **C.R. Wahlestedt:** Research Grant; 1RO1MH08373301. Ownership Interest; CURNA.

Nanosymposium

831. Genetics and Brain Imaging in Psychiatric Illness

Location: Room 4

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 831.4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 5RC1MH090912

5R01MH064498

Title: Using simultaneous transcranial magnetic stimulation and functional magnetic resonance imaging to probe the integrity of cortico-thalamic circuits

Authors: *Y. GULLER[1], A. SHACKMAN[2], E. FEREDOES[3], G. TONONI[2], M. E. MEYERAND[2], B. R. POSTLE[2];
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Abstract: Disrupted cortico-thalamic circuitry is thought to be a critical component of the pathophysiology underlying schizophrenia. Task-based functional magnetic resonance imaging (fMRI) has been used to probe thalamic integrity, but can be confounded by group differences in attention and performance. Accordingly, we used transcranial magnetic stimulation (TMS) to more directly assess thalamic physiology. We applied single-pulse TMS to the motor cortex and concurrently measured thalamic blood oxygen level-dependent (BOLD) response with fMRI. This technique exploits the ability of TMS to remotely perturb the thalamus via poly-synaptic connections. We compared the thalamic and cortical hemodynamic responses elicited by TMS to those evoked by button pressing.
TMS Targeting: Just prior to the combined TMS-fMRI session, each participant's previously acquired high resolution structural MRI was used to identify the M1 hand area.
Concurrent TMS-fMRI Session: In the scanner participants completed TMS runs and button pressing runs. TMS was delivered using an MR-compatible figure 8 coil attached to a custom built plastic arm. T2*-weighted BOLD images were collected using a 3 Tesla GE750 scanner. To minimize artifact we used a custom pulse sequence that allowed TMS

to be delivered during a 230ms gap in slice acquisition. Standard image acquisition parameters and processing steps (using VoxBo and AFNI) were followed. A priori anatomical regions of interest were identified (based on structural scans) for the thalamus and central sulcus.
We conducted a comparison of the TMS-evoked thalamic hemodynamic response function (HRF) to that evoked by button press and by a sham TMS condition. The TMS and button press conditions evoked a larger thalamic response than the sham TMS condition. Visual inspection revealed that the HRFs to TMS and the BP task were similar in the thalamic and central sulcus ROIs; this similarity was most apparent for the rising edge. In the TMS condition the HRF returned to baseline faster and had a larger undershoot than in the button press task. This suggests that the blood-delivery process during TMS and the button press task occurs at a similar rate but the return of vasculature to baseline does not. The rising edge is considered a more stable response parameter than the falling edge. These results will be compared to a group of participants diagnosed with schizophrenia to evaluate the integrity of cortico-thalamic circuitry in health and mental illness.

Disclosures: Y. Guller, None; **A. Shackman**, None; **E. Feredoes**, None; **G. Tononi**, None; **M.E. Meyerand**, None; **B.R. Postle**, None.

Nanosymposium

831. Genetics and Brain Imaging in Psychiatric Illness

Location: Room 4

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 831.5

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH T32 MH019879-15 (AK)

NIH R03 DA023462-01 (JWB)

AFOSR FA9550-07-1-0454 (JWB)

a NARSAD Young Investigator Award and the Sydney R. Baer, Jr. Foundation (JWB)

Title: Impaired error-likelihood prediction in medial prefrontal cortex in schizophrenia

Authors: *A. KRAWITZ[1], T. S. BRAVER[3], D. M. BARCH[3], J. W. BROWN[2];

[1]Indiana Univ., BLOOMINGTON, IN; [2]Indiana Univ., Bloomington, IN; [3]Washington Univ., St. Louis, MO

Abstract: Patients with schizophrenia have deficits in a variety of executive control processes including working memory in dorsolateral prefrontal cortex and performance monitoring in medial prefrontal cortex (MPFC). Typically, the deficits in MPFC have been interpreted using theories of error detection or conflict monitoring that focus on the evaluation of performance. However, new work supports a unifying view of MPFC function in terms of predictions of response-outcome association followed by evaluations of those predictions. Here we investigate whether predictions of error likelihood and subsequent evaluations of error unexpectedness are impaired in MPFC in schizophrenia. We used a rapid event-related fMRI design with a modified version of the change-signal task combined with a delayed match-to-sample task. Subjects encoded a cue into working memory in order to perform the delayed match-to-sample task. The cue was also paired with high or low error-likelihood conditions in the change-signal task. The design allowed us to compare patients to controls and dissociate deficits in working memory, error detection, prediction of error likelihood (greater predictive activity for high error-likelihood, when errors and correct outcomes are both likely, than low error-likelihood, when only correct outcomes are likely), and evaluation of error unexpectedness (greater evaluative activity after error commission in the low error-likelihood condition, when errors were not expected, than in the high error-likelihood condition, when errors were expected).
Consistent with past work, patients showed poorer working memory and reduced error-detection signals in MPFC. However, the view of this error-detection deficit as solely reflecting an underlying dysfunction in performance monitoring was brought in to question by our further findings. Comparing subgroups matched on working memory, patients showed deficits in error-likelihood prediction in MPFC at the time of the predictive cue, and error-unexpectedness evaluation at the time of response. However, mediation of the evaluative error-unexpectedness signals by the predictive error-likelihood signals was intact. These findings are consistent with an underlying dysfunction in the prediction of response-outcome associations that cannot solely be explained by working memory deficits. Critically, the inaccurate predictions can lead to atypical evaluation signals despite an intact outcome evaluation mechanism in MPFC.

Disclosures: A. Krawitz, None; **T.S. Braver**, None; **D.M. Barch**, None; **J.W. Brown**, None.

Nanosymposium

831. Genetics and Brain Imaging in Psychiatric Illness

Location: Room 4

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 831.6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: K24 MH001848

R21 MH075944

Title: Hyperactivation of anterior and posterior cingulate in Bipolar I euthymic subjects with comorbid ADHD during a response inhibition task

Authors: *J. D. TOWNSEND[1], S. Y. BOOKHEIMER[2], J. J. MCGOUGH[2], T. D. MOODY[3], L. FOLAND-ROSS[4], J. FISCHER[2], L. L. ALTSHULER[2];
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Abstract: Bipolar Disorder (BP) and Attention Deficit Hyperactivity Disorder (ADHD) share some common features such as poor attention and response inhibition. BP and ADHD co-occur frequently. This the first fMRI study comparing comorbid BP/ADHD subjects to BP, ADHD and control subjects. We predicted that comorbid subjects would demonstrate the most pronounced dysfunction in anterior cingulate and dorsolateral prefrontal cortex (DLPFC) during a response inhibition task compared to other patient groups and to controls.

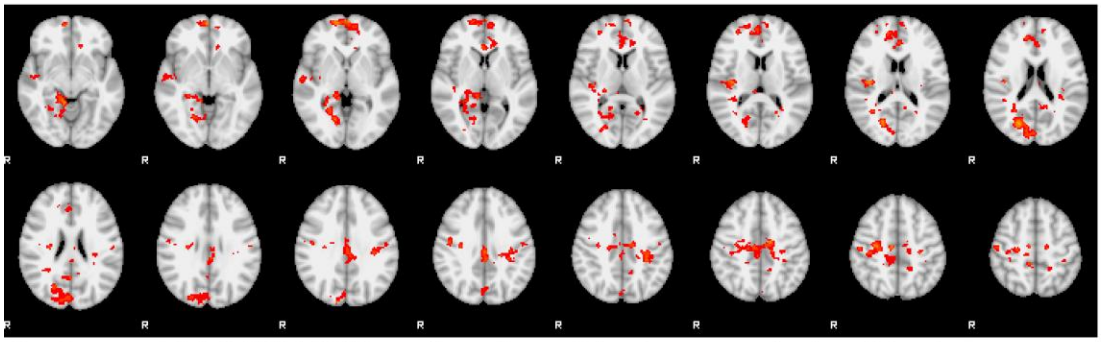
Subjects were scanned using the GoNoGo paradigm, a response inhibition task known to robustly activate frontal regions including the cingulate, frontal and subcortical regions in normal control subjects. 3T fMRI data was collected for 16 BP I euthymic subjects (12M/4F, age=38.1 ± 12.2), 16 comorbid BP I euthymic/ADHD subjects (9M/7F, age= 36.4 ± 14.1), 16 ADHD subjects (10M/6F, age= 35.7± 10.8) and 16 control subjects (10M/6F, age=36.9±14.4).

Within-group analyses revealed that control subjects activated typical response inhibition regions including bilateral inferior frontal cortex (BA47), bilateral middle frontal gyrus (BA10), left DLPFC, the cingulate, bilateral parietal regions (BA40), and subcortical regions. Euthymic subjects activated similar regions in bilateral BA47, right BA10, right DLPFC, cingulate, and right BA40. ADHD subjects showed activations in right BA47, right BA10 , right DLPFC and right BA40. Comorbid BP/ADHD euthymic subjects showed significant activations in bilateral BA47, right BA10, right DLPFC, right BA40 and cingulate.

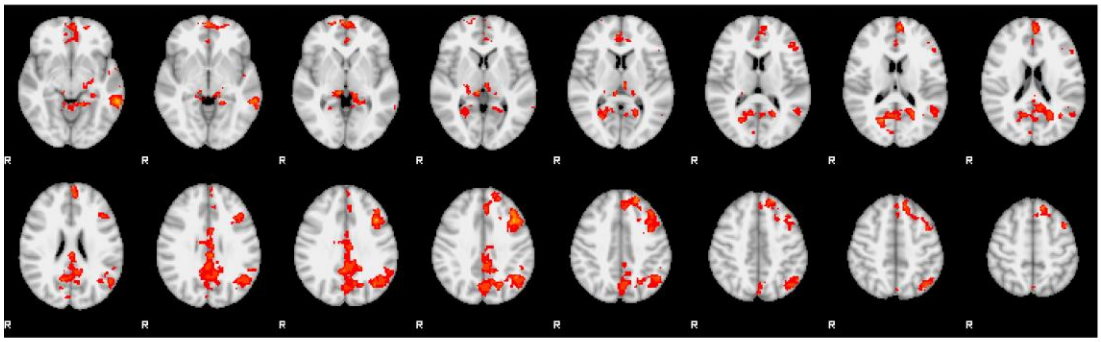
Between-group results demonstrate a striking pattern of hyperactivation of the anterior and posterior cingulate and BA10 in the BP/ADHD group compared to BP only, ADHD only and control subjects.

These data suggest that subjects comorbid for BP and ADHD demonstrate distinct hyperactivity in brain regions that serve attention and evaluative functions like responding to sensory stimuli. These dysfunctions may, in part, explain their continued poorer prognosis even when mood symptoms resolve in euthymia.

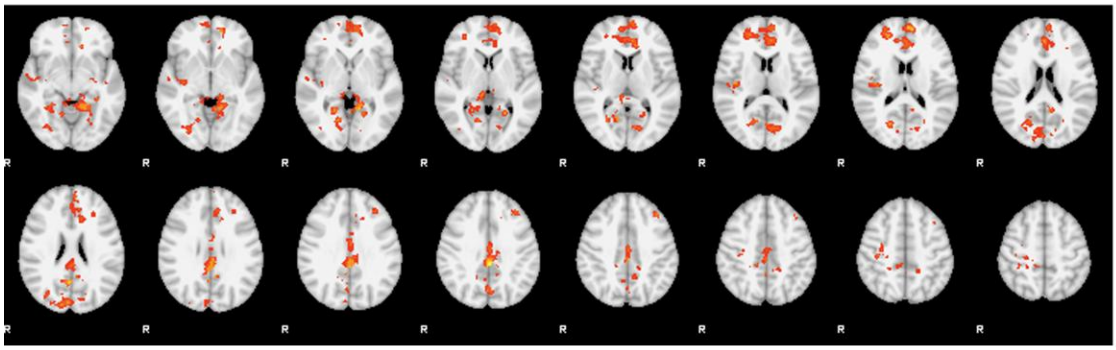
Fig. 1: Comorbid BP/ADHD subjects (n=16) showed hyperactivation in anterior and posterior cingulate compared to BP euthymic (n=16), ADHD (n=16) and normal control subjects (n=16) [Z>1.7, p=0.05, corrected).



A. Comorbid BP/ADHD euthymic subjects > control subjects



B. Comorbid BP/ADHD euthymic subjects > BP euthymic subjects



C. Comorbid BP/ADHD euthymic subjects > ADHD subjects

Disclosures: J.D. Townsend, None; **S.Y. Bookheimer**, None; **J.J. McGough**, None; **T.D. Moody**, None; **L. Foland-Ross**, None; **L.L. Altshuler**, None; **J. Fischer**, None.

Nanosymposium

831. Genetics and Brain Imaging in Psychiatric Illness

Location: Room 4

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 831.7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Intramural funding

R01 HD050735

P41 RR013642

Title: Longitudinal mapping of brain development in healthy siblings of patients with childhood-onset schizophrenia

Authors: *X. HUA[1], N. GOGTAY[3], S. LEE[2], C. BOYLE[2], R. STIDD[3], A. CHAVEZ[3], J. L. RAPOPORT[3], J. N. GIEDD[3], L. S. CLASEN[3], A. W. TOGA[2], P. M. THOMPSON[2];
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Abstract: Childhood-onset schizophrenia (COS) is a rare but severe form of mental illness, impeding a patient's ability to interpret reality and their social environment. Children with COS start to develop hallucinations and other psychotic symptoms before age thirteen. Earlier studies revealed accelerated cortical gray matter (GM) loss and slowed white matter (WM) growth rates in COS compared to normally developing children. The cause of COS is not well-understood but it likely involves a complex interplay between genetic and environmental factors. To better understand how genetic risk for COS impacts brain development in healthy siblings of COS patients, we longitudinally scanned a group of 34 healthy siblings of COS patients (mid-interval age: 17.7±5.0 years; 19 males and 15 females) and 57 controls (16.9±5.3; 28 males and 29 females). Two 3D brain MRI scans were acquired with a 1-4 year interval. All siblings and controls had no history of psychotic symptoms; all had an IQ higher than 70.
3D maps of brain tissue growth were created with tensor-based morphometry. Healthy siblings of COS patients showed slower white matter growth rates than controls, but differences were significant only for the parietal white matter, after multiple comparisons correction. A voxel-wise multiple regression model including age, sex, diagnosis, and a sex x age interaction term, revealed that age effects on growth rates differed between the COS siblings and controls, in a distributed pattern of brain regions including the temporal lobes (GM+WM, critical P=0.0002), occipital WM (critical P=0.011), parietal WM

(critical *P*=0.0036), and temporal WM (critical *P*=0.0016), corrected for multiple comparisons using the false-discovery rate procedure. Growth rates change differently with age in the two groups. Although both groups were normal and showed no psychotic symptoms, they may follow different pathways of brain development, offering insight into how COS-related genes impact brain growth in relatives of patients.
†X Hua and N Gogtay contributed equally to this work.

Disclosures: X. Hua, None; **N. Gogtay**, None; **S. Lee**, None; **C. Boyle**, None; **R. Stidd**, None; **A. Chavez**, None; **J.L. Rapoport**, None; **J.N. Giedd**, None; **L.S. Clasen**, None; **A.W. Toga**, None; **P.M. Thompson**, None.

Nanosymposium

831. Genetics and Brain Imaging in Psychiatric Illness

Location: Room 4

Time: Wednesday, November 17, 2010, 1:00 pm - 3:00 pm

Program Number: 831.8

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH MH079201, MH060451, MH067996

NIH RR017701

Singapore NMRC/TCR/003-GMS/2008

Singapore MOE T208A3126

NARSAD

The Lennon Family Foundation

Canadian CIHR CPRF

Title: A functional alternative splicing mutation in human tryptophan hydroxylase-2

Authors: *X. ZHANG[1,2], P. J. NICHOLLS[2], G. LAJE[3], T. D. SOTNIKOVA[2,4], R. R. GAINETDINOV[2,4], P. R. ALBERT[5], G. RAJKOWSKA[6], C. A. STOCKMEIER[6], D. C. STEFFENS[2], M. C. AUSTIN[6], F. J. MCMAHON[3], R. R. KRISHNAN[2], M. A. GARCIA-BLANCO[2], M. G. CARON[2];
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Abstract: The brain serotonergic (5-HT) system plays an essential role in the physiological functions of the central nervous system and dysregulation of its homeostasis has been implicated in many neuropsychiatric disorders. The tryptophan hydroxylase-2 (*TPH2*) gene is the rate-limiting enzyme in brain serotonin synthesis, thus an ideal candidate gene for understanding the role of dysregulation of brain serotonergic homeostasis. Here, we characterized a common, but functional single nucleotide polymorphism (SNP *rs1386493*) in the *TPH2* gene, which decreases efficiency of normal RNA splicing, resulting in a truncated TPH2 protein (TPH2-TR) by alternative splicing. TPH2-TR, which lacks TPH2 enzyme activity, dominant-negatively affects full-length TPH2 function, causing reduced serotonin production. The predicted mRNA for TPH2-TR is present in postmortem brain of *rs1386493* carriers. The rs13864923 variant does not appear to be overrepresented in either global or multiplex depression cohorts. However, in combination with other gene variants linked to 5-HT homeostasis, this variant may exhibit important epistatic influences.

Disclosures: X. Zhang, None; **P.J. Nicholls**, None; **G. Laje**, None; **T.D. Sotnikova**, None; **R.R. Gainetdinov**, None; **P.R. Albert**, None; **G. Rajkowska**, None; **C.A. Stockmeier**, None; **D.C. Steffens**, None; **M.C. Austin**, None; **F.J. McMahon**, None; **R.R. Krishnan**, None; **M.A. Garcia-Blanco**, None; **M.G. Caron**, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA018181 (NIDA)

Title: Do metabolites of oxycodone mediate analgesic, rewarding and aversive effects of oxycodone?

Authors: *V. R. BATRA, L. M. SCHROTT;
Pharmacology,Toxicology&Neuros, LSU Hlth. Sci. Ctr., SHREVEPORT, LA

Abstract: Oxycodone, a semi-synthetic opioid analgesic is associated with side effects of

abuse potential and nausea. Oxidative metabolism of oxycodone gives rise to noroxycodone, the major metabolite and oxymorphone, a minor metabolite. While the antinociceptive effects of these metabolites have been investigated, their potential contribution to the development of abuse potential and emetic side effects remains unknown. The present study determined whether antinociceptive doses of metabolites were involved in abuse potential as assessed by reward in a condition place preference (CPP) paradigm or emetic potential as assessed by the pica response. Oxymorphone (0.1, 0.5 and 1.0mg/kg), noroxycodone (25 and 50mg/kg) or oxycodone (15mg/kg) were administered to adult Sprague-Dawley rats. Using the hot plate, antinociception was assessed every 15 min for 4hr. Oxycodone and higher doses of oxymorphone (0.5 and 1.0mg/kg) induced maximal antinociception of about 80-100% for 3 to 3.5 hr post-treatment. In contrast, 50mg/kg of noroxycodone led to a modest antinociceptive response of approximately 50% only at 30min. Rats administered oxymorphone or noroxycodone were assessed for CPP as an index of reward. In comparison to oxycodone, no dose of oxymorphone induced CPP. Similarly, noroxycodone (50mg/kg) also did not induce CPP. This suggested that antinociceptive doses of the metabolites do not mediate the central effect of reward. We then investigated if antinociceptive doses of metabolites induce the aversive response of pica behavior. Bedding and kaolin intake were assessed as indices of pica. Rats were administered oxymorphone (0.1, 0.5 and 1.0mg/kg) or noroxycodone (50mg/kg) 1 hr prior to a 3hr pica test session. Higher doses of oxymorphone (0.5 and 1.0m/kg) induced pica response of bedding intake. Oxymorphone (0.1mg/kg) and noroxycodone (50mg/kg) did not induce the pica response. In addition, higher doses of oxymorphone also modulated kaolin intake. In conclusion, oxymorphone is an active metabolite of oxycodone that mediates the aversive but not the rewarding effects at the antinociceptive dose. Future studies will dissect out the contribution of parent drug or its active metabolite in mediating the aversive response of pica.

Disclosures: V.R. Batra, None; **L.M. Schrott**, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.2

Topic: C.17. Drugs of Abuse and Addiction

Support: DA027128

DA024030

Title: The AMPA-GluR2 subunit is co-localized with the mu-opioid receptor in dendritic compartments of central nucleus of the amygdala neurons and plays a role in opioid addictive behaviors

Authors: *M. J. GLASS[1], Q. XU[3], M. A. BECKERMAN[1], C. E. INTURRISI[2]; [1]Neurol. and Neurosci., [2]Pharmacol., Cornell Univ. - Weill Med. Col., NEW YORK, NY; [3]Dept. of Anesthesiol., UCSD, LaJolla, CA

Abstract: Glutamate signaling in the central nucleus of the amygdala (CeA) plays an important role in coordinating sensory processes, emotion, and memory with goal directed behaviors critically involved in opioid addiction. Many important actions of glutamate are mediated by activation of GluR2 subunit-expressing AMPA receptors lacking calcium permeability. However, the ultrastructural relationship between GluR2 expressing AMPA receptors and mu-opioid receptors in the mouse CeA is unknown. In addition, the role of functional CeA GluR2 in opioid addictive behaviors is unclear. These issues were addressed by a multidisciplinary strategy employing single and dual labeling immunocytochemical light and electron microscopy, conditional gene deletion, and behavioral analysis. A single labeling ultrastructural study using immunoperoxidase or immunogold markers revealed that approximately 10% of all GluR2 expressing CeA neuronal profiles were somata, whereas 80% were dendritic. Only 10% of labeled profiles were axons or axon terminals, while there was little appreciable glial labeling. Dendritic profiles expressing GluR2 were frequently contacted by unlabeled axon terminals. Approximately 50% of these formed non-synaptic appositions, 35% formed asymmetric excitatory-type contacts, and 15% formed symmetric inhibitory-type synapses. Dual labeling ultrastructural analysis showed frequent co-localization of GluR2 and the mu-opioid receptor in CeA neuronal profiles. Most of these structures were dendrites (approximately 80%), with few examples of dually labeled axon terminals (<5%). A neurotropic recombinant adeno-associated virus that expressed the enzyme Cre recombinase was microinjected into the CeA of adult floxed GluR2 mice. This treatment produced a local deletion of postsynaptic GluR2. Phenotypic analysis of bilateral CeA GluR2 knockout animals indicated that this protein was required for the normal expression of learned affective behaviors associated with morphine exposure. These results indicate that non-calcium permeable GluR2 expressing AMPA receptors and mu-opioid receptors are strategically positioned for the postsynaptic co-modulation of excitatory signaling in CeA neurons. Moreover, functional postsynaptic GluR2 plays an important role in opioid addictive behaviors.

Disclosures: M.J. Glass, None; **C.E. Inturrisi**, None; **M.A. Beckerman**, None; **Q. Xu**, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.3

Topic: C.17. Drugs of Abuse and Addiction

Support: NET grant # 79919 from CIHR

Title: Behavioral and neurochemical correlates of relapse vulnerability to cocaine and oxycodone seeking in laboratory rats

Authors: *F. LERI[1], Y. ZHOU[2], M. KREEK[2], C. ALLEN[1], A. LEVY[1,2];
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Abstract: Two studies were designed to explore behavioral and neural correlates of relapse vulnerability using an animal model whereby all subjects are exposed to identical amounts of drug. Sprague-Dawley male rats (n=96 cocaine study; n=75 oxycodone study) received a forced-swim test, tests of locomotion reactivity to a novel environment and to cocaine (15 mg/kg IP) or oxycodone (0.25 mg/kg SC), and a hot-plate test. Then, they were implanted with intravenous catheters and tested for operant responding to an audio-visual cue associated with passive intravenous infusions of cocaine (150 infusions, 0.05 mg/kg/inf) or oxycodone (500 infusions, 0.1 mg/kg/infusion). Finally, following extinction of the response, animals were tested for reinstatement induced by priming injections of cocaine (15 mg/kg IP) or oxycodone (0.25 mg/kg SC), and by foot-shock stress. Three days following the conclusion of operant testing, rats are challenged with cocaine (15 mg/kg IP) or oxycodone (0.25 mg/kg SC), and sacrificed within 90 min to collect brain and plasma.
Low and high drug seekers were identified by median split on responses emitted on reinstatement (prime + stress). In the cocaine study, high drug seekers displayed lower levels of active escape behaviors in the forced-swim test, as well as higher activity in a novel environment. In the oxycodone study, high drug seekers displayed lower pain threshold. Interestingly, high drug seekers in both studies displayed higher levels of plasma corticosterone and lower levels of orexin mRNA expression in the lateral hypothalamus after the acute drug challenge.
From these data, it is concluded that although behavioural correlates of relapse vulnerability can differ across drug classes, neurochemical adaptations that develop in particular sub-groups of individuals may be common. Why these differences develop remains unclear, although our studies in rats suggest that amount of drug exposure is not a critical factor.

Disclosures: F. Leri, None; **C. Allen**, None; **A. Levy**, None; **Y. Zhou**, None; **M. Kreek**,

None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.4

Topic: C.17. Drugs of Abuse and Addiction

Support: Centre National de la Recherche Scientifique

Institut de la Santé Et de la Recherche Médicale

Université de Strasbourg

Ministère de l'Enseignement Supérieur et de la Recherche

European Union (GENADDICT/FP6 005166)

Fondation pour la Recherche Médicale

National Institutes of Health (NIAAA AA-16658 and NIDA DA-16768)

Title: 5-HT1A receptor signaling is impaired by chronic morphine treatment in mice: an autoradiographic study

Authors: P.-E. LUTZ, C. GOELDNER, A. A. PRADHAN, *K. BEFORT, B. KIEFFER;
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Abstract: Serotonergic (5-HT) neurons regulate many physiological functions. These neurons originate in the dorsal raphe nucleus (DRN) and notably project to the medial prefrontal cortex (mPFC). The 5-HT1A receptor (5-HT1AR), a seven transmembrane domain receptor coupled to Gi/o-proteins, is strongly expressed in DRN and in mPFC. This receptor crucially regulates emotional behaviors in both humans and rodents. However little is known about the function of 5-HT1AR in anxiety or depressive states associated with opiate addiction, in particular during protracted abstinence.
Here we induced dependence to morphine (a prototypical opiate) in mice with twice daily injections of ascending doses of morphine over 6 days. Using agonist-stimulated [35S]-

GTPγS autoradiography, we investigated the effect of morphine on 5-HT1AR signaling at three time points: 2 hours (chronic group), 1 week (1-week abstinence group) or 4 weeks (4-week abstinence group) after the last morphine injection.
In the chronic group, we observed that (R)-(+)-8-Hydroxy-2-dipropylamino-tetralin hydrobromide (8-OH-DPAT)-induced [35S]-GTPγS binding was increased in mPFC, with no change in the DRN, suggesting that 5-HT1AR is hypersensitive at this time point. As 5-HT1AR activation in the mPFC inhibits 5-HT neurons, this effect could contribute to the reported decreased firing rate of 5-HT neurons during morphine withdrawal.
In the 1-week abstinence group, 8-OH-DPAT-induced [35S]-GTPγS binding was decreased selectively in the DRN while this effect was no longer detectable in the 4-week abstinence group. Desensitization of this receptor was reported in two rodent models of depression, the chronic mild stress and chronic social defeat. 5-HT1AR desensitization could therefore be implicated in emotional alterations which develop during opiate abstinence in our animal model (Goeldner at al., under revision).
Altogether we show for the first time that 5-HT1AR function is sequentially modified during morphine abstinence, first in mPFC and then in DRN. These effects could trigger neuronal adaptations at the transcriptional or circuitry levels within the 5-HT system and contribute to the incubation of behavioral deficits revealed after 4 weeks of abstinence (Goeldner et al, under revision).

Disclosures: P. Lutz, None; **K. Befort**, None; **B. Kieffer**, None; **A.A. Pradhan**, None; **C. Goeldner**, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.5

Topic: C.17. Drugs of Abuse and Addiction

Support: Department of Veterans Affairs Merit Review

Title: Induction of prefrontal cortical and amygdalar brain derived neurotrophic factor in opiate conditioned place preference in mice

Authors: *G. B. KAPLAN[1], S. C. HEINRICHS[3], K. A. LEITE-MORRIS[2], A. J. YOUNG[3], K. A. MOORE[4], M. D. GUY[3];
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Abstract: Synaptic remodeling develops with repeated exposure to drugs of abuse such as opiates. Such modifications of brain substrates as a result of repeated opiate exposure is adaptive for the organism. Brain-derived neurotropic factor (BDNF) has been shown to increase the survival of neurons, increase synaptic efficiency and alter related behavioral responses. This study examined neuroadaptive changes in BDNF after repeated morphine treatment in a model of conditioned context place preference in C57BL/6 mice. The apparatus consisted of two chambers with distinctively different flooring paired with pre-session administration of saline (10 ml/kg sc) or morphine (10 mg/kg sc). Following a preference test (Day 1, 15 min/session) mice were exposed to four morphine and four saline conditioning sessions (Days 2-9, 50 min/session). Time spent in morphine-associated (CS+) and saline-associated (CS-) chambers was measured subsequently during post-conditioning preference testing (Day 10). Brains were harvested 24 hours following the preference test (Day 11) in order to quantify a long-lasting BDNF response. Quantitative immunohistochemical studies assessed relative BDNF protein levels in various limbic and cortical regions which play a role in conditioned drug reward including prefrontal cortical cortical, striatal, and amygdalar subregions. Relative to saline-controls, morphine treatment induced a conditioned place preference (200+ sec more time spent in the CS+ compartment) which was accompanied by an increase in BDNF levels in several brain regions, notably a significant 50% increase in the infralimbic cortex, a brain region which mediates appetitive conditioning responses, and in basolateral amygdala, also important in conditioning. These findings suggest that distinct regional activation of neural BDNF accompanies the acquisition of conditioned opiate drug reward. As an mechanism in neuroplasticity, BDNF has potentially important therapeutic roles for addiction-related disorders.

Disclosures: G.B. Kaplan: Research Grant; Department of Veterans Affairs. **S.C. Heinrichs:** None. **K.A. Leite-Morris:** None. **A.J. Young:** None. **K.A. Moore:** None. **M.D. Guy:** None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH T32-MH014654-32

NIH P20-DA025995

Title: The effect of chronic morphine exposure on dendritic spine density in mouse hippocampus

Authors: L. M. AMBROSE-LANCI[1], C. SHEEKEY[1], L. HAN[1], G. G. SMITH[3], S. E. ARNOLD[1], R. J. BUONO[3], W. H. BERRETTINI[1], *T. N. FERRARO[2];
[1]Dept Psychiatry, Univ. Pennsylvania, Philadelphia, PA; [2]Dept Psychiatry, Univ. Pennsylvania, PHILADELPHIA, PA; [3]Coatesville Veteran's Affairs Med. Ctr., Coatesville, PA

Abstract: Chronic administration of mu-opioid receptor (MOR) agonists produces morphological changes in hippocampal neurons that may contribute to the development of opioid dependence (OD). The present study was designed to test the hypothesis that chronic morphine exposure causes a reduction in dendrtic spine densities in mouse hippocampus as measured using immnuohistochemical (IHC) analysis of synaptopodin (SP), an actin-binding protein associated with dendritic spines. Male B6, D2 and MOR knockout (MuKO) mice underwent surgery for s.c. implantation of a 25 mg morphine (or placebo) pellet and were euthanized 4 days later. Brains were fixed in paraformaldehyde, sliced coronally and processed for IHC. SP expression was quantified in two regions of hippocampus, CA3 and dentate gyrus (DG), in multiple sections from each mouse. Images were captured using a light microscope and analyzed using Image-Pro Plus software. Optical density values were averaged within each treatment group and normalized to sham controls. Morphine-treated B6 (n = 2) and D2 (n = 3) mice demonstrated a 25% and 29% reduction in SP expression, respectively, in CA3 compared to sham controls. Surprisingly, morphine-treated MuKO mice demonstrated a 50% increase in SP expression in CA3 compared to MuKO sham controls ($t(11) = 9.587$, $p<0.0001$; n = 6-7). We hypothesize that this effect is due to morphine action at delta or kappa opioid receptors and will attempt to distinguish subtype specific roles in future studies. In contrast to CA3, results so far suggest no differences in SP expression levels in DG between treatment groups for any strains. Overall, these preliminary data support previous studies demonstrating changes in dendritic integrity after chronic morphine exposure and begin to define the pattern of changes observed in the hippocampal complex following chronic morphine exposure via s.c. pellet in B6 and D2 mice. The relative abundance of MOR on CA3 pyramidal neurons provides an anatomical substrate for opioid-induced changes in dendritic morphology, although selective effects in CA3 may also be indirect via DG input. Recent evidence that MOR interacting proteins, such as Gpr177, which controls Wnt secretion, contribute to morphine-induced neuroplasticity suggests a possible mechanism for further investigation. Ultimately, we hope that elucidation of the functional relationship between MOR and interacting proteins, such as Gpr177, will provide new insight into the association between opioid-induced changes in neuronal morphology within the hippocampal complex and development of OD.

Disclosures: L.M. Ambrose-Lanci, None; **C. Sheekey**, None; **L. Han**, None; **G.G. Smith**, None; **S.E. Arnold**, None; **R.J. Buono**, None; **W.H. Berrettini**, None; **T.N.**

Ferraro, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.7

Topic: C.17. Drugs of Abuse and Addiction

Support: 1K12ZDH55885-01 (MWF)

DHHS/NIH/NIDA IRP (AZ, TSS)

P50DA015369 (RES)

DA020751 (ACR)

MUSC NARC Pilot Project (ACR)

Title: Persistent inflammatory pain alters heroin-seeking in rats

Authors: *M. W. FELTENSTEIN[1], A. ZAPATA[2], T. S. SHIPPENBERG[2], R. E. SEE[1], A. C. RIEGEL[1];
[1]Dept Neurosci, Med. Univ. South Carolina, CHARLESTON, SC; [2]Integrative Neurosci. Section, NIH, NIDA Intramural Res. Program, Baltimore, MD

Abstract: The mesolimbic dopamine (DA) pathway is involved in the assessment and response to salient, unexpected events, regardless of its valence (positive or negative). Addictive opiate analgesics as well as painful stimuli can activate this pathway via changes in DA inputs from the ventral tegmental area (VTA). Although interactions between opiates, pain, and the relapse to drug-seeking are predicted, it remains unclear precisely how persistent pain influences ongoing opiate self-administration and relapse. Experimentally, relapse can be studied using the extinction-reinstatement model in animals. In this model, exposure to previously drug-paired cues, stress, or a drug priming injection leads to reinstatement of extinguished drug-seeking behavior as indexed by an increase in responding on the previously drug-paired lever in the absence of primary reinforcement. Thus, the current study examined whether heroin self-administration and subsequent relapse would be altered in response to persistent inflammatory pain associated with intraplantar hindlimb injection of complete Freund's adjuvant (CFA; 25

ul). Male rats pressed a lever for heroin (0.25 ug/50 ul/infusion, IV) paired with a light+tone stimulus during 14 daily, 2 hr sessions. Following extinction of responding, the ability of heroin-paired cues, the anxiogenic a2-noradrenergic receptor antagonist, yohimbine (2.5 mg/kg, IP; alone and in combination with the heroin-paired cues), and a priming injection of heroin (0.25 mg/kg, SC) were examined for their ability to reinstate heroin-seeking. Animals received CFA or sham injection 1d prior to the beginning of heroin self-administration or reinstatement testing. Although no differences were noted during self-administration, CFA animals under both conditions showed a trend towards an overall enhancement in reinstatement. Separate electrophysiological and microdialysis data also demonstrated that persistent pain not only produced a long-lasting enhancement in VTA DA neuron firing, but also changed basal and drug-evoked DA dynamics in the nucleus accumbens. Taken together, these data imply that persistent nociceptive stimulation during opiate administration can influence the subsequent responsiveness to salient stimuli associated with relapse to drug seeking.

Disclosures: M.W. Feltenstein, None; **R.E. See**, None; **A.C. Riegel**, None; **A. Zapata**, None; **T.S. Shippenberg**, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.8

Topic: C.17. Drugs of Abuse and Addiction

Support: VA Merit Award-BLR&D

NIH grant DA027843

NIH grant DA010475

Title: Reversible inactivation of basolateral amygdala abolishes acquisition and expression of one-trial conditioned morphine reward and conditioned withdrawal from acute morphine dependence

Authors: *G. SCHULTEIS[1], D. W. CHIANG[2];
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Abstract: The basolateral amygdala (BLA) is critical for fear conditioning, and conditioned rewarding effects of drugs of abuse. Excitotoxic lesions of the BLA prior to conditioning prevent the formation of conditioned associations between discrete cues and precipitated withdrawal from chronic morphine dependence (Schulteis et al. 2000, Nature, 405, 1013). The present study dissected the role of the BLA in acquisition and/or expression of both conditioned opioid withdrawal and the acute rewarding effects of morphine. Male Wistar rats implanted with bilateral guide cannula targeting the caudal BLA received a single injection of morphine (10 mg/kg subcutaneous [SC]) followed 30 min later min confinement to a visually and texturally unique compartment of a place conditioning apparatus for 20 min; confinement to the opposite compartment after vehicle injection occurred 24 hr prior to or after this conditioning trial. Other groups received 10 mg/kg morphine followed 4 hr later by naloxone (1 mg/kg SC) and 20 min confinement to one compartment. For both conditioned place preference (CPP) to morphine and aversion (CPA) to naloxone-precipitated withdrawal paradigms, a trial preceded by vehicle in place of morphine and/or naloxone occurred 24 hr prior to or after the drug pairing. All rats were allowed to freely explore the entire apparatus 24 hr prior to the first and again 48 hr after the final conditioning trial, with the difference in time spent on the drug- or withdrawal-paired side from pre-conditioning to test trial serving as the index of CPP or CPA, respectively. A single injection of morphine produced reliable CPP (+155 $\pm$ 30 s), and a single pairing of naloxone 4 hr post-morphine produced reliable CPA (- 165 $\pm$ 37 s). Both the CPP to morphine and the CPA to precipitated withdrawal were completely reversed by bilateral infusion of lidocaine (1 µl of 4% solution) 15 min prior to either the conditioning trial or the test trial. The abolition of acquisition and expression of CPP and CPA was not due to state-dependent learning, as lidocaine given prior to both conditioning and test trials still blocked the conditioned response. Injections dorsal to the BLA into the border between caudal portion of the central amygdala and the lateral amygdala, or more dorsal still into the caudate, did not affect acquisition or expression of CPP or CPA. Therefore, the BLA is necessary for both the acquisition and expression of conditioned responses to a single bout of morphine intoxication or a single episode of precipitated withdrawal from acute opioid dependence. *Supported by VA Merit Award, NIDA grant DA010475, and the Center on Interoceptive Dysregulation of Addiction (DA027843).*

Disclosures: G. Schulteis, None; **D.W. Chiang**, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.9

Topic: C.17. Drugs of Abuse and Addiction

Support: Grants-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: BiP, an endoplasmic reticulum chaperone, plays an important role on the development of morphine tolerance

Authors: *T. AOE[1], T. DOBASHI[1], S. TANABE[1], M. YASURAOKA[1], T. YAMAMOTO[2], O. SAITO[3], S. GOTO[4], H. JIN[1];
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Abstract: Morphine is a potent analgesic, but the molecular mechanism for tolerance formation after repeated use is not fully understood. Chronic morphine tolerance may be derived from adaptations in the intracellular signal transduction of post-MOR activation. Proteins destined for secretory pathways, such as cell surface receptors including MOR, are inserted into the endoplasmic reticulum (ER), where their folding or degradation intermediaries interact with molecular chaperones. Recent studies have suggested that chronic ER stress might modulate intracellular signaling pathways, resulting in several chronic disorders, such as type II diabetes and interstitial pneumonia. Binding immunoglobulin protein (BiP) is an ER chaperone that is central to ER function. We examined knock-in mice expressing a mutant BiP with the retrieval sequence deleted in order to elucidate physiological processes that are sensitive to BiP functions. We tested the thermal antinociceptive effect of morphine in heterozygous mutant BiP mice in a hot plate test. Paw withdrawal latencies before and after a single administration of morphine were not significantly different between the wild-type and mutant BiP mice. Repeated morphine administration caused the development of morphine tolerance in the wild-type mice. The activation of glycogen synthase kinase 3b (GSK3b) was associated with morphine tolerance, since an inhibitor of GSK3b prevented it. On the other hand, the mutant BiP mice showed less morphine tolerance, and the activation of GSK3b was suppressed in their brain. These results suggest that BiP may play an important role in the development of morphine tolerance. Furthermore, we found that a chemical chaperone which improves ER protein folding capacity also attenuated the development of morphine tolerance in wild-type mice, suggesting a possible clinical application of chemical chaperones in preventing morphine tolerance.

Disclosures: T. Aoe, None; **T. Dobashi**, None; **S. Tanabe**, None; **M. Yasuraoka**, None; **T. Yamamoto**, None; **O. Saito**, None; **S. Goto**, None; **H. Jin**, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.10

Topic: C.17. Drugs of Abuse and Addiction

Support: DA 19959

Title: Opioid antagonist efficacy in morphine precipitated withdrawal

Authors: *D. M. NAVANI, P. MADIA, S. SIROHI, B. C. YOBURN;
Pharmaceut. Sci., Col. of Pharmacy, St. John's Univ., Jamaica, NY

Abstract: Opioid ligands can be classified as agonists, neutral antagonists or inverse agonists. In previous studies, we have shown that 6β-naltrexol can antagonize the precipitation of morphine withdrawal by inverse opioid agonists (e.g., naltrexone). In the present study, we examined if the effects of 6β-naltrexol on naltrexone-induced morphine withdrawal were dose-dependent and characterized the time action profile of naltrexone and 6β-naltrexol to precipitate morphine withdrawal. Mice were implanted s.c with a morphine pellet (25 mg) for 3 days, injected with saline or 6β-naltrexol (0.5-10.0 mg/kg) and 70 minutes later (time of peak effect), injected with naltrexone (0.00625-0.4mg/kg) and observed for withdrawal jumping for 15min (N=5-10/dose). Low doses of 6β-naltrexol (0.5, 1.0mg/kg) shifted the withdrawal dose response function (ED_{50}) for naltrexone to the right by 2.3- and 3.5-fold, respectively, whereas a higher 6β-naltrexol dose (2.5mg/kg) did not significantly alter the potency of naltrexone. The highest dose of 6β-naltrexol (10mg/kg) increased the potency of naltrexone. In short, as the dose of 6β-naltrexol increased, efficacy shifted from that of a neutral antagonist to an inverse agonist. In earlier studies, we informally observed that some mice injected with 6β-naltrexol continued to display withdrawal jumping well after the 15min observation period. Therefore, in the next experiment the time action profile for both naltrexone and 6β-naltrexol induced jumping was studied. Mice were implanted with a morphine pellet (25 mg) for 3 days, injected (N=9-10/dose) with 6β-naltrexol (1.0-10 mg/kg) or naltrexone (0.025-0.1mg/kg) and observed for withdrawal jumping for up to 85min. 6β-naltrexol treated mice displayed dose-dependent jumping for the full observation period; whereas naltrexone treated mice did not jump after 45min. These data were analyzed using area under the curve. Naltrexone was ≈ 67-fold more potent (EC_{50}) than 6β-naltrexol and the E_{max} for naltrexone was ≈ 2.5-fold less than that for 6β-naltrexol. These data indicate that while naltrexone retains its greater potency over a longer observation period, it produces less overall jumping due to a shorter time-action profile. Taken together, these results suggest that the efficacy of 6β-naltrexol is dose-dependent; behaving as a neutral antagonist at low doses and as an inverse agonist at higher doses. Furthermore, while 6β-naltrexol is less potent than naltrexone, 6β-naltrexol produces a

surprisingly long-lasting and robust precipitated withdrawal syndrome. This may have consequences on the potential clinical utility of 6β-naltrexol.

Disclosures: D.M. Navani, None; **P. Madia**, None; **S. Sirohi**, None; **B.C. Yoburn**, None.

Nanosymposium

832. Opioids: Neural and Behavioral Effects

Location: Room 7B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 832.11

Topic: C.17. Drugs of Abuse and Addiction

Support: DA 19959

Title: Opioid tolerance and [^{35}S]GTPγS binding in mouse spinal cord

Authors: *P. A. MADIA, B. C. YOBURN;
Pharmaceut. Sci., Col. of Pharmacy, St. John's Univ., Jamaica, NY

Abstract: Among potential substrates for opioid tolerance, are reduction in receptor density (downregulation), desensitization and uncoupling of the receptor from downstream signaling. The [^{35}S]GTPγS binding assay can be utilized to examine the mechanisms of opioid tolerance that are proximal to the receptor. In this study, agonist stimulated [^{35}S]GTPγS binding was evaluated in mouse spinal cord membranes following induction of tolerance. Mice (N=5/group) were implanted s.c. for 7 days with a morphine pellet (75 mg) or continuously infused for 7 days s.c. using minipumps with oxycodone (117 mg/kg/day); hydromorphone (32 mg/kg/day) or etorphine (250 µg/kg/day). All treatments have been shown to produce substantial analgesic tolerance in *in vivo* tests. Controls were implanted with a placebo pellet. DAMGO dose-dependently increased [^{35}S]GTPγS binding in control and treated membranes. There was no significant change following morphine, oxycodone or hydromorphone treatment in E_{max} or EC_{50} for DAMGO stimulated [^{35}S]GTPγS binding. However, following continuous treatment with etorphine, there was a decrease in the E_{max} (≈30% reduction in stimulation), but no change in the EC_{50}. Since this etorphine dose reliably downregulates µ-opioid receptor density, but morphine, oxycodone and hydromorphone do not, we hypothesized that the [^{35}S]GTPγS assay may be more sensitive to opioid tolerance based on receptor density changes than on tolerance mediated by receptor desensitization in mouse spinal cord. To test this proposal, 2 protocols that up- and downregulate opioid receptor density were

studied. Spinal cord membranes from opioid antagonist treated mice (7 day, 10mg s.c. naltrexone pellet) and mice treated acutely with the irreversible μ opioid receptor antagonist clocinnamox (0.32-12.8mg/kg) were examined for changes in DAMGO stimulated [^{35}S]GTPγS binding. Chronic naltrexone upregulates, while clocinnamox downregulates μ opioid receptor density. Naltrexone increased the [^{35}S]GTPγS E_{max} (48%) but did not change the EC_{50}. μ opioid receptor depletion following clocinnamox dose-dependently reduced the E_{max} (49 % maximal decrease) but did not change the EC_{50}. Overall, only treatments that induced receptor upregulation (naltrexone) or downregulation (etorphine, clocinnamox) produced corresponding changes in DAMGO stimulated [^{35}S]GTPγS binding. Opioid treatment protocols that do not regulate opioid receptor density, but reliably produce tolerance in *in vivo* assays, did not alter DAMGO stimulated [^{35}S]GTPγS binding. These data suggest that the [^{35}S]GTPγS assay in spinal cord homogenate is relatively insensitive to μ-opioid receptor desensitization.

Disclosures: P.A. Madia, None; **B.C. Yoburn**, None.

Nanosymposium

833. Control of Behavior by Neurotransmitter Signaling

Location: Room 6F

Time: Wednesday, November 17, 2010, 1:00 pm - 2:45 pm

Program Number: 833.1

Topic: C.18. Behavioral Pharmacology

Support: National Council for Scientific and Technological Development (CNPq)

Title: Modulatory effects of dopaminergic stimulation on iron-induced recognition memory deficits

Authors: *N. SCHRÖDER[1,2], J. PRESTI-TORRES[1], V. A. GARCIA[1], F. S. SCALCO[1], M. N. M. DE LIMA[1,2];
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Abstract: Excess of iron in the brain has been implicated in the pathogenesis of several human neurodegenerative diseases, for example Alzheimer's (AD) and Parkinson's (PD) disease. It has been shown that the neonatal period is critical for the establishment of normal iron content in the adult brain and it is also known that aging alters the cerebral distribution of this metal. We have previously demonstrated that neonatal administration of iron severely impairs recognition memory in adult rats. Thus, the aim of the present

study was to determine if dopaminergic stimulation by SKF 38393 (a dopamine D1 receptor agonist) and GBR 12935 (a dopamine reuptake inhibitor) could reverse iron-induced recognition memory deficits. In order to do that, male Wistar rats received vehicle (5% sorbitol in water) or iron (10.0 mg/Kg) orally from postnatal days 12 to 14. These animals were submitted to a novel object recognition memory task when they reached the age of 6 months. Vehicle- and iron-treated animals received an intraperitoneal injection of vehicle (1% DMSO in saline solution) or SKF 38393 (5.0 mg/Kg) or GBR 12935 (5.0 or 10.0 mg/Kg) immediately after training in the novel object recognition task. Object recognition task consisted of a 5-min training trial, when they explored two copies of the same object. In retention test trials, one of the objects was replaced by a novel object. Results have indicated that SKF 38393 (5.0 mg/Kg) and GBR 12935 (10.0 mg/Kg) attenuated iron-induced recognition memory deficits.

Disclosures: N. Schröder: Employment; Pontifical Catholic University. Research Grant; National Council for Scientific and Technological Development (CNPq), Eurofarma Laboratories Ltd.. Ownership Interest; Partner, NeuroAssay Ltd.. Consultant/Advisory Board; NeuroAssay Ltd.. **J. Presti-Torres:** None. **V.A. Garcia:** None. **F.S. Scalco:** None. **M.N.M. de Lima:** None.

Nanosymposium

833. Control of Behavior by Neurotransmitter Signaling

Location: Room 6F

Time: Wednesday, November 17, 2010, 1:00 pm - 2:45 pm

Program Number: 833.2

Topic: C.18. Behavioral Pharmacology

Support: NIDA Grant DA025158

Title: Serotonin at the serotonin 2A receptor signals through a βarrestin2/PI3-K/Src/AKT complex in vivo

Authors: *C. L. SCHMID[1,2], L. M. BOHN[1];
[1]Mol. Therapeut., The Scripps Res. Inst., Jupiter, FL; [2]Neurosci. Grad. Studies Program, The Ohio State Univ., Columbus, OH

Abstract: Disregulation of the serotonin 2A receptor (5-HT2AR) is implicated in numerous mental health disorders, including schizophrenia, anxiety and depression. Further, serotonergic hallucinogens, such as lysergic acid diethylamide (LSD) and dimethyltryptamines (DMT), produce their psychotomimetic effects through 5-HT2AR

activation. Previously we have demonstrated that serotonin and 5-HT2AR agonist, (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI), differ in their necessity for the regulatory protein βarrestin2 to induce their biological effects. Here we assess how differential regulation by βarrestin2 effects the activation of downstream signaling cascades for serotonin and its hallucinogenic metabolites, the N-methylated tryptamines. We utilize βarrestin2 knockout mice to compare signaling profiles in vivo, by assessing the head twitch response (which serves as a behavioral model for selective 5-HT2AR activation), and ex vivo, by assessing kinase activation in primary cortical neuronal cultures. We demonstrate that serotonin leads to 5-HT2AR-mediated phosphorylation of the serine/threonine kinase AKT in the mouse frontal cortex and in primary neuronal cultures. Furthermore, we show that this activation requires the formation of a signaling complex that includes βarrestin2, PI3-K, Src and AKT and that inhibition of this complex disrupts the serotonin-mediated head twitch response. In contrast, N-methyl-serotonin and the N-methylated tryptamine, 5-MeO-DMT, do not induce phosphorylation of AKT in cortical neurons and induces the head twitch response regardless of βarrestin2 or inhibition of AKT expression. We also show that in the absence of βarrestin2, the head twitch response is enhanced in response to both of these compounds, suggesting that while βarrestin2 positively mediates serotonin signaling at the 5-HT2AR, it may dampen N-methylated tryptamine signaling at this receptor. Elucidating the divergence in the 5-HT2AR-mediated signaling cascades for serotonin and hallucinogenic compounds, such as the N-methylated tryptamines could have significant impacts upon drug discovery efforts for the treatment of these mental health disorders.

Disclosures: C.L. Schmid, None; **L.M. Bohn**, None.

Nanosymposium

833. Control of Behavior by Neurotransmitter Signaling

Location: Room 6F

Time: Wednesday, November 17, 2010, 1:00 pm - 2:45 pm

Program Number: 833.3

Topic: C.18. Behavioral Pharmacology

Support: DA020129

Title: Defining the role of norepinephrine in cannabinoid-induced aversion and anxiety

Authors: *A. F. CARVALHO[1,3], E. VAN BOCKSTAELE[2];
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Braga, Portugal

Abstract: In the central nervous system, cannabinoids have been shown to regulate neurotransmitter release, control the hypothalamic-pituitary-adrenal *axis and impact several physiological systems, such as food intake, pain and emotion perception.* Manipulation of the cannabinoid system using exogenous compounds has been explored as a potential therapeutic for several disorders; however some severe side effects have been reported. Understanding the neural circuits and neurochemical substrates impacted by cannabinoids will provide a better means of gauging their actions within the central nervous system that may contribute to the expression of unwanted side effects. Previous work from our lab had shown that the cannabinoid receptor (CBr) agonist WIN 55,212-2 is able to induce changes in noradrenergic transmission in limbic structures such as prefrontal cortex (PFC) and the nucleus accumbens (Acb). Moreover, we have previously reported that norepinephrine in the nucleus accumbens (Acb) is critical for WIN 55,212-2-induced aversion, as measured by the place conditioning paradigm. In the present study, we further explore the role of norepinephrine in cannabinoid-induced behaviors. More specifically, we investigate whether norepinephrine in the limbic forebrain of rats is important for the anxiety induced by WIN 55,212-2 (3.0mg/kg, i.p.). Lesion of noradrenergic neurons in the Acb and bed nucleus of the stria terminalis (BNST) was achieved by the intracerebral injection of the toxin saporin conjugated with an antibody that recognizes the enzyme dopamine-beta-hydroxylase (DBH). This toxin yields a specific lesion of noradrenergic neurons. The anxiogenic effects of WIN 55,212-2 were then measured in the elevated zero maze. The results show that depletion of noradrenergic innervation of the Acb and BNST did not reduce the anxiogenic properties of WIN 55,212-2. These results, together with our previous findings, suggest that the anxiogenic and aversive properties of the CBr agonist WIN 55,212-2 are differentially regulated, with the aversive effects being dependent on noradrenergic transmission within the Acb and the anxiogenic effects being regulated by a, yet to be determined, alternative mechanism/circuit.

Disclosures: A.F. Carvalho: None. **E. Van Bockstaele:** None.

Nanosymposium

833. Control of Behavior by Neurotransmitter Signaling

Location: Room 6F

Time: Wednesday, November 17, 2010, 1:00 pm - 2:45 pm

Program Number: 833.4

Topic: C.18. Behavioral Pharmacology

Support: NIH/NIDA R01 DA003240

NIH/NIDA R01 DA019942

NIH/NIDA K05 DA015267

NIH/NIDA F31 DA023301

Title: Impulsivity under Go/No-go and delay discounting tasks for food and/or cocaine in adolescent vs adult rats

Authors: *M. E. CARROLL, S. F. NAVIN, J. J. ANKER;
Univ. Minnesota, Minneapolis, MN

Abstract: Adolescents and females show increased vulnerability to drug dependence, and this may be due to heightened levels of impulsivity. In this study, adolescent and adult male and female rats were compared on a Go/No-go (GNG) task for food and iv cocaine to determine the influence of age and sex on impulsive lever responding (impaired response inhibition). Rats were trained to self-administer food or iv cocaine (0.4 mg/kg) under an FR 1 schedule during daily Go/No-go sessions consisting of 3 45-min periods of food or cocaine reinforcement (Go) that alternated with 2 15-min periods of non-reward (No-go) or extinction. Go and No-go components were signaled by different stimuli. In another study male and female adolescent and adult rats were compared on impulsive choice for food under an adjusting delay discounting (DD) task in which responding on one lever produced a food pellet immediately and responding on the other lever produced 3 pellets after a delay that started at 6 sec then increased by 1 sec after each response on the delay lever or decreased by 1 sec after each response on the immediate lever. Results showed that adolescents self-administered significantly more iv cocaine and responded more than adults during the Go period, and they responded significantly more than adults during the No-go period indicating an age difference in impaired response inhibition. These results were consistent with previous data showing elevated extinction responding (impaired inhibition) in adolescent vs adult rats. In the present study adolescents also showed more impulsive choice for food than adults in the DD task. There were no age or sex differences in the GNG task for food, and there were no sex differences in impulsivity for food and/or drug in the GNG or DD tasks. These results suggest that adolescent rats are more impulsive than adults for food in both tasks and for cocaine in the GNG task.

Disclosures: M.E. Carroll: None. **S.F. Navin:** None. **J.J. Anker:** None.

Nanosymposium

833. Control of Behavior by Neurotransmitter Signaling

Location: Room 6F

Time: Wednesday, November 17, 2010, 1:00 pm - 2:45 pm

Program Number: 833.5

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: ESF GARFS7479

ESF GARFS0062J

Title: Does wfs1 deletion interfere with motivational circuits?

Authors: *S. KOKS, A. TERASMAA, K. MEHEVITS, V. MATTO, E. VASAR; Univ. of Tartu, Tartu, Estonia

Abstract: Wfs1 deficient mice display reduced motor activity in the open-field test. In addition, we have described reduced sensitivity to the amphetamine in mutant animals compared to the wild-type mice. The aim of present study was to analyse the efficiency of the morphine actions in the wfs1 deficient mice. We measured motor activity of mice after the administration of different doses (0.5, 1, 2.5, 5, 10, 20 mg/kg) of morphine, performed GTP binding autoradiography after stimulation with mu-specific opioid agonist DAMGO ([D-Ala2, N-MePhe4, Gly-ol]-enkephalin) and finally, we evaluated dopamine (DA) release in the striatum of mice with microdialysis. Morphine administration significantly increased the total travelled distance in wild-type mice. The response of mutant mice to the morphine was weaker and in case of 10 mg/kg the difference was statistically significant (genotype effect $F(2, 132)=7.5$, $p<0.01$). We performed in vitro DAMGO induced GTP binding autoradiography in order to evaluate the activation of the m-opioid receptors. In wfs1 mutant mice, DAMGO induced significantly stronger activation of G-protein in the Substantia Nigra of mutant mice ($t=2.213$, $p<0.05$). Microdialysis also indicated some significant differences related to the genotype of animals. The concentrations of DA of the baseline samples were 0.8 nM to 1.4 nm for the wild type animals and 0.6 to 1.3 nM for the Wfs1 KO mice. Repeated measures ANOVA revealed a significant time ($F(1,11)= 18.9$, $p<0.001$) and genotype ($F(1,11)= 14.6$, $p<0.01$) effect, as well as an interaction between these factors ($F(1,11)= 10.6$, $p<0.01$). 10 mg/kg morphine injection increased the striatal DA output in all tested wild type animals (up to 160% from baseline) while in the KO animals only a minor increase (up to 105% from baseline, with a large within group variation) was found. Time-point-by-time-point post hoc analysis (20 - 120 min) considered this difference as statistically insignificant. The application of the [K+]-rich modified Ringer solution increased the striatal DA output in wild type animals (up to 3000%) but the increase of the striatal DA output of Wfs1 KO mice was less pronounced yielding only up to 450% from the baseline values. There was a statistically significant difference between these two mice groups ($p<0.01$ and $p<0.001$ for the time points 160 and 180 min, respectively). Our results show that the Wfs1 KO mice have reduced behavioral sensitivity to opioid

peptides and this finding correlates with neurochemical changes. More detailed analysis of the mechanisms behind these findings needs to be done in further experiments.

Disclosures: S. Koks, None; **A. Terasmaa**, None; **K. Mehevits**, None; **V. Matto**, None; **E. Vasar**, None.

Nanosymposium

833. Control of Behavior by Neurotransmitter Signaling

Location: Room 6F

Time: Wednesday, November 17, 2010, 1:00 pm - 2:45 pm

Program Number: 833.6

Topic: C.18. Behavioral Pharmacology

Support: Pathfinder Award of the UK Medical Research Council (MRC, G0401099)

and Clinical Neuroscience Institute (BCNI), jointly funded by MRC and Wellcome Trust (G0001354)

Title: Performance-dependent effects of oral methylphenidate on midbrain D2/D3 autoreceptor regulation

Authors: *N. DEL CAMPO[1,5], T. D. FRYER[6,2], Y. T. HONG[6,2], R. SMITH[6,2], R. TAIT[5], S. R. CHAMBERLAIN[1,5], J. DOWSON[1], J.-C. BARON[2,5], F. I. AIGBIRHIO[2,6,5], T. W. ROBBINS[5,3], B. J. SAHAKIAN[5,1], U. MULLER[4,5];
[1]Dept. of Psychiatry, [2]Dept. of Clin. Neurosciences, [3]Dept. of Exptl. Psychology, [4]Univ. of Cambridge, Cambridge, United Kingdom; [5]Behavioural and Clin. Neurosci. Inst., Cambridge, United Kingdom; [6]Wolfson Brain Imaging Ctr., Cambridge, United Kingdom

Abstract: Background: Based primarily on the clinical effectiveness of psychostimulants in the treatment of attention deficit/hyperactivity disorder (ADHD), catecholamines have long been implicated in the manifestation of ADHD symptoms and associated cognitive deficits. However, the precise neurobiological mechanisms underlying the disorder and its treatment remain poorly understood.
Objective: To determine the specificity of ADHD-related methylphenidate effects on nigro-striatal dopaminergic activity in conjunction with an objective measure of sustained attention.
Methods: 16 adult ADHD patients and 16 matched controls were scanned with positron emission tomography and [18F]fallypride, a high affinity D2/D3 receptor radioligand,

after oral methylphenidate (0.5 mg/kg) and placebo in a randomised, double-blind, cross-over design. [18F]fallypride binding potential in regions manually defined on magnetic resonance images was calculated using a reference tissue model. The rapid visual information processing task was administered on both occasions.
Results: Methylphenidate improved sustained attention in participants from both ADHD and control groups who performed worse at baseline (p=0.02). This enhancing effect of methylphenidate in low performers was accompanied by smaller drug-induced increases in midbrain dopamine levels (p=0.007) than those observed in high performers. On placebo, low performers had reduced D2/D3 receptor availability in left pre-commissural caudate compared to high performers (p=0.035).
Conclusions: Our results are concordant with a continuum model of ADHD. They suggest that cognitive deficits associated with ADHD may be better predictors of the underlying dopaminergic dysregulation than the disorder itself, revealing a hitherto neglected role of midbrain D2/D3 autoreceptor regulation in determining the therapeutic effects of oral methylphenidate.

Disclosures: **N. Del Campo:** Consultant/Advisory Board; Cambridge Cognition. **T.D. Fryer:** None. **Y.T. Hong:** None. **R. Smith:** None. **R. Tait:** None. **S.R. Chamberlain:** Cambridge Cognition, P1Vital, and Shire Pharmaceuticals. **J. Dowson:** None. **J. Baron:** None. **F.I. Aigbirhio:** None. **T.W. Robbins:** Cambridge Cognition, Pfizer, Eli Lilly, GlaxoSmithKline, Allon Therapeutics, Lundbeck and Pangenics, Cambridge Cognition. Ownership Interest; Cambridge Cognition and Springer-Verlag. **B.J. Sahakian:** Speakers Bureau/Honoraria; of Psychological Medicine. Ownership Interest; Cambridge Cognition and CeNeS. Consultant/Advisory Board; Novartis, Shire, GlaxoSmithKline, Eli Lilly, Boehringer-Ingelheim and Cambridge Cognition. **U. Muller:** Other Research Support; Janssen-Cilag. Speakers Bureau/Honoraria; Bristol-Myers Squibb, Eli Lilly, Janssen-Cilag, Lundbeck, Pharmacia-Upjohn, and UCB Pharma.

Nanosymposium

833. Control of Behavior by Neurotransmitter Signaling

Location: Room 6F

Time: Wednesday, November 17, 2010, 1:00 pm - 2:45 pm

Program Number: 833.7

Topic: C.18. Behavioral Pharmacology

Support: work supported by NIDA/IRP, NIH/DHHS funds

Title: Benztropine analogs as atypical dopamine transporter inhibitors: Place conditioning and dopamine microdialysis studies in rats

Authors: *G. TANDA[1], A. THOMAS[1], S.-M. LI[1], M.-F. ZOU[2], A. EBBS[1], J. GREEN[1], L. GARCES-RAMIREZ[1], A. H. NEWMAN[2], J. L. KATZ[1];
[1]Psychobiology Section, [2]Medicinal Chem. Section, NIDA, BALTIMORE, MD

Abstract: Inhibition of the dopamine transporter (DAT) is the primary pharmacological action of cocaine related to its abuse liability. However, the DAT is also a target of drugs with potential therapeutic efficacy as medications for cocaine-addiction. Several benztropine (BZT) analogs have been classified as atypical DAT blockers due to their high affinity for the DAT combined with lower efficacy in producing behaviors like those produced by typical DAT inhibitors. In the present study place conditioning with the BZT analogues, 4-Cl-BZT, 4-4-di-Cl-BZT, MFZ 4-86, and MFZ 4-87, was assessed in Sprague Dawley rats, and compared with their stimulation of extracellular dopamine (DA) levels in the nucleus accumbens shell (NAS) using microdialysis coupled with electrochemical detection. The NAS was studied as acute administration of virtually all drugs abused by humans, including psychostimulants, selectively or preferentially increase DA levels in that area compared to other DA terminal areas. All drugs tested significantly and dose-dependently (1-10 mg/kg, i.p.) stimulated extracellular DA levels in the NAS with a longer duration of action compared to cocaine. Maximum peak DA effects for BZT analogs and cocaine (10 mg/kg) were similar. However, the onset of action, and the maximal peak effect were reached later in the time course for BZT analogs than for cocaine. Place conditioning was induced by cocaine (10 mg/kg i.p.) but not the BZT analogs (0.3-10 mg/kg i.p.) administered immediately before the conditioning sessions. Further, the BZT analogs were inactive in producing place conditioning when administered at 45 or 90 minutes before sessions to accommodate their slower onset of effects on DA levels, In summary, the present BZT analogs significantly stimulated DA levels in a brain area involved in the reinforcing effects of drugs. However they did not induce place conditioning, a behavioral effect shared by drugs of abuse confirming their differentiation from typical DAT inhibitors such as cocaine.

Disclosures: G. Tanda: None. **A. Thomas:** None. **S. Li:** None. **M. Zou:** None. **A. Ebbs:** None. **J. Green:** None. **L. Garces-Ramirez:** None. **A.H. Newman:** None. **J.L. Katz:** None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.1

Topic: D.04. Vision

Support: NRSA 1F32EY018279

NSF BCS 0920865

Title: Characterizing face representations in the ventral stream: Effects of physical variability and distance from the average face

Authors: *N. DAVIDENKO[1], D. REMUS[2], K. GRILL-SPECTOR[2];
[2]Psychology, [1]Stanford Univ., STANFORD, CA

Abstract: fMRI research has identified regions in the human fusiform gyrus (FFA) and inferior occipital gyrus (IOG) that respond selectively to faces, but the mechanisms by which neurons in these regions represent different faces is highly debated. A prominent view posits that face-selective neurons employ a norm-based representation, responding more strongly to distinctive faces that deviate from the average face in particular directions in face space. However, in humans, evidence for this view is based on block-design fMRI experiments in which the within-block physical variability of face stimuli is not controlled across different blocks. If blocks of distinctive faces also contain more physically variable faces than blocks of typical faces, a larger BOLD response to distinctive blocks may indicate less adaptation during these high-variability blocks rather than preferential tuning to distinctive faces. In 3 studies (8-10 subjects each), we measured responses of FFA, IOG, and an object-selective region (LO) using fMRI at 3T. We manipulated distance from the average face (distinctiveness) independently from the physical variability of face stimuli using a perceptually validated silhouette face space. When physical variability and distinctiveness co-varied (Study 1), we replicated previous studies and found stronger responses during distinctive (and high-variability) blocks in FFA and IOG. However, we found the same pattern in LO, suggesting general adaptation to low-variability blocks rather than evidence for a face coding mechanism. In contrast, when physical variability was held constant across blocks (Study 2), responses in FFA and IOG (but not LO) were stronger during typical face blocks, providing new evidence of a typicality preference in FFA and IOG. A key prediction of the norm-based model is that blocks of faces sampled from multiple directions in face space relative to the average face should activate a larger neural population and thus yield a stronger BOLD response than blocks of faces sampled from a single direction. Contrary to this prediction, we found that FFA and IOG responses did not differ across these two types of blocks when physical variability was matched (Study 3). Our results highlight the importance of controlling the physical variability of stimuli when investigating the functional properties of ventral visual cortex. Further, responses in FFA and IOG across all 3 studies can be explained by an exemplar-tuned neural population that allocates more neurons with sharper tuning to frequently experienced, typical faces near the average face. We propose similar coding principles may underlie the representation of other visual categories.

Disclosures: N. Davidenko, None; **D. Remus**, None; **K. Grill-Spector**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.2

Topic: D.04. Vision

Support: Max-Planck Institute

Title: The face selective activity in ventral temporal lobe in macaques

Authors: *S.-P. KU[1], A. S. TOLIAS[2], N. K. LOGOTHETIS[1], J. GOENSE[1];
[1]MPI Biol Kybernetics, Tuebingen, Germany; [2]Baylor Col., Houston, TX

Abstract: Face perception is one of the most crucial abilities for social animals like humans and nonhuman primates. fMRI-, lesion- and electrophysiology studies in humans and monkeys have indicated the existence of a dedicated and wide-spread face-processing network. In humans the most robust face-selective brain areas are fusiform face area (FFA), occipital face area (OFA) and superior temporal sulcus (STS). However, in monkeys the strongest face selectivity is found predominantly in STS, and no reliable face selectivity has been reported in fusiform gyrus and occipital temporal region. These differences may be a species difference, or they may be due to technical difficulties, because in monkeys the fusiform gyrus and ventral occipital-temporal area are located in regions that are difficult to map with fMRI due to susceptibility artifacts from the ear canal. Here we used an optimized imaging protocol at 7T, which does not suffer from the usual signal loss in inferior temporal areas. We investigated the functional organization of face processing in 5 awake or anesthetized macaques while the subjects viewed faces, fruit, houses and fractal patterns.
We found face-specific BOLD responses in STS, anterior medial temporal sulcus (AMTS), the regions anterior and lateral to AMTS and amygdala, consistent with previous fMRI and electrophysiology results. But in addition, entorhinal cortex (EC), ventral TE (posterior to AMTS), and hippocampus also contain face selective patches. These areas have not been reported to be face-selective in monkeys before, although they were shown to be responsive to faces with fMRI or intracortical recording in humans. The results indicate that there is much more extensive face selective brain activity than earlier studies have found in monkey ventral temporal lobe and suggests a large degree of similarity between the human and monkey face-processing network.

Disclosures: S. Ku, None; **J. Goense**, None; **N.K. Logothetis**, None; **A.S. Tolias**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.3

Topic: D.04. Vision

Title: Adaptation reveals: Faithful representation of face views is achieved in object-centered representations prior to viewer-centered ones

Authors: *B. NOUDOOST[1,2], H. ESTEKY[2];
[1]Stanford Univ., STANFORD, CA; [2]Cognitive sciences, Inst. for theoretical studies in physics and mathematics, Tehran, Iran, Islamic Republic of

Abstract: Here we studied the neural basis of view invariant face recognition by examining impact of face-view adaptation on the responses of inferior temporal neurons of two passively fixating monkeys. We recorded responses of 131 neurons to a set of 20 face views evenly spaced between -180° to 180° face views. Shape selectivity and face view tuning of neurons were determined in a passive fixation paradigm. Then the responses of the same neurons to the same set of face views were recorded following 5 seconds of adaptation with either front or left profile views. The normalized neural responses with peak tuning at or close to that of the adapter were significantly decreased after adaptation. Adaptation with non-optimal view resulted in response enhancement. Also adaptation resulted in shift of view tuning away from the adapted view point. The distance between the adapter and the peak of tuning was increased after adaptation. The shift in tuning peak was maximal when adapter was close (≤90°) to the peak whereas no significant shift was observed for far (>90°) adaptations. In addition, based on responses of neurons to any specific stimulus after adaptation we estimated the most likely stimulus which would drive that response, given the stimulus-response relationships before adaptation.using the responses without adaptation we determined the expected stimulus based on responses after adaptation (optimal decoding method). We found that after adaptation, the decoded stimuli were shifted away from the adapter. For example, after adaptation with a left profile view, a half-profile stimulus would be decoded as a stimulus more similar to front view. This repulsive shift of neuronal representation was observed in both adaptation conditions. We also found evidence of perceptual repulsive shift in two monkeys in a separate task requiring monkeys to categorize face views before and after adaptation. The consistency of our findings in monkey psychophysics with the observed

repulsive shift of representation in IT neurons suggests these neurons as a neural correlate of face view representation. Furthermore, we found that neurons responding to a wide range of face views (invariant neurons) exhibit signatures of repulsive aftereffect in their response earlier than neurons responding to specific views. This suggests the involvement of view invariant neurons in representation of face views even prior to view selective neurons. This result adds an important constraint to models of object recognition and suggests that view-invariant representation of objects is achieved earlier than, and probably independent from, view-selective representation.

Disclosures: B. Noudoost, None; **H. Esteky**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.4

Topic: D.04. Vision

Support: NIH 1R01EY019702

Searle Foundation

the Klingenstein Foundation

NSF CAREER BCS-0847798)

Feodor Lynen Research Fellowship from the Humboldt Foundation

Irma T Hirschl & Monique Weill-Caulier Trusts award

Title: Representations of multiple objects in the macaque face processing system

Authors: *A. F. EBIHARA[1,2], D. Y. TSAO[2], W. A. FREIWALD[1];
[1]The Rockefeller Univ., New York, NY; [2]Caltech, Pasadena, CA

Abstract: Primates excel in recognizing faces in cluttered scenes. It has even been proposed that faces "pop out" of arrays of objects (1). Face recognition is thought to be mediated by specialized face processing areas. In the macaque monkey, six bilateral face-selective regions have been found in the temporal lobe by functional magnetic resonance

imaging (fMRI). These regions, termed face patches, consist of one posterior, two middle and three anterior face patches, named after their anatomical location along the temporal lobe. They are structurally connected to form a dedicated face-processing network. Anatomical location and functional properties of neurons in different face patches suggest a hierarchical arrangement of face patches, along which both invariance and selectivity properties increase. Remarkably, even at an early processing level of the system, many neurons have large receptive fields (of more than ten degrees diameter), and face selectivity is maintained even at the largest eccentricities. Thus, in natural environments these receptive fields will most often encompass multiple stimuli simultaneously.
Here we investigated the question of how multiple stimuli are represented by neurons in the middle face patches. Face patches were localized by fMRI and targeted with recording electrodes, guided by structural MRI. We recorded responses to a range of stimuli including individual faces and objects at different locations of the receptive field, and combinations of these stimuli. This allowed us to determine stimulus selectivity and response latency in different parts of the receptive field and test whether the response to multiple stimuli approaches the average or the maximum of the responses to the individual stimuli (2). We found that simple models of response combination could not account for the responses to multiple stimuli we observed. First, the magnitude of the response to stimulus pairs did not follow directly from the responses to individually presented stimuli. Rather spatial location within the receptive field turned out to be strong determinant of response magnitude. Second, the temporal profile of the response to stimulus pairs could exhibit a dynamics not anticipated by responses to individual stimuli. We present recording results from the middle face patches and discuss them in the context of current models of hierarchical shape processing in inferotemporal cortex.
1. O. Hershler, S. Hochstein, Vision Research 45, 1707 (2005).
2. I. Lampl, D. Ferster, T. Poggio, M. Riesenhuber, Journal of Neurophysiology 92, 2704 (2004).

Disclosures: A.F. Ebihara, None; **D.Y. Tsao**, None; **W.A. Freiwald**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.5

Topic: D.04. Vision

Support: NIH 1R01EY019702

Searle Foundation

the Klingenstein Foundation

NSF CAREER BCS-0847798

Title: Contrast tuning in face cells - Evidence for region based encoding

Authors: *S. OHAYON[1], D. TSAO[1], W. FREIWALD[2];
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Abstract: Several state-of-the-art computer vision systems for face detection, e.g., Viola-Jones [1], rely on region-based features that compute contrast by adding and subtracting average image intensity within different regions of the face. This is a powerful strategy due to the invariance of these features across changes in illumination (as proposed by Sinha [2]). The computational mechanisms underlying face detection in biological systems, however, remain unclear. We set to investigate the role of region-based features in the macaque middle face patch, an area that consists of face-selective neurons. We presented a sequence of face stimuli parameterized by the intensity level within each of 11 regions, allowing us to determine the tuning for contrast between different face regions. First, we found that fully inverting the contrast across all face regions reduced firing rate by 50% on average. We then analyzed the sensitivity of cells to the contrast relationship between specific pairs of regions within a face. We found that individual neurons were tuned to subsets of contrast relationships between pairs of face regions. The sign of tuning for these relationships was strikingly consistent across the population (for example, almost all neurons preferred a lower average intensity in the eye region relative to the nose region). Furthermore, the pairs and polarity of tuning were fully consistent with Sinha's proposed ratio-template model of face detection [2]. Non-face images from the CBCL dataset that contained correct contrast polarities in pre-defined regions (facial parts) did not elicit increased firing in face-selective neurons, suggesting that the neurons are not only computing averaged intensity according to a fixed template, but are also sensitive to the specific shape of features within a region.
[1] Robust Real-time Object Detection, Paul Viola and Michael Jones. Second International Workshop on Statistical and Computational Theories of Vision - Modeling, Learning, Computing, and Sampling. Vancouver, Canada, July, 2001.
[2] Qualitative Representations for Recognition, Pawan Sinha. Proceedings of the Second International Workshop on Biologically Motivated Computer Vision, Tubingen, November, 2002.

Disclosures: S. Ohayon: Research Grant; NIH 1R01EY019702, Searle Foundation, the Klingenstein Foundation, NSF CAREER BCS-0847798. **D. Tsao:** None. **W. Freiwald:** None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.6

Topic: D.04. Vision

Support: NIMH IRP

Title: Representation of facial motion and emotional expression in macaque superior temporal sulcus

Authors: N. FURL[1], F. HADJ-BOUZIANE[2], N. LIU[2], B. AVERBECK[3], *L. G. UNGERLEIDER[4];
[1]Lab. of Neuropsychology, [2]Lab. of Brain and Cognition, [3]NIMH Lab. of Neuropsychology, NIMH-NIH, Bethesda, MD; [4]NIMH-NIH, BETHESDA, MD

Abstract: Many animals, including humans, use faces to recognize critical social cues. These cues are highly transient, as faces continually move, making the identification of accurate social cues particularly challenging. Humans may rely on neural populations in the superior temporal sulcus (STS) specialized for representing changeable facial attributes, like expressions. However, the relationship between facial motion and these representations has proven difficult to specify. Monkeys provide an interesting model system, as many areas in STS respond strongly (sometimes selectively) to faces and, importantly, also manifest motion sensitivity. We therefore quantified information about individual expression categories coded in motion-sensitive areas, face-selective and face-responsive areas of macaque STS.
We recorded high resolution fMRI (1.56 × 1.56 ×1.5 mm) from 3 male *Macaca Mulatta* in a GE 3T scanner (TR=2 s) and used MION (8-10 mg/kg) as a contrast agent to increase signal to noise ratio. We first acquired a "face-patch" localizer, where monkeys viewed blocks (40 s + 20 s fixation) containing 20 different static color photographs of neutral monkey faces, places, or objects. The main experiment comprised 6 types of blocks (36 s + 10 s fixation), where monkeys viewed either movies or static images of 3 familiar monkey faces, expressing either threatening, submissive or neutral emotions (i.e., 6 image/movie categories). We show sensitivity to facial dynamics in several areas in bilateral STS, including MT and FST. From single trial fMRI responses in these motion-sensitive voxels, we decoded information about facial motion and expression. We used leave one session out cross-validation to test a regularized multinomial regression model on the six motion/expression categories. The model classified fMRI responses above chance using motion-sensitive voxels, face-responsive voxels (faces > baseline)

and face-selective voxels (faces > objects/places). From the model's confusions, we visualized category similarity spaces using multidimensional scaling and found orthogonal dimensions which discriminated: dynamic versus static faces, the three static expressions, and the three dynamic expressions. We conclude that the macaque STS is highly sensitive to facial dynamics, codes both motion and expression categories, and can code dynamic and static expressions using dissimilar response patterns. These results raise interesting questions about motion influences on face representations in humans and monkeys.

Disclosures: N. Furl, None; **L.G. Ungerleider**, None; **F. Hadj-Bouziane**, None; **N. Liu**, None; **B. Averbeck**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.7

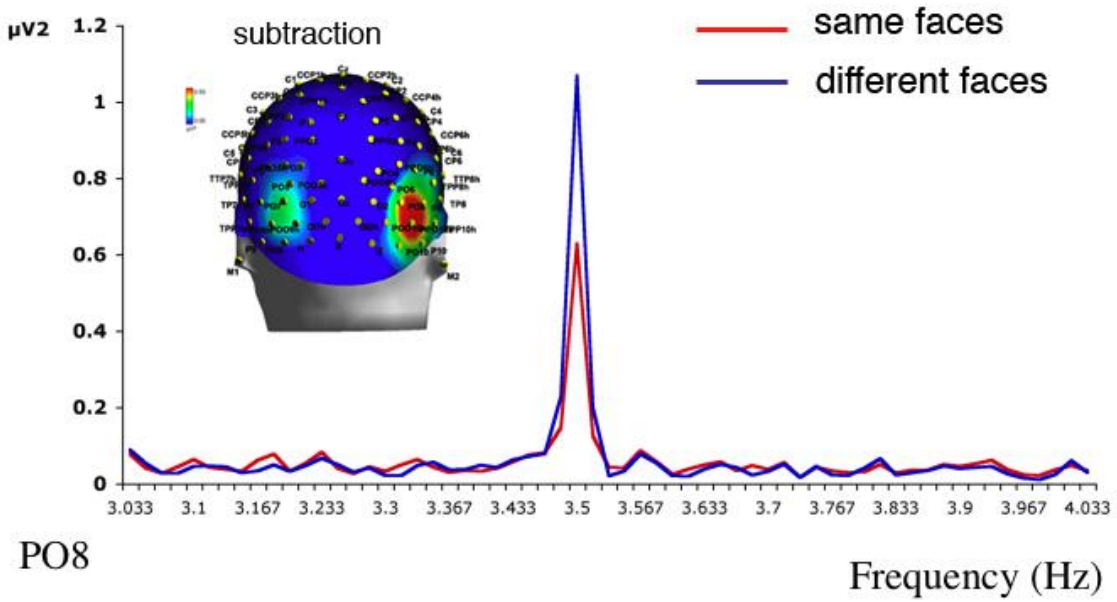
Topic: D.04. Vision

Title: Robust representation of individual faces in the right human occipito-temporal cortex: Evidence from steady-state visual evoked potentials

Authors: *B. ROSSION, A. BOREMANSE;
Univ. catholique Louvain, Louvain-la-Neuve, Belgium

Abstract: Over recent years, visual adaptation has been used as a tool to probe the response properties of face-sensitive areas of the visual cortex in neuroimaging studies (e.g., Grill-Spector et al., 2006), as well as in scalp event-related potentials studies that aim to clarify the time-course of sensitivity to facial features and their integration in the human brain (e.g., Jacques et al., 2007). However, this approach is often limited by low signal-to-noise ratio (SNR), as well as the ambiguity of measurement and quantification of adaptation effects. Here we tested the sensitivity of the visual system to face identity using steady-state visual evoked potentials (SSVEP, Regan, 1966). Twelve subjects were submitted to a 90s sequence of faces presented at a constant rate (3.5Hz or faces/second) while high-density electroencephalogram (EEG) was recorded (128 channels). Fast-Fourier Transform (FFT) of EEG data showed a clear and specific response at the fundamental frequency (3.5Hz) and its harmonics (7Hz, 10.5Hz⋯) over posterior electrode sites. EEG power at 3.5Hz over a few contiguous occipito-temporal channels of the right hemisphere was much larger when face identity changed at that rate than when

the same face was repeated throughout the sequence. Significant effects of face identity adaptation were found in every single participant following a few minutes of EEG recording only. This effect was not due to low-level feature repetition, since it was observed despite large changes of face size and was focused over the right lateral occipital cortex rather than low-level visual areas. Moreover, the larger response to different than identical faces disappeared when they were presented upside-down. This first demonstration of SSVEP adaptation to face identity in the human brain confirms previous observations using a much simpler, faster and higher SNR approach. It offers a promising tool to study the sensitivity to processing of visual features in individual faces in various populations presenting a much lower SNR of their electrical brain responses (e.g., infants, brain-damaged patients).



Disclosures: B. Rossion, None; **A. Boremanse**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.8

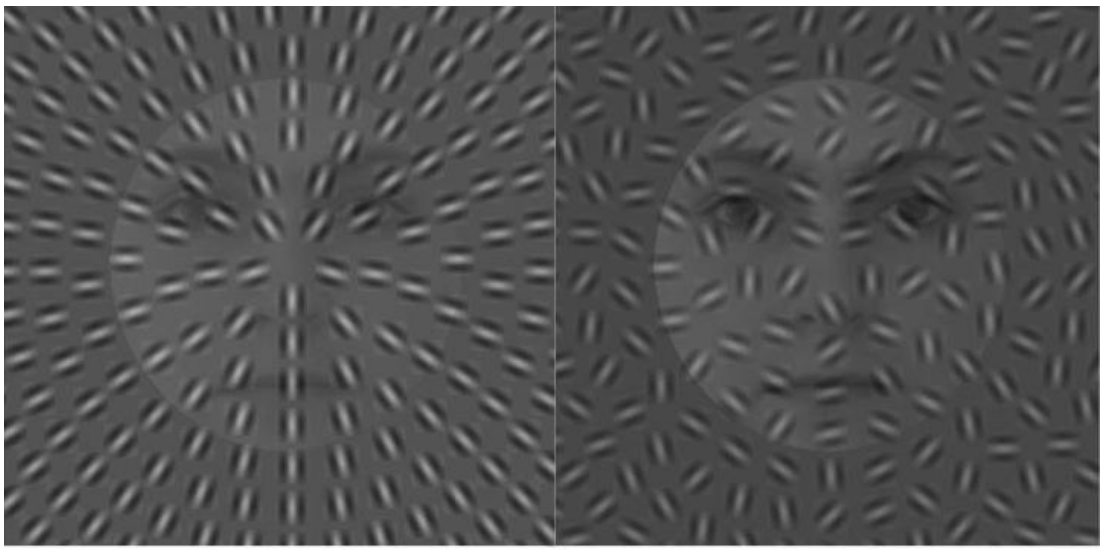
Topic: D.04. Vision

Support: Deutsche Forschungsgemeinschaft Grant KO 3918/1-1

Title: Task-irrelevant stimulus coherence determines face - noise interactions: An ERP study

Authors: *G. KOVÁCS[1,2], G. VOLBERG[2], Z. VIDNYÁNSZKY[3], M. ZIMMER[1]; [1]Tech. Univ. Budapest, Budapest, Hungary; [2]Inst. Psychology, Univ.Regensburg, Regensburg, Germany; [3]Neurobionics Res. Group, Hungarian Acad. of Sci., Budapest, Hungary

Abstract: Adding noise to a stimulus affects its processing. Recent studies have revealed that adding Gaussian or phase noise to a face stimulus affects the N170 and P200 components of the event related potentials (ERP), suggesting that the extraction of task-relevant information is related to these time-windows. However, we know little about the dependence of this process on the properties of the noise stimulus. In the current study, we used ERP recordings during a face gender-discrimination task. Subjects (n=14) were presented androgenous faces with added, trial-unique noise fields, composed of Gabor-patches (see attached Figure). To systematically vary the coherence of the noise stimulus we changed the orientation of the individual Gabor elements and presented either randomly oriented Gabor fields (RND) or patterns composed of aligned Gabor patches, forming radial lines (COL). Behavioral results showed that the difficulty of the gender discrimination task was similar in COL and RND. We found, that the face-sensitive N170 component of the ERP decreased over the right hemisphere by the addition of the noise pattern similarly for RND and COL when compared to a clear face stimulus. On the other hand, the later P200 component, that may reflect the increased sensory processing of noisy stimuli, was affected differentially by noise: RND increased its amplitude over left occipital sites when compared to COL or to clear face stimuli. These results suggest that the collinearity of the noise stimulus affects the site and time-period of relevant information extraction: while both RND and COL modulate the N170 similarly their effect is different on the P200 component. This suggests that the extraction of task-relevant information from random noise fields lasts longer than from coherent noise. Control experiments, performed on the same stimuli, but orienting the attention of the subjects to the Gabor stimuli showed no significant modulation of the N170 and P200 components, emphasizing the role of top-down processes in the extraction of relevant information.



Disclosures: G. Kovács, None; **G. Volberg**, None; **Z. Vidnyánszky**, None; **M. Zimmer**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.9

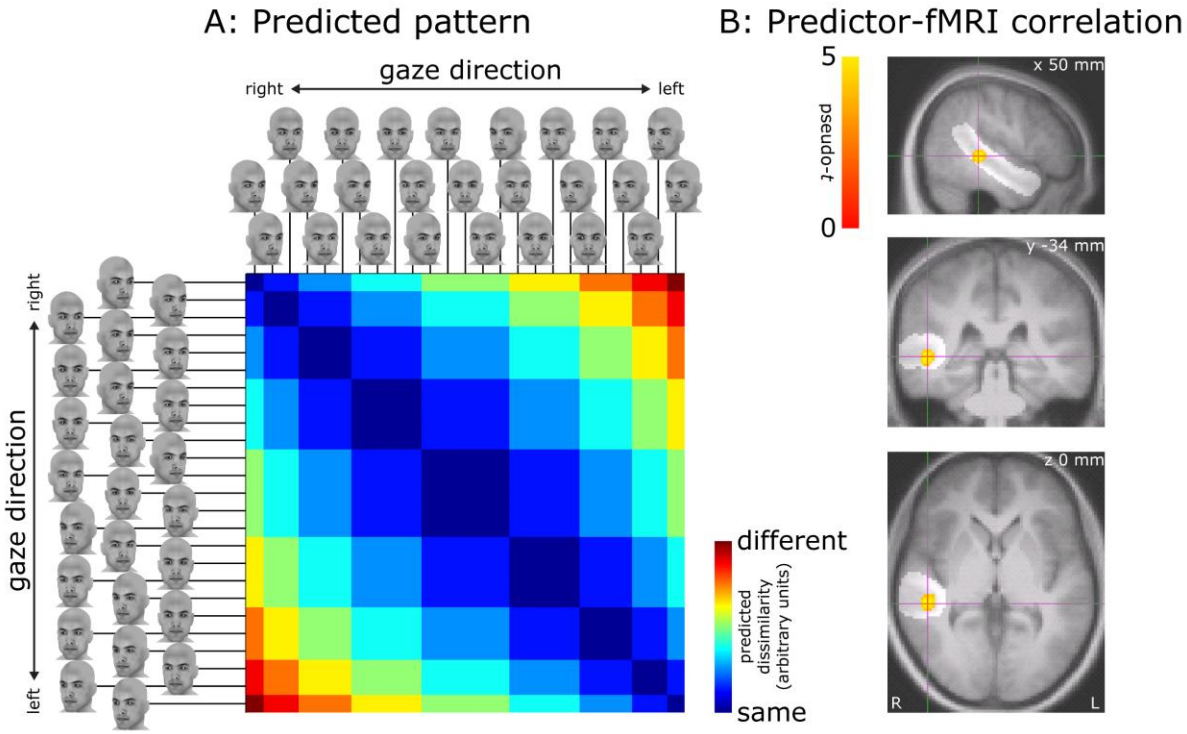
Topic: D.04. Vision

Title: Superior temporal sulcus extracts perceived gaze direction from combinations of head orientation and eye position

Authors: *J. D. CARLIN[1], A. J. CALDER[1], N. KRIEGESKORTE[1], J. B. ROWE[1,2,3]; [1]MRC Cognition and Brain Sci. Unit, Cambridge, United Kingdom; [2]Dept. of Clin. Neurosciences, Univ. of Cambridge, Cambridge, United Kingdom; [3]MRC Behavioural and Clin. Neurosciences Inst., Cambridge, United Kingdom

Abstract: In humans and macaques, superior temporal sulcus (STS) responds in a view-specific manner to manipulations to head orientation or eye position relative to the head (from here on, eye position). The function of this representation may be to code the direction of social attention in terms of perceived gaze direction (Perrett et al., 1992, *Phil*

Trans R Soc B), rather than coding the component head orientation and eye position cues. However, human data to support this hypothesis has been lacking. We carried out a rapid event-related functional MRI experiment in which participants viewed heads with 25 unique combinations of head orientation and eye position (head and eye position cues both appeared in 5 10-degree increments between left 20 and right 20). We used a novel combination of multivariate searchlight mapping and correlation-based representational similarity analysis of the voxel activation patterns evoked by each condition. Our method allowed systematic comparisons of the observed pattern of between-condition voxel correlations and the patterns produced by a set of hypothesis-guided predictors for gaze direction, head orientation, and eye position. We found that the right STS representation of the 25 head/eye combinations is guided by perceived gaze direction. No significant relationship appeared between the voxel patterns in this region and alternative predictors based on head view or eye position in isolation. Furthermore, the patterns in this region were significantly better accounted for by a gaze direction predictor that allows for subtle graded similarities between adjacent gaze directions, as compared to a predictor that merely distinguishes left, direct, and right gaze. Our results indicate that the representation of faces in human STS is not reducible to coding of head or eye position in isolation. Instead, the STS region integrates these cues to form a graded representation of perceived gaze direction.



Disclosures: J.D. Carlin, None; **A.J. Calder**, None; **N. Kriegeskorte**, None; **J.B. Rowe**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.10

Topic: D.04. Vision

Title: The behavioral neuroscience of perceptual and decisional aspects of performance in the composite face task

Authors: *M. J. WENGER[1], R. J. VON DER HEIDE[2], J. L. BITTNER[2], D. FITOUSI[2]; [1]Psychology, Pennsylvania State Univ., UNIVERSITY PK, PA; [2]Psychology, Pennsylvania State Univ., University pk, PA

Abstract: A key construct in the study of the perception of faces is the notion of configurality: a tightly-bound internal representation of the perceptual information available in the stimulus, also referred to as a holism or gestalt. Generally, this construct is applied to the encoded perceptual representation, rather than to how internal decisional mechanisms are applied to the representation in order to determine behavior. However, theoretical analyses, based on the application of multidimensional signal detection theory, have suggested that the behavioral regularities indicative of perceptual holisms can be obtained by a variety of combinations of perceptual and decisional factors. In order to try to disentangle the roles of perceptual and decisional factors contributing to one of these regularities---the composite face effect---Kuefner and Rossion (2009) used electrophysiological results (the N170 and the lateralized readiness potential, LRP) to suggest that the composite face effect---and, by extension, the hypothesized holistic or configural processing of faces---is driven solely by perceptual factors. The goal of the present study was to show that the results of Kuefner and Rossion are actually one of a set of possible outcomes. We do this by examining performance of individual observers, all of whom completed three experimental tasks involving the same stimuli. The first was a replication of Kuefner and Rossion's composite face task, allowing examination of critical neurophysiological regularities suggestive of perceptual sources. The second was an implementation of the Eriksen flanker task, allowing explicit consideration of aspects of decisional sources, with the addition of EEG data. The third was a complete identification task, in which both perceptual and decisional sources could be assessed, coupled with EEG data. Results illustrate how the set of possibilities for the perceptual and decisional sources of the composite face effect predicted can be expressed and measured in the performance of individual observers.

Disclosures: M.J. Wenger, None; **R.J. Von Der Heide**, None; **J.L. Bittner**, None; **D. Fitousi**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.11

Topic: D.04. Vision

Title: How behavior and visual attention influence face-sensitive event-related potentials

Authors: *G. RIGHI[1], S. S. JESTE[2], C. A. NELSON[1];
[1]Div. of Developmental Med., Childrens Hosp. Boston, Boston, MA; [2]Semel Inst., UCLA, Los Angeles, CA

Abstract: Studies using event-related potentials (ERP) have identified several components that are sensitive to different aspects of face processing. However, debate continues in the literature regarding what aspects of processing modulate the morphology of these components. The aim of this study was to examine how face sensitive ERP components are modulated by behavioral performance and by the location of attention allocation when viewing a face.
Twenty adult participants were asked to judge facial identity in the context of upright and inverted faces in a delayed match-to-sample task while ERPs and eye movements were recorded. Behavioral performance, defined as accuracy of facial identity recognition, was also recorded. ERPs were sampled with a 128-electrode cap and extracted from continuous EEG. Eye movements were quantified in two ways. First, we measured the percentage of looking time spent on the eye region, mouth region, and other portions of a face across all trials and both experimental conditions. We also measured the average proportion of time spent on different regions of the face over the duration of the stimulus presentation at 60 Hz, in order to preserve the dynamic structure of the eye movement data. Behavioral performance was quantified using d' prime as measure of accuracy. Correlational analyses were conducted in order to explore the relation between amplitude and latency of the ERP signal and behavioral performance. In the first analysis we focused on the characteristics of a well-established face-sensitive ERP component, the N170. The results showed that the difference in latency in the N170 between upright and inverted face trials correlated with the size of the behavioral inversion effect across subjects. There was also a marginally significant correlation between N170 latency and % time spent looking at the eyes. No significant correlations were found between N170 amplitude and behavioral performance, nor between N170 amplitude and % time spent looking at the eyes. The second analysis was designed to use the dynamic structure of

both the eye movement data and of the ERP data by correlating these two sets of time-series across a 500ms time window. This analysis revealed a relationship between the patterns of eye movements and amplitude variations in time windows similar to those in which face-sensitive ERP components have been identified.
Overall these results suggest a relationship between both behavioral performance and the location of visual attention when viewing a face and the ERP signal, with latency primarily related to behavioral performance and amplitude primarily related to patterns of eye movements.

Disclosures: G. Righi, None; **S.S. Jeste**, None; **C.A. Nelson**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.12

Topic: D.04. Vision

Support: NSF BCS0923763

SBE-0542013

Title: Using high resolution DTI to detect impaired brain connectivity in congenital prosopagnosia

Authors: *L. ZEIFMAN[1], W. EDDY[2], M. BEHRMANN[3];
[2]Statistics, [3]Psychology, [1]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Congenital prosopagnosia is a disorder affecting up to 2.5% of the population that is characterized by impaired recognition of faces. The nature of the neural deficit underlying the disorder remains poorly understood. In congruence with the biological evidence that face perception is mediated by a widely distributed network, Thomas and colleagues (2009) demonstrated for the first time a significant reduction in fractional anisotropy within the inferior longitudinal fasciculus and within the inferior fronto-occipito fasciculus, using Diffusion Tensor Imaging (DTI) with 6 directions and a multiple Regions of Interest (ROI) approach for analysis. Here, we follow up with a study using high resolution DTI (50 non-collinear directions and 5 b=0 scans) to further our knowledge of impaired brain connectivity among prosopagnosic individuals. We used the iteratively reweighted least squares algorithm, which takes the variance structure of the

signals into account and imposes a constraint on the eigenvalues to guarantee non-negative definite estimates, to estimate the tensor parameters, which we found to be superior to other existing estimation methods. Rather than choosing regions of interest and performing tractography, we used data-driven non-parametric statistical approach with minimal preprocessing to assess differences in brain connectivity among prosopagnosics in comparison to the control population. We expect to detect the same differences found in the previous study, and perhaps additional, previously undocumented differences in brain connectivity and/or fiber structure. To the best of our knowledge, this is the only such study currently being done.

Disclosures: L. Zeifman, None; **W. Eddy**, None; **M. Behrmann**, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.13

Topic: D.04. Vision

Support: Burroughs-Wellcome Career Development Award

Title: Confounding of prototype and similarity effects in fMRI studies of face and object representation

Authors: *G. K. AGUIRRE;
Univ. Pennsylvania, PHILADELPHIA, PA

Abstract: Psychological models suggest that perceptual similarity can be divided into geometric effects, such as metric distance in stimulus space, and non-geometric effects, such as stimulus-specific bias or prototype. In separate neuoimaging studies, these effects of stimulus similarity and prototype representation have been sought in neural coding for faces and objects (e.g., Jiang et al., 2006, Neuron, 50, 159-172; and Loffler et al., 2005, Nature Neuroscience, 8, 1386-1390). Practically, stimulus similarity is observed as a graded recovery from neural adaptation with ever greater dissimilarity between a pair of stimuli. A prototype effect, in contrast, is a larger absolute response to a stimulus which is distant from the center of a stimulus space. In standard neuroimaging paradigms, however, these two effects are confounded and can be mistaken for one another. Stimuli which are more distinctive are less subject to adaptation from perceptual neighbors. Therefore, a putative prototype effect may simply result from greater

adaptation of prototypical stimuli by other stimuli in the experiment. Conversely, stimulus pairs which are the most perceptually distant from one another, and therefore expected to show the greatest recovery from adaptation, are disproportionately drawn from the extremes of the stimulus space. Therefore, a putative neural similarity effect may be driven by an underlying prototype representation.
These effects may be dissociated properly in the context of a counter-balanced experimental design (e.g., continuous cary-over; Aguirre 2007). A linear model may then be used to simultaneously estimate the otherwise confounded effects of similarity and prototype. I will show how this may be done both with event-related potential and fMRI data, and consider the interpretation of prior reports in the literature in light of these observations.

Disclosures: G.K. Aguirre, None.

Nanosymposium

834. Encoding of Visually Presented Faces II

Location: Room 5B

Time: Wednesday, November 17, 2010, 1:00 pm - 4:30 pm

Program Number: 834.14

Topic: D.04. Vision

Support: Temporal Dynamics of Learning Center SBE-0542013

NIH Grant 1 F32 Ey019445-01

NIH Grant 2 RO1 EY013441-06A2

Title: Category learning stretches diagnostic shape dimensions in human visual cortex

Authors: *J. R. FOLSTEIN, I. GAUTHIER, T. J. PALMERI;
Vanderbilt Univ., Nashville, TN

Abstract: Learning to categorize objects can selectively enhance the ability to perceive differences along object dimensions that are diagnostic for categorization. These selective increases in discriminability can be measured in discrimination tasks administered after category learning is completed, suggesting that category learning causes lasting changes in the visual system. The neural locus of these behavioral changes has not been clearly identified. Some previous studies have found that while category learning generally sharpens overall neural tuning for objects, the response of visual neurons does not depend

on learned categories. However, the paradigms used in these studies did not provide behavioral evidence for any selective increases in visual discriminability along diagnostic dimensions, so the absence of evidence for changes in neural discriminability in the visual system is perhaps not surprising.
We created a set of morphed cars differing along two dimensions, each defined by a morph-line between two parent cars. Having first demonstrated that category learning causes selective increases in visual discriminability along diagnostic dimensions in this space, we next conducted an fMRI study designed to identify increased neural sensitivity to diagnostic dimensions in visual cortex. Participants were trained to categorize the space of morphed cars according to a counterbalanced category boundary. Immediately after the training session, participants were scanned in a fast event-related fMRI adaptation design in which they judged whether similar pairs of cars occupied identical or slightly different positions relative to fixation. The critical pairs differed along either the diagnostic dimension (diagnostic pairs) or the non-diagnostic dimension (non-diagnostic pairs). Following the scanning session, participants performed a visual discrimination task on the same pairs of cars. Diagnostic pairs were easier to visually discriminate than non-diagnostic pairs. In terms of fMR-adaption, diagnostic pairs adapted less than non-diagnostic pairs in the left ventral temporal lobes near area pFs. Diagnostic pairs that crossed the category boundary also adapted less than diagnostic pairs that did not cross the category boundary in a more posterior area, likely overlapping area V4. These results suggest that when category learning causes dimensional modulation that is evidenced behaviorally, it can also cause selective and lasting increases in neural sensitivity to diagnostic dimensions in the visual system.

Disclosures: J.R. Folstein, None; **I. Gauthier**, None; **T.J. Palmeri**, None.

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.1

Topic: D.05. Visual Sensory-motor Processing

Support: SNSF 120652

Title: Reach and eye position modulate hand grasping signals in macaque premotor area F5

Authors: *S. J. LEHMANN[1,3], H. SCHERBERGER[2];

[1]Abt. Neurobiologie, [2]Neurobio., DPZ / German Primate Ctr. Goettingen, Goettingen, Germany; [3]Inst. of Neuroinformatics, ETH / Uni, Zurich, Switzerland

Abstract: The ventral premotor cortex (area F5) in the macaque brain has been shown to encode hand grasping movements, in particular the shape of fingers, in order to execute different grip types. In the present work we investigated the influence of different spatial factors on the grasp encoding in area F5 while monkeys performed a delayed grasping task. We systematically varied the initial hand, eye, and target position in space to study the influence of these signals on the coding of grasp type in F5.
Macaque monkeys were trained to grasp a handle with one of two grip types (either a precision grip or a power grip) while the position of the handle and the eye fixation light were systematically varied in three vertical and three horizontal positions (11 cm spacing). All task conditions were presented randomly interleaved, and eye position was monitored with an optical eye tracker. Grasping trials consisted of four epochs (fixation, cue, memory, and movement) during which the monkey had to maintain eye fixation of a red LED in the dark. To initiate the task, the animal placed its left hand at the start position and fixated the red LED. In the following cue epoch (1000ms duration), the handle position was revealed (target illumination) and the grasp type instructed by the color cue of a second LED (green: power grip; orange: precision grip). After another delay of about one second during which the animal could plan but not execute the movement, a 'go' signal was presented that required the animal to execute the grasp movement while maintaining eye fixation. All correct trials were rewarded with a small amount of fluid.
Preliminary results from one animal revealed the existence of F5 neurons that encode the spatial grasp target position as well as the eye fixation position in addition to the grip type. From 20 neurons recorded so far, 80% (n=16) were significantly tuned for grip-type (1-way ANOVA, $p<0.05$). Of these we found during the planning epoch 6 neurons (37.5%) modulated by the target position and 8 neurons (50%) by the eye fixation position. In contrast, these values were distinctly reduced during movement execution (target modulation: 25%, fixation: 37.5%).
These results suggest a strong modulation of grip-type specific grasping signals in F5 by the target location and eye fixation position. These modulated signals seem to encode grasp type with gain factors for target position in spatial and retinal coordinates that could reflect multiplexed representations in the same network. Interestingly, these spatial representations are dynamically reduced during grasp movement execution.

Disclosures: S.J. Lehmann: Research Grant; Swiss National Science Foundation (SNSF). **H. Scherberger:** None.

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.2

Topic: D.05. Visual Sensory-motor Processing

Support: NIH

Title: Causal evidence for effector specificity in early visuomotor areas LIP and PRR

Authors: *E. A. YTTRI[1], Y. LIU[2], C. WANG[2], L. H. SNYDER[2];
[1]Dept Anat. & Neurobiol, Washington Univ. St Louis, SAINT LOUIS, MO; [2]Dept Anat. & Neurobiol, Washington Univ. St Louis, Saint louis, MO

Abstract: The lateral intraparietal area (LIP) and the parietal reach region (PRR), both in the posterior parietal cortex, are involved in visuomotor transformations, but their exact roles remain controversial. Both areas are active when a visual target appears, and show sustained activity during a delay period prior to a movement to that target. LIP is very active in the delay period preceding a planned saccadic eye movement, and somewhat less active in the delay period preceding a planned reaching movement. Three interpretations of this have been suggested. First, LIP could be completely effector specific, playing a role in saccades but not in reaching. In this scenario, the activity recorded prior to a reach would be non-functional or would serve a function unrelated to the actual reach. Second, LIP could be incompletely effector specific, playing a major role in saccades and a minor role in reaches. Third, LIP could be completely effector non-specific. In this scenario, LIP activity reflects the subject's attentional locus or a salience map of the visual field. The reduced activity prior to a reach may reflect the possibility that subjects pay less attention to a planned reach target than to a planned saccade target. To distinguish among these possibilities, we used muscimol to reversibly lesion either LIP or PRR, an asked whether saccades, reaches or both types of movements were affected. The results were very clear: LIP lesions delay the initiation of saccades and have no effect on reaches, while PRR lesions delay the initiation of reaches and have no effect on saccades. Interestingly, LIP lesions did influence reaches when the animals were allowed to first look at the target before reaching for it. We believe that in this case, the reaching movement "waits" for the saccade system, and so the direct effect of the lesion on the saccade reaction time has an indirect effect on the reach reaction time. These results are important for several reasons. First, they resolve a long-standing issue regarding the functional specificity of parietal areas with regard to particular effectors. More importantly, they underscore the fact that measurements of neuronal activity are only one of several tools that must be used in order to understand brain function.

Disclosures: E.A. Yttri, None; **Y. Liu**, None; **C. Wang**, None; **L.H. Snyder**, None.

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.3

Topic: D.06. Eye Movements

Support: Adapt-Eye Grant N° ANR-06-NEURO-001

Title: Involvement of the parietal cortex in the adaptation of scanning voluntary saccades: An fMRI-guided TMS study

Authors: *M. PANOUILLERES, P. GERARDIN, L. HUDRY, R. SALEMME, C. URQUIZAR, D. PELISSON;
INSERM U864 Espace Et Action, Bron Cedex, France

Abstract: Saccades are fast, accurate and conjugated movements of the eyes. They are involved in perception of our environment by allowing to foveate objects of interest. Saccade accuracy is thus crucial for visual perception and its maintenance in a dynamic world is achieved thanks to sensory-motor adaptation processes. Two types of saccades can be defined: reactive saccades elicited by a sudden target presentation and voluntary saccades which are generated when scanning a stable visual environment. Even if the scanning voluntary saccades are the most common saccades in our daily-life, investigations of the adaptation of these saccades started only recently. Some evidence from behavioural studies suggest that the neural substrates of saccadic adaptation are different for reactive saccades and for voluntary saccades (Pelisson et al, Neurosci and Biobehav Rev 2010), with a recently postulated role of the parietal cortex in the adaptation of scanning voluntary saccades (Cotti et al, J Physiol 2009). The aim of our study was to use Transcranial Magnetic Stimulation (TMS) to assess the role of the parietal cortex during the adaptation of voluntary saccades and to try to establish the critical timing of action of this area in saccadic adaptation. Before starting the TMS sessions, each subject performed a functional MRI scan while executing pro- and anti-saccades toward visible targets. Areas involved in oculomotor behaviour were detected by contrasting BOLD signals measured in these oculomotor tasks versus the baseline task (central fixation). Then the Talairach coordinates of the activated cluster in the posterior intraparietal sulcus (pIPS) were extracted. A neural navigator system was used to specifically target this posterior area of intraparietal sulcus with the TMS coil. Amplitude shortening adaptation of scanning voluntary saccades was induced using the double-step target paradigm (Alahyane et al, Brain Res 2007) and a single-pulse TMS (at 120 % of motor threshold) was applied in each trial at a specific timing relative to primary saccade onset. In separate sessions, TMS pulses over pIPS occurred at 30, 60 or 90ms after

primary saccade onset. For the control sessions, TMS was applied over the Vertex at 30, 60 and 90ms after saccade onset. Preliminary results (6 subjects) show that TMS over the pIPS did not modify the amplitude of voluntary saccades. However, the voluntary saccades adaptation was decreased when TMS was applied over the pIPS as compared to vertex TMS. Recruitment of further subjects will allow us to test the crucial timing of the involvement of the posterior intraparietal sulcus in the adaptation of scanning voluntary saccades.

Disclosures: M. Panouilleres, None; **P. Gerardin**, None; **L. Hudry**, None; **R. Salemme**, None; **C. Urquizar**, None; **D. Pelisson**, None.

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.4

Topic: D.05. Visual Sensory-motor Processing

Support: Grant-in-Aid for Scientific Research on Priority Areas 20020002

Grant-in-Aid for Scientific Research (C) 22500348

Title: Neuronal activity in the periarcuate cortex during reaching by eyes and/or hand

Authors: *K. KURATA;
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Abstract: In reaching toward an object, human and nonhuman primates execute coordinated hand and eye movements. For generation of the behavior, our brain transforms location of the visuospatial target into motor commands specialized for eye and hand movements. However, it is not clear how the visuospatial transformation takes place to be utilized for control of different effectors in brain. Thus, we trained monkeys to make reaching movements by either eye or hand, or both, when visuospatially identical targets were presented on a LDC by aligning a cursor that indicated their hand position, and focused on the periarcuate cortex including the ventral premotor cortex (PMv) and the frontal eye field (FEF). In the task, we presented two types of instruction signals serially during a preparatory period: one of four visuospatial cues to indicate location of the forthcoming target and one of three color cues for effectors. The monkeys moved a hand held cursor with their right hand on a 45 x 30 cm digitizing tablet whose positions

and visual cues were displayed on the LCD. We also monitored eye movements using an infrared oculometer. Within a central holding zone for hand movements, a fixation spot for eyes was presented. After 0.6 s holding period, the first instruction signal (IS) for either an effecter (eye, hand, or both) or a target was presented. Further after 0.6 s, the second IS for an effecter or a target was presented. Orders for the two types of ISs were randomized. After the second preparation period, the central spot turned to blue to initiate the instructed movements. If the monkey correctly reached the target, a drop of juice was delivered as a reward.
We found distinct types of set- and movement-related neuronal activities in the periarcuate cortex before and during eye and/or hand movements. First, we recorded set-related neuronal activity in PMv that reflects serial transformations to prepare for eye and/or hand movements. Second, the FEF activities were mostly specific to saccadic eye movements, whereas those in the caudal areas depended on the task types (eye only, hand only, or both). Furthermore, we found a trend that neuronal activities in the FEF preceded the saccadic eye movements, but those of the cortex immediately caudal to the FEF lagged the eye movement onsets. Based on the findings, we suggest that the FEF is specialized for saccades, whereas the caudal areas including the fundus of the arcuate sulcus and the PMv contribute to (1) preparation for and execution of coordinated reaching movements and (2) monitoring required movements in the trials with different effectors.

Disclosures:

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.5

Topic: D.05. Visual Sensory-motor Processing

Title: From hand to eye: Oscillatory activity gates sensorimotor transformations

Authors: *V. N. BUCHHOLZ, O. JENSEN, W. MEDENDORP;
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Abstract: Synchronization of neuronal populations can be found in topographically organized sensory maps during stimulus encoding, as well as in regions involved in motor planning, like the posterior parietal cortex. However, because the spatial relationship between sensory stimuli and motor actions changes with body posture, the

transformation between them cannot depend on anatomical connections alone. Oscillatory activity has been suggested to implement selective routing of information contained in spikes, a mechanism that can be adjusted on small time scales. Here we investigated the temporal and spatial patterns of oscillatory activity during sensorimotor transformations for saccades. Under continuous recording of magneto-encephalographic data, subjects fixated either to the left or right of the body midline, while planning a saccade to a remembered tactile stimulus applied to an invisible fingertip, located either to the left or right of the fixation point. After stimulation, we found increased synchronization in the gamma band (~80 Hz) in primary somatosensory areas (S1) contralateral to the stimulated fingertip, in agreement with somatotopic processing. At the same time, increased gamma power was also found in the intraparietal sulcus (IPS) and lateral occipital cortex, at slightly higher frequencies (~100 Hz), but showing gaze-dependent processing. Modulations in the lower frequency bands reflected anticipatory effects. Prior to the tactile stimulation, there was a suppression of the alpha (8-12 Hz) and beta (18-30 Hz) band activity in S1 contralateral to the hand to be stimulated, whereas after stimulation alpha band suppression retuned to gaze-dependent processing in the IPS. Beta band activity showed somatotopic processing in S1 throughout the trial. Power-to-power correlations across trials revealed a change of functional connectivity between S1 and IPS for the two gaze positions, supporting the hypothesis that oscillations play a role in flexible routing of stimulus information. We conclude that gamma band activity can operate in two differently organized spatial maps at the same time, reflecting the dynamic routing of information between topographic maps that operate with different spatial reference frames.

Disclosures: V.N. Buchholz, None; **O. Jensen**, None; **W. Medendorp**, None.

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.6

Topic: D.05. Visual Sensory-motor Processing

Support: Leaders Opportunity Fund from the Canadian Foundation for Innovation (CFI)

Research Training Centre at The Hospital for Sick Children

Title: The effect of anisometropic amblyopia on online control during visually guided reaching

Authors: *E. NIECHWIEJ[1], H. C. GOLTZ[1,2], M. CHANDRAKUMAR[1], Z. A. HIRJI[1], A. M. F. WONG[1,2];
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Abstract: ***Introduction:*** Impairment of spatiotemporal visual processing in amblyopia has been studied extensively, but its effects on visuomotor tasks have been examined rarely. Visual feedback plays a major role in regulating goal directed arm movements. Here, we investigate how the visual deficits in amblyopia affect online control of visually guided reaching movements.
Methods: Twelve patients with anisometropic amblyopia and 12 visually normal subjects reached and touched a target presented on a computer monitor during binocular and monocular viewing. Hand movements were recorded with the Optotrak infrared system. Each trial began with a central fixation cross. After a variable delay, the target appeared randomly at 5 or 10 deg to the left or right of fixation. The degree of online control was assessed by calculating the coefficient of determination (R^2) which relates the spatial position of the limb at 25% (early), 50% (middle) and 75% (late) in the trajectory to the endpoint position. A low R^2 value indicates that online feedback was used to attenuate errors in the initial motor plan.
Results: Mean end-point accuracy was comparable between patients and control subjects in all viewing conditions; however, mean precision was significantly worse ($p=0.041$) when patients viewed with the amblyopic eye (4.2 mm) in comparison to fellow eye (3.6 mm) or binocular viewing (3.3 mm), and in comparison to control subjects (binocular 3.3 mm, monocular 3.7 mm). Patients exhibited significantly higher R^2 values ($p=0.015$) in all viewing conditions, which were particularly evident in the late trajectory (0.41 in patients vs 0.28 in control subjects).
Conclusions: When visual feedback was available, visually-normal subjects exhibited a higher peak acceleration and extended the duration of deceleration phase, which presumably allowed them to utilize online visual feedback of the hand as it approached the target in order to improve end-point accuracy and precision. Our data indicate that patients reached a similar level of accuracy and precision when viewing binocularly or with the fellow eye but used a different control strategy: they used primarily the initial motor plan and relied less on online visual feedback during the latter phase of the reach trajectory.

Disclosures: E. Niechwiej, None; **H.C. Goltz**, None; **M. Chandrakumar**, None; **Z.A. Hirji**, None; **A.M.F. Wong**, None.

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.7

Topic: D.05. Visual Sensory-motor Processing

Title: Effects of a gradual visuomotor rotation on error corrections while moving away from and towards a home target

Authors: *L. A. MROTEK[1], P. KRAEGEL[2];
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Abstract: Visuomotor rotations are often used to examine how subjects adapt to unusual environments. We examined how subjects corrected errors during a gradual visuomotor rotation both across and within trials. Every trial began with a cursor in the center of the monitor at a home dot and the subject could manipulate the cursor position using a standard mouse which could not be seen. The subject initiated a trial by clicking the mouse button and then a target dot appeared in one of ten locations located circularly around the home dot. The subject was to smoothly and quickly move the cursor on the monitor to the target dot and back to the home location. The home location and target dot were visible throughout every trial, and the cursor was visible on 90% of the trials. Over 170 trials the cursor motion was gradually rotated counter-clockwise to 50 degrees from the actual mouse motion. The subjects then completed 80 trials at a constant 50 degree rotation. We found that the maximal speed of the movements away from and back to the home location did not differ, nor did the speed differ as the visuomotor rotation increased. The maximal position error when the subjects moved away from the home location increased as the visuomotor rotation increased, but this change was not significant until the rotation was greater than 40 degrees. Furthermore, when the visuomotor rotation remained consistent at 50 degrees the error was reduced back to a level similar to the smaller rotation magnitudes. When the subjects returned to the home target location, the maximal position error was comparable at all visuomotor rotations. Maximum position error was also significantly larger while the subjects moved away from the home location compared to the return movement. We found differences in maximal movement speed and position error for different movement directions; however we believe that these differences are mainly due to arm dynamics. It seems that within trials subjects incorporated errors during the initial movement into predictions so that they could adjust to the environment for the second portion of the movement. However if the data are examined across trials, overall these corrections did not entirely account for the visuomotor rotation.

Disclosures: L.A. Mrotek, None; **P. Kraegel**, None.

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.8

Topic: D.05. Visual Sensory-motor Processing

Title: Dynamic reaching adjustment during continuous body perturbation is markedly improved by visual motion

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[2]Shimojo ERATO project, JST, Kanagawa, Japan; [3]Tokaigakuen Univ., Nagoya, Japan

Abstract: During arm reaching movement in our daily life, our body is not always stabilized. We can extend our arm to a cup on a table even during jogging and to a ball in playing tennis, baseball, and rugby football etc, suggesting dynamic adjustment mechanisms in our visually guided reaching. Because of slow adjustment of the voluntary control and insufficient vestibular-based compensation, additional quick reaction mechanisms should be equipped in the involuntary visuomotor control. In the last two decades, several visuomotor studies demonstrated that particular type of visual background motion applied during arm-reaching induces a quick manual response in the visual motion direction (Gomi 2008 Curr Opinion Neurobiol.), suggesting that visual motion which is usually in the opposite direction to the body motion may improve the reaching accuracy. However, it is not yet clear whether reaching error caused by body fluctuation can be visually reduced substantially.
To directly consider this issue, we have examined the effect of visual motion induced by body motion on the reaching movements in this study. Participants was asked to produce arm-reaching toward a visual target shown on the screen, with seating on a saddle which was continuously moved during reaching. Three types of visual background were presented during the task; 1) short-lifetime (33ms) dynamic-random dots (S-RD), 2) long-lifetime (330ms) dynamic random dots (L-RD), and 3) black screen (no visual background, control). In L-RD condition, the participants could receive rich visual motion information associated with the body motion because of enough duration of dots presentation, while they did not in the S-RD condition.
In the result, the endpoint variances was significantly different in those conditions. The horizontal variation in L-RD condition was significantly smaller than those for S-RD and black screen conditions. In addition, the reaching trajectory variation in L-RD condition was also smaller than the other two conditions whereas the hand path relative to the shoulder deviated more in the L-RD than in the S-RD condition. Since these visual stimuli did not affect reaching accuracy in static postural conditions, the results obviously suggest that the background visual motion induced by body fluctuation substantially

contributes to the online reaching adjustment.

Disclosures: H. Gomi, None; **K. Kadota**, None; **N. Abekawa**, None.

Nanosymposium

835. Guidance of Movements in Space

Location: Room 33C

Time: Wednesday, November 17, 2010, 1:00 pm - 3:15 pm

Program Number: 835.9

Topic: D.05. Visual Sensory-motor Processing

Support: Office of Naval Research NHRC60842

Gift from the Swartz Foundation

Title: Brain dynamics associated with navigation in 3-D space

Authors: *K. GRAMANN[1], B. RIECKE[2], S. WING[1], T.-P. JUNG[1], E. VIIRRE[1];
[1]UCSD, LA JOLLA, CA; [2]Simon Fraser Univ., Surrey, BC, Canada

Abstract: Spatial navigation is a complex task requiring integration of multisensory information on the navigator's movement in space based on distinct reference frames (egocentric or allocentric reference frames). Even though different reference frames contribute to successful spatial orienting, several factors influence the use of one or the other system. One factor is an individual proclivity to use specific reference frames during spatial orienting [Gramann et al. 2005; Riecke et al. 2008; Iaria et al. 2003]. Most studies on spatial navigation investigated spatial orienting in 2D space demonstrating that visual flow information on heading changes is sufficient to update one's position and orientation. In contrast, updating yaw and pitch rotation based on visual flow seems to be severely limited [Vidal et al. 2004]. However, no study investigated the influence of individual proclivities in using distinct reference frames during orienting in three-dimensional virtual environments. That is, do our preferred strategies for 2D navigation extend into the third dimension? And how do the underlying brain dynamics support this? We investigated homing performances of subjects preferentially using an egocentric or an allocentric reference frame during passages through three-dimensional space with heading changes in yaw and pitch. The participants' task was to keep up orientation during passages through star fields devoid of landmarks with heading changes in the horizontal or the vertical plane. At the end of a passage participants had to adjust a homing arrow to point back to the origin. High density EEG was recorded and analyzed

using Independent Component Analysis (ICA) and subsequent clustering of independent components (ICs).
Participants preferentially using an egocentric or an allocentric reference frame revealed comparable homing accuracy for heading changes in pitch and yaw. Importantly, approximately half of the subjects with a proclivity for an egocentric reference frame used the preferred frame for horizontal heading changes only but switched to an allocentric reference frame for heading changes in the pitch plane. The brain dynamics underlying spatial orienting for horizontal heading changes replicated previous results with increased activity in a wide spread cortical network during the passage [Gramann et al. 2009]. Heading changes in pitch were associated with increased activity in a wide-spread network with differences in task-related spectral perturbations as compared to heading changes in yaw. We discuss common patterns and differences in brain dynamics associated with the use of distinct reference frames for heading changes in pitch and yaw.

Disclosures: K. Gramann, None; **B. Riecke**, None; **S. Wing**, None; **T. Jung**, None; **E. Viirre**, None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.1

Topic: D.17. Voluntary movements

Support: McGovern Institute for Brain Research

Charles A King Trust

Title: A theory of biological sensorimotor learning as distinguishable from robotics

Authors: *R. J. AJEMIAN[1], A. D'AUSILIO[2], H. MOORMAN[3], E. BIZZI[1];
[1]MIT, Cambridge, MA; [2]Italian Inst. of Technol., Genoa, Italy; [3]Univ. of California at Berkeley, Berkeley, CA

Abstract: Viewing a passing shot hit on the run by a professional tennis player or a downhill putt into a crosswind by a professional golfer, one realizes that humans are capable of remarkable feats of adaptive dexterity that exceed the current capacities of robotic manipulators. Some of the ability is no doubt attributable to robust biological actuators (i.e., muscles) which maintain certain properties, such as active stiffness, that

are not easy to replicate in artificial actuators. However, biological mechanisms of adaptive control must also function exceedingly well to enable this level of performance. Yet, neurons are noisy computing elements whose signals propagate slowly, while computer chips are virtually noiseless with signal transmission velocities approaching the speed of light. How, then, are our brains so good at adaptive control? Here we propose a theory of horizontal computing in the highly parallel central nervous system, as it contrasts with vertical computing in robotic control. This theory is based upon three main assumptions: 1) the brain is extremely noisy; 2) synapses are constantly being modified at rapid rates (hyperplasticity); and 3) the nervous system is highly redundant at all levels. With these assumptions, we build a model of sensorimotor learning that seeks to explain unique properties of biological motor control, such as the Contextual Interference effect, Negative Transfer, and the need for task-specific warm-up.

Disclosures: R.J. Ajemian, None; **E. Bizzi**, None; **H. Moorman**, None; **A. D'Ausilio**, None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.2

Topic: D.16. Posture and Gait

Support: NSBRI Cooperative Agreement NCC9-58

Title: The role of adaptation in body load-regulating mechanisms during locomotion

Authors: T. M. RUTTLEY[1], *J. C. HOLT[3], A. P. MULAVARA[4], J. J. BLOOMBERG[2]; [1]Intl. Space Station Program Sci. Office, [2]Neurosci. Motion Lab., NASA Johnson Space Ctr., Houston, TX; [3]Otolaryngology, Univ. of Rochester|910006481|0, Rochester, NY; [4]Johnson Space Center- Neurosci. Motion Lab., Natl. Space Biomedicine Res. Inst., Houston, TX

Abstract: Body loading is a fundamental parameter that modulates motor output during locomotion, and is especially important for controlling the generation of stepping patterns, dynamic balance, and termination of locomotion. Load receptors that regulate and control posture and stance in locomotion include the Golgi tendon organs and muscle spindles at the hip, knee, and ankle joints, and the Ruffini endings and the Pacinian corpuscles in the soles of the feet. Increased body weight support (BWS) during

locomotion results in an immediate reorganization of locomotor control, such as a reduction in stance and double support duration and decreased hip, ankle, and knee angles during the gait cycle. Previous studies on the effect during exposure to increased BWS while walking showed a reduction in lower limb joint angles and gait cycle timing that represents a reorganization of locomotor control. Until now, no studies have investigated how locomotor control responds after a period of exposure to adaptive modification in the body load sensing system. The goal of this research was to determine the adaptive properties of body load-regulating mechanisms in locomotor control during locomotion. We hypothesized that body load-regulating mechanisms contribute to locomotor control, and adaptive changes in these load-regulating mechanisms require reorganization to maintain forward locomotion. Head-torso coordination, lower limb movement patterns, and gait cycle timing were evaluated before and after a 30-minute adaptation session during which subjects walked on a treadmill at 5.4 km/hr with 40% body weight support (BWS). Before and after the adaptation period, head-torso and lower limb 3D kinematic data were obtained while performing a goal directed task during locomotion with 0% BWS using a video-based motion analysis system, and gait cycle timing parameters were collected by foot switches positioned under the heel and toe of the subjects' shoes. Subjects showed adaptive modification in the body load-regulating mechanisms that included increased head movement amplitude, increased knee and ankle flexion, and increased stance, stride, and double support time, with no change in the performance of the task with respect to that measured before exposure to BWS. These changes in locomotor control are opposite to that reported during 40% BWS exposure and indicative of an after-effect after removal of the adaptive stimulus. Therefore, it is evident that just 30 minutes of 40% BWS during locomotion was sufficient to induce adaptive modifications in the body load sensing systems that contribute to reorganization of sensory contributions to stable locomotor control.

Disclosures: T.M. Ruttley, None; **A.P. Mulavara**, None; **J.C. Holt**, None; **J.J. Bloomberg**, None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.3

Topic: D.17. Voluntary movements

Support: NIH Grant NS37422

Title: A role of cerebellum in maximizing rewards during visuomotor adaptation task

Authors: *J. IZAWA[1], S. HEMMINGER[2], R. SHADMEHR[2];
[1]Grad. Sch. of Information Systems, The Univ. of Electro-Communications, Chofu-Shi/ Tokyo, Japan; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Motor learning has been assumed as a process to reduce "error" between the observed trajectory and the baseline trajectory in order to compensate for the perturbation. However, a recent result (Izawa, 2008) suggests that the principle of motor learning is to maximize rewards (i.e. task achievement). When we redefine motor learning as an optimization process, theory suggests that the neural architecture of motor learning may be composed of two systems: action selection updated by the reward-prediction error and forward model adaptation via sensory prediction error. How are these two systems embedded in the brain? Here we considered a group of patients who had a genetic disorder that led to loss of Purkinje cells in the cerebellum. We hypothesized that the cerebellum is important for predicting a visual consequence of action, and that basal ganglia is important for selecting action. If this is true, the cerebellar patients should be able to update their action (learning from reward prediction error) but be impaired at updating their forward model (learning from sensory prediction error). Subjects held a manipulandum with their right hand and made ballistic movements through a target. Their limb was covered by the screen while a cursor was projected on the screen to provide the visual feedback of the hand. The rotation of the cursor position was introduced gradually. Before and after subjects experienced this adaptation, we tested localization of movement direction. In the localization task, there was no visual target or visual cursor for the shooting movement. Rather, subjects were free to choose any goal. After they made the shooting without visual feedback, they were asked to make a pointing movement with their left hand toward the estimated right hand movement direction. With this paradigm, we quantified whether adaptation to the visuomotor rotation resulted in an updating of the estimate of hand position. The gradual introduction of the perturbation produced robust adaptation in the cerebellar patients. However, we found that despite the adaptation, the localization error was clearly larger in the control subjects than the cerebellar patients. This indicated that adaptation in the control subjects led to an updating of a forward model, but this updating was impaired in the cerebellar subjects. This result suggests that the system with which the brain responds to visuo-motor discrepancy is composed of two mechanisms: forward model and action selection. We found that the cerebellum is responsible for updating the forward model, which in turn suggests that the other neural substrate is responsible for updating action selection.

Disclosures: J. Izawa, None; **S. Hemminger**, None; **R. Shadmehr**, None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.4

Topic: D.16. Posture and Gait

Title: An adaptive state estimation model for postural control

Authors: *T. J. KLEIN[1,2], J. JEKA[3], A. M. LEWIS[2];
[1]Tucson, AZ; [2]Univ. of Arizona, Tucson, AZ; [3]Univ. of Maryland, Baltimore, MD

Abstract: Human postural balance incorporates sensory data from multiple sources, including visual, vestibular, and proprioceptive sources. Under time-varying noise conditions, humans appear to adaptively reweight sensory data. In the case of upright postural balance, human response is tested by using a moving visual reference field to create the illusion of motion. These human subject studies show that in humans, noisy sensory data is rapidly downweighted during a disturbance, then gradually upweighted when it becomes stable again. The underlying mechanisms governing sensory reweighting are not understood. Of particular interest is whether the nervous system incorporates an internal model of the body which could be used to perform state estimation.
Operating on the hypothesis that an internal model exists, we propose a model sensory reweighting mechanism using an adaptive Kalman filter. The adaptive filter estimates noise covariances in real time and modifies the Kalman gain for each channel such that the gain is downweighted when noise is high. We test this model in simulation and in a bipedal robot model and show that it is capable of adapting to time-varying noise conditions. This model successfully reproduces the response of human beings in which visual motion is rapidly downweighted, then gradually upweighted following a disturbance, supporting the hypothesisproviding evidence consistent with the hypothesis that an internal body model and state estimator may beis involved in human balance.

Disclosures: T.J. Klein: None. **J. Jeka:** None. **A.M. Lewis:** None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.5

Topic: D.17. Voluntary movements

Title: Grip force adaptation during a reaching task reflects uncertainty on environmental dynamics

Authors: *A. M. HADJIOSIF, J. B. BRAYANOV, M. A. SMITH;
Harvard Univ., Cambridge, MA

Abstract: To skillfully manipulate a object we need to simultaneously control its motion and prevent it from slipping from grasp. Grip forces (GF) have been shown to reflect internal models of object dynamics. However, previous work focused on the ability of GF to adapt to changes in the mean value of dynamics experienced in a novel environment. Here we show that the variability we experience independently contributes to GF control and with stronger effects than the mean. A highly uncertain, variable environment will demand higher GF levels in order to maintain grasp in a worst case scenario; however, optimal manipulatory forces should in most cases be essentially independent of the amount of variability in the environment and, instead, reflect the mean perturbation strength.

Here we created a variable environment with a static offset force throughout the baseline and a family of a viscous force-fields (FFs) in the training period. The strength of the viscous FF changed from one trial to the next, drawn from a Gaussian distribution with mean zero. We then compared blocks with four different variability levels. We found that GF levels systematically increased with environmental variability, whereas, MF levels showed essentially no modulation with variability levels, as expected, given that the mean of the viscous FF was not changed. Interestingly, we found that grip force patterns were modulated in a temporally-specific manner that anticipated the amount of force variability throughout the movement.

We proceed to show that the modulation of GF with uncertainty can explain adaptation phenomena even in cases where a constant perturbation is learned. In this case, uncertainty is introduced at the onset of a new perturbation and diminishes with prolonged exposure to it. We show that this framework can explain previous results where GF appeared to adapt faster than MF in a task in which variability-induced GF modulation was aligned with mean-induced GF modulation by studying two different viscous FFs - one in which adaptation to the mean and variability were in the same direction and one in which these adaptations opposed one another. This allowed us to disambiguate the variability-induced and mean-induced GF adaptations. We observed that the component of GF corresponding to the mean FF displayed essentially the same timecourse as the adaptation of MF ($r = 0.96$). Synthesis of the data revealed that GF was modulated about 1.8x more strongly by the standard deviation of the applied FF than by its mean. This ratio corresponds to a 96% (one-sided) confidence interval, suggesting that the motor system takes variability into account adapting GF to roughly maintain a 95% confidence against slippage.

Disclosures: A.M. Hadjiosif, None; **J.B. Brayanov**, None; **M.A. Smith**, None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.6

Topic: D.17. Voluntary movements

Support: NIH Grant NS037422

Title: Seeing is not always believing: Sensitivity to visual and proprioceptive errors

Authors: *M. K. MARKO, A. M. HAITH, M. D. HARRAN, R. SHADMEHR; Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Motor adaptation appears to be driven by sensory prediction error, but how do we respond to errors across different modalities like vision and proprioception? It is unclear what the relative contribution of error in each modality is, and how multiple sources of sensory feedback are integrated in our brain to guide adaptation. We measured motor learning in 22 subjects by giving a combination of force and visual perturbations to their reaching movements. Through this combination of perturbations, sensory feedback in individual trials was manipulated so that the visual prediction error was either larger, the same as or smaller than the proprioceptive prediction error. We observed that decreasing visual prediction error relative to proprioceptive error lead to a decrease in adaptation. However, increasing visual error relative to proprioceptive error did not increase adaptation. For very large errors, increasing visual prediction error actually led to a decrease in the extent of adaptation. This sigmoidal pattern of learning can be attributed to either the discrepancy between the two modalities, or the total error in the movement. Because the sigmoidal sensitivity to error was apparent when there was no discrepancy between the modalities, and there was no clear discrepancy dependent pattern when the total error was held constant, the data suggests that sigmoidal sensitivity to error is due to the difference between predicted and observed sensory consequences of motor commands, and not the difference between prediction errors among the various sensory modalities. We can explain these results through a Bayesian model in which adaptation corresponds to inferring the size of a perturbation when the subject is uncertain about the variance of their observation noise. As the size of an error increases, the posterior confidence in each observation decreases and the sensitivity to error is therefore reduced. In other words, we learn relatively less from large errors because such errors reduce our confidence about the sensory feedback.

Disclosures: M.K. Marko: None. **A.M. Haith:** None. **M.D. Harran:** None. **R. Shadmehr:** None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.7

Topic: D.17. Voluntary movements

Support: NIH Grant 1F31NS058275-01A2

NIH Grant DP1 OD003646

Title: Assessment of lower extremity motor adaptation via an extension of the force field adaptation paradigm

Authors: *I. CAJIGAS[1,3], M. T. GOLDSMITH[4], M. A. SMITH[5], E. N. BROWN[3,2], P. BONATO[3];
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Abstract: Ambulation is a very important part of everyday life and its absence has a detrimental effect on an individual's quality of life. Until technologies are developed to allow restoration of damaged neural tissue back to its original state, physical therapy (which aims to restore function by establishing new motor-cortical connections among the remaining neurons) remains the most viable option for patients. While meta-analysis of a clinical trials While systematic analyses have demonstrated the effectiveness of physical therapy in gait retraining compared to placebo, comparison of of superiority among distinct strategies in restoring motor function remains are equivocal. This is likely due to the multitude of approaches, underlying pathophysiology of diseases, and assessment scales used. To address the latter of these issue, this issue we introduce a new clinical lower extremity measure of lower extremity short-term learning motor (adaptation) that may help guide an individuals' course of lower extremity rehabilitation. We develop an extension of the Force Field Adaptation Paradigm, originally developed to quantitatively assess upper extremity motor adaptation, to the lower extremity. The algorithm is implemented on the only FDA approved lower extremity gait orthosis (Lokomat, Hocoma HG) and utilized to assess short term motor adaptation in healthy

adult subjects. Establishing an understanding of how healthy adults' motor systems adapt to external perturbations will be important to understanding how the how the adaptive mechanisms involved in gait are altered by disease.

Disclosures: I. Cajigas, None; **M.T. Goldsmith**, None; **M.A. Smith**, None; **E.N. Brown**, None; **P. Bonato**, None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.8

Topic: D.17. Voluntary movements

Support: US-ISRAEL BSF grant 2007195

NINDS grant 1R01NS053581

Title: Space-time separability in the sensory-motor system

Authors: *F. A. MUSSA-IVALDI[1], A. PRESSMAN[2], L. S. SIMO[3], A. KARNIEL[2]; [1]Dept Physiol, Physical Med., Northwestern U./RIC, CHICAGO, IL; [2]Biomed. Engin., Ben Gurion Univ. of the Negev, Beer Sheva, Israel; [3]Physiol., Northwestern Univ., Chicago, IL

Abstract: It is self evident that our brains can represent spatial concepts- such as distances between points- and temporal concepts - such as the duration of an interval. Are these representations of space and time mutually independent? The laws of classical, Galilean, physics describe the universe as a four-dimensional affine space, whose points are space-time events. Ordinary space, in this context, is a three-dimensional subspace of the space-time continuum, describing all events that are simultaneous to any given event. Since simultaneity in classical physics is coordinate independent, space and time are separable entities. However, this description implies that space is defined by simultaneity and therefore, any alteration of simultaneity may lead to some consequent alteration of space. In sensory-motor processes, simultaneity can be altered either by natural occurrences, which affect conduction delays across different sensory modalities, or artificially, through the interaction with virtual reality environments. We developed a simple computer game where a player must repeatedly hit a moving ball, as in a game of pong. In this game, we introduced a delay between sensory events (the visual scene, the

collision forces, etc) and the action of the players. After subjects adapted to the time delay we tested their proprioceptive sense of space by asking them to perform reaching movements in the absence of visual feedback. We found that the exposure to delayed sensory-motor information resulted in a systematic recalibration of proprioceptive space. We considered three competing models to account for this recalibration: a) a model that uses the information on the visuomotor mismatch throughout the entire game period, b) a model that compensates for the average amount of visuomotor mismatch but only at discrete events, i.e. the collisions between hand and moving ball, and c) a Bayesian model that also focuses on discrete collision events but does so by optimally combining a prior and a likelihood distribution for the visuomotor mismatch. Our findings refute the first hypothesis. A comparison of the second and third hypothesis by their likelihood ratio provides the stronger support to the Bayesian model. We discuss the implications of this finding with respect to the influence of temporal simultaneity on the formation of continuous representations of proprioceptive space.

Disclosures: F.A. Mussa-Ivaldi, None; **A. Pressman**, None; **L.S. Simo**, None; **A. Karniel**, None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.9

Topic: D.17. Voluntary movements

Support: FORTUNE Program of the University Hospital Cologne

Title: Motor skill learning within the speech network- a combined behavioral and fMRI study

Authors: *I. G. MEISTER[1], S. C. LIPSKI[1], M. GRICE[2], G. R. FINK[1];
[1]Dept of Neurology, Univ. Hosp. Cologne, Cologne, Germany; [2]Linguistics, Univ. of Cologne, Cologne, Germany

Abstract: Speech is among the most complex motor behaviors of man. The acquisition of a new articulatory gesture requires the implementation of intricate motor programs coordinating over hundred bilateral muscles with millisecond precision. Whereas the cortical network subserving speech has been characterized, the representational plasticity within the cerebral speech network during the process of motor skill learning is unknown

so far.
The present study combined behavioral measures and functional Magnetic Resonance Imaging (fMRI) to investigate the learning processes underlying the acquisition of an articulatory gesture which did not belong to the repertoire of subjects' native language German.
14 healthy subjects trained the utterance of the two voiced plosives /gb/ in initial position of nonwords over two weeks. This CC cluster violates phonotactic restrictions of German. As control, a phonotactically valid CVC cluster with a vocal in between the consonants (/gib/) was uttered. The learning process was characterized by three fMRI sessions before training, after one week of training and after the end of the two-week training. On the behavioral level, learning was characterized by the duration of the epenthetic schwa vowel, which was inserted by all subjects between the voiced plosives at the beginning of the training to avoid utterance of the phonotactically impossible CC cluster.
Analysis of the behavioral data revealed that subjects made significant learning progress and achieved increasing automaticity during training. The functional imaging data (analysis using FSL, significance threshold $p<0.05$, corrected (FDR)) showed a decrease of fMRI activation within bilateral inferior frontal and ventral primary motor regions and left superior temporal cortex during the learning process. Furthermore, interaction analysis showed a significant effect within left putamen, left posterior insula, left posterior superior temporal gyrus and supramarginal gyrus, which was driven by the fMRI activation during utterance of the novel CC cluster during the first scan prior to motor learning.
The results of the present study showed that speech motor skill learning involves a predominantly left hemispheric network including insula, basal ganglia and posterior perisylvic regions. These regions have been associated with speech perception (thereby providing feedback during speech production) and speech motor planning. We assume that these results reflect the progress of motor skill learning, which according to recent models is characterized by initial reliance on perceptual feedback and later development of feedforward models with increasing proficiency.

Disclosures: I.G. Meister, None; **S.C. Lipski**, None; **M. Grice**, None; **G.R. Fink**, None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.10

Topic: D.17. Voluntary movements

Support: NIH Grant R01 DC004855

NIH Grant R01 DC006435

American Heart Association

Title: Plasticity in structural and functional connectivity underlying recovery after stroke as detected by MEG, fMRI, and probabilistic fiber tracking

Authors: *K. P. WESTLAKE[1], M. BUCCI[2], L. B. HINKLEY[2], A. GUGGISBERG[3], R. HENRY[2], S. NAGARAJAN[2];
[1]UCSF, SAN FRANCISCO, CA; [2]Dept. of Radiology and Biomed. Imaging, UCSF, San francisco, CA; [3]Div. of Neurorehabilitation, Dept. of Clin. Neurosciences, Univ. Hosp. Geneva, Geneva, Switzerland

Abstract: Introduction: After cerebral ischemia, disruption and subsequent reorganization of functional and structural connections can occur both locally and remotely to the lesion. In this study, we aimed to identify plasticity of these connections with respect to motor recovery of the upper extremity after stroke using a multi-modal brain imaging approach. Methods: We tested subjects demonstrating motor involvement of the hand following an ischemic stroke in the territory of the middle cerebral artery. Using magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI), we recorded spontaneous brain activity while subjects lie in an awake, resting state. An adaptive spatial filtering technique was applied to MEG data and a mean value of imaginary coherence was then computed for each voxel paired with all other brain voxels as a measure of functional connectivity (fcMEG) in the 8-12Hz frequency range. For fMRI data, probabilistic independent component analysis (PICA) was carried out in FSL MELODIC v3.09 to obtain functional connectivity maps within a sensorimotor network (fcfMRI). For fiber tracking of structural connections within a motor network, we applied probabilistic QBall fiber tracking algorithm to HARDI data. Seed regions for the motor network were defined using MEG regions of activation during a button press task of the affected and unaffected hands. To identify recovery, a composite measure of change scores was calculated using the upper extremity Fugl-Meyer sensorimotor Scale, modified Rankin, timed Wolf Motor Function subscale, and grip strength. Subjects were assessed twice with 8-12 weeks between visits.
The relationship between changes in brain imaging metrics and the composite recovery score was assessed using Pearson's correlation coefficients. Results: Functional connectivity changes (fcMEG and fcfMRI) demonstrated a shift towards the ipsilesional motor network with fcMEG changes primarily observed as a reduction in connectivity in the contralesional hemisphere. Our data also provide evidence of the structural changes underlying this shift in connectivity and support a bilaterally distributed process of plasticity. Transcallosal connections between the two primary motor (M1) cortices and between the lesioned MI and contralesional supplementary motor cortex, not present at timepoint one, were identified at timepoint two. Plasticity within these networks appears related to upper extremity recovery. Conclusions: Together, these findings show that

plasticity after stroke affects distributed neural networks and defines brain regions in which the extent of network participation underlies the recovery process.

Disclosures: K.P. Westlake: None. **M. Bucci:** None. **L.B. Hinkley:** None. **A. Guggisberg:** None. **R. Henry:** None. **S. Nagarajan:** None.

Nanosymposium

836. Adaptive Control of Movement

Location: Room 1B

Time: Wednesday, November 17, 2010, 1:00 pm - 3:45 pm

Program Number: 836.11

Topic: D.17. Voluntary movements

Support: Whitaker International Fellows and Scholars Program

European Union, under FP7 project HUMOUR

Title: Stiffness versus intermittent control in an unstable bimanual task

Authors: *D. J. SAHA[1], P. MORASSO[2,3];
[1]Northwestern Univ., CHICAGO, IL; [2]Robotics, Brain, and Cognitive Sci., Italian Inst. of Technol., Genoa, Italy; [3]Univ. of Genoa, Genoa, Italy

Abstract: The concept of how subjects choose from various control strategies is becoming increasing relevant in the study of motor control. Knowledge of the factors that affect the choice of a control strategy is critical for the development of work equipment that minimizes injury and musculoskeletal disorders.
Previous studies tested the plausibility of a number of motor control strategies based on different mechanisms, such as continuous feedback, stiffness modulation, and intermittent control, in a variety of tasks. Since the feasibility of each strategy is strongly dependent on the nature of the task and the specific environmental conditions, no firm conclusion could be drawn on the identification of an optimal stabilization strategy for unstable tasks. The goal of this preliminary study was to design a task that allowed two biologically plausible solutions: 1) a stiffness strategy and 2) an intermittent stabilization strategy. Specifically, we wished to characterize the spatiotemporal patterns in the two cases and identify the factors that dictate which strategy will be chosen for stabilization. A haptic, bimanual manipulandum has been used to implement an unstable task, which requires subjects to stabilize a mass-load under the action of a saddle force field with two non-linear springs. Subjects learn to position the mass at various target points by

adjusting the rest length, and thus the stiffness of the two springs. Since the springs are nonlinear, the stiffness increases as the springs are stretched. Subjects can stabilize the mass by either 1) applying large forces to stretch the springs and increase the mechanical stiffness of the system (stiffness strategy) or by 2) applying force impulses and intermittently adjusting the position of the mass (intermittent strategy). The former control policy is simple but fatiguing, while the latter one is less fatiguing but computationally more challenging.
Preliminary results indicate that human subjects can adopt one strategy or the other. The choice is correlated with the bimanual configuration of their hands as well as the dynamics experienced during the task. Subjects that align their hands along the stable manifold apply an intermittent strategy, while subjects that align their hands along the unstable manifold apply a stiffness strategy. Moreover, the dynamics of the mass during the first few trials were critical in determining the strategy applied throughout the experiment. Subjects that experienced large mass velocities along the unstable manifold often applied an intermittent strategy, whereas subject that experienced moderate velocities during the first few trials applied the stiffness strategy.

Disclosures: D.J. Saha, None; **P. Morasso**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.1

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DC03681

Title: Reassessing the pathway for intelligible speech

Authors: *G. S. HICKOK;
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Abstract: Sophie Scott and colleagues published an influential series of papers in the last decade, which described functional imaging experiments that contrasted various forms of intelligible and unintelligible speech stimuli. The primary finding from this work was that a left anterior superior temporal region responded preferentially to intelligible speech. This led to the view that the pathway for intelligible speech projects anterior from primary auditory areas within the left hemisphere. Here I will reassess the evidence for

this view both from the perspective of the neuropsychological literature and from two new functional imaging studies using the same intelligibility manipulations to those in the Scott et al. experiments. The new studies found a bilateral pattern of activation that includes posterior temporal regions as well as anterior regions. I conclude that the pathway for intelligible speech is bilaterally organized with posterior temporal regions supporting lexical-phonological processing and anterior regions supporting higher-order integrative functions.

Disclosures:

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.2

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DC03681

Title: Neuroanatomy of speech perception in noisy conditions: A lesion study

Authors: *C. ROGALSKY[1], T. LOVE[2], S. SHIVAPOUR[3], D. DRISCOLL[4], S. W. ANDERSON[3], G. HICKOK[5];
[1]Dana and David Dornsife Cognitive Neurosci. Imaging Ctr., USC, Los Angeles, CA; [2]San Diego State Univ. & Univ. of California, San Diego, CA; [3]Univ. of Iowa, Iowa City, IA; [4]NINDS/NIH, Bethesda, MD; [5]Univ. of California, Irvine, CA

Abstract: Recent imaging and transcranial stimulation studies suggest that inferior frontal regions may contribute to speech perception in degraded, difficult situations. In addition, numerous studies have demonstrated a tight link between sensory and motor speech processes. However, few studies have assessed the effects of lesions to inferior frontal regions on degraded speech perception. The present study explored this possibility by measuring speech comprehension abilities of patients with left hemisphere focal chronic lesions (n = 21) and age-matched controls. Subjects completed a psycholinguistic battery to assess their phonological, lexical, and sentence-level speech comprehension and production abilities. This battery included auditory word-to-picture matching tasks in which an auditory single word was presented alone, and in white noise 14 db above the root mean squared of the word. The task was to select which picture in a 4-item array corresponds to the word presented. The picture array contained the target, as well as a

phonological, semantic, and unrelated foil picture. For purposes of group analysis, lesion patients performing at least two standard deviations below the mean control subject performance on a given task were categorized as impaired, and patients performing within one standard deviation were categorized as unimpaired. Seemingly in support of frontal regions contributing to speech perception in noisy situations, patients with impaired speech comprehension in noise had overlapping lesions in Broca's area (pars triangularis & pars opercularis), as well as posterior superior temporal gyrus (STG), and supramarginal gyrus (SMG). However, a subtraction of the regions lesioned in the unimpaired performers from the regions lesioned in these impaired performers reveals that Broca's area lesions are present in both groups, whereas the temporo-parietal lesions are predominately associated with impaired performance. Furthermore, Broca's area lesions were not associated with a greater decrease in performance between the "no noise" and "noise" conditions than that of the control subjects. Analyses of error types indicate that the rate of phonological errors is not correlated with any specific lesion pattern, but that the semantic and unrelated error rates are driving the temporo-parietal lesion pattern associated with impaired speech comprehension in noise, as well as the similar pattern correlated with a greater decline in performance between the "no noise" and "noise" conditions. These preliminary results suggest that speech comprehension in noisy conditions may engage Broca's area, but is not dependent upon Broca's area.

Disclosures: C. Rogalsky, None; **T. Love**, None; **S. Shivapour**, None; **D. Driscoll**, None; **S.W. Anderson**, None; **G. Hickok**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.3

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DC008072-01A2

NIH Grant DC008171-01

Title: Differential processing of perception and stimulus changes in auditory streaming

Authors: *K. T. HILL[1], L. M. MILLER[2];
[1]UC Davis, DAVIS, CA; [2]UC Davis, Davis, CA

Abstract: Auditory streaming is an important process that allows humans to segregate and group auditory objects. While the behavioral properties of streaming have been well studied, there is relatively little knowledge about its neural mechanisms. We presented subjects with continual ABA tone sequences that alternated perceptually, in a bistable manner, between grouped and segregated streams. Using electroencephalography (EEG), we identified the neural markers of both changes in perception (grouped vs segregated) and changes due solely to stimulus attributes. We found two novel neural markers that are driven by a change in perception. First, the neural response is significantly earlier to the middle, captured tone in the ABA triplets when the stimuli are grouped relative to segregated, suggesting faster processing of sounds within a stream. Second, we observed an anterior negative amplitude difference for grouped sounds at ~200ms after the triplet begins. In addition, stimulus-related changes in activity show a spatio-temporal progression distinct from changes due to perception. Funding for the project was provided by the National Institute on Deafness and Other Communication Disorders (NIDCD).

Disclosures: K.T. Hill: None. **L.M. Miller:** None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.4

Topic: F.01. Human Cognition and Behavior

Title: Cortical spectral dynamics of load effect in phoneme verbal reproduction

Authors: *A. B. HERMAN[1], A. FINDLAY[2], M. VERTINSKI[2], J. HOUDE[3], S. VINOGRADOV[2], S. NAGARAJAN[2];
[1]Radiology, [2]UCSF, San Francisco, CA; [3]UCSF, San Francsico, CA

Abstract: In this MEG study of phoneme repetition, we make a significant contribution to our understanding of the temporal, spatial and spectral properties of the neural processes associated with the phonemic components of speech encoding, memory and production. Subjects (n=17) performed a verbal repetition task in which they heard a pre-recorded two or four phoneme sequence and were instructed to repeat the phoneme sequence, while MEG neural responses were recorded. Source localization followed by time-frequency analysis of cortical oscillatory power was performed using CTF and NUTMEG software packages. Stimulus and response-locked activations were computed

to identify the general phoneme repetition network. Activation contrasts were computed between the four and two phoneme trials for both the encoding and repetition stages to examine the influence of increased cognitive load on changes in oscillatory cortical activity. Group analyses with permutation tests were performed to asses statistical significance. Our results from analyzing correct trials reveal a network of brain regions associated with a load-effect in phoneme-encoding and production including the superior, medial and posterior temporal, ventral premotor, Broca's dorsolateral prefrontal cortical areas. While encoding and repetition resulted in power changes in both low and high frequency bands, the most significant load effects were found in the high gamma (50-115 Hz) band, lateralized to the left side. Furthermore, when incorrect trials were included in the analysis, the ratio of high gamma power in these areas between the 4 and 2 phoneme conditions showed statistically significant correlation with difference in the accuracy rates between the 4 and 2 phomene conditions. These neural-behavioral correlations at the very least demonstrate that gamma power changes reflect encoding and production accuracy, and may be required for accurate repetition of speech sounds.

Disclosures: A.B. Herman, None; **A. Findlay**, None; **M. Vertinski**, None; **S. Vinogradov**, None; **S. Nagarajan**, None; **J. Houde**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.5

Topic: F.01. Human Cognition and Behavior

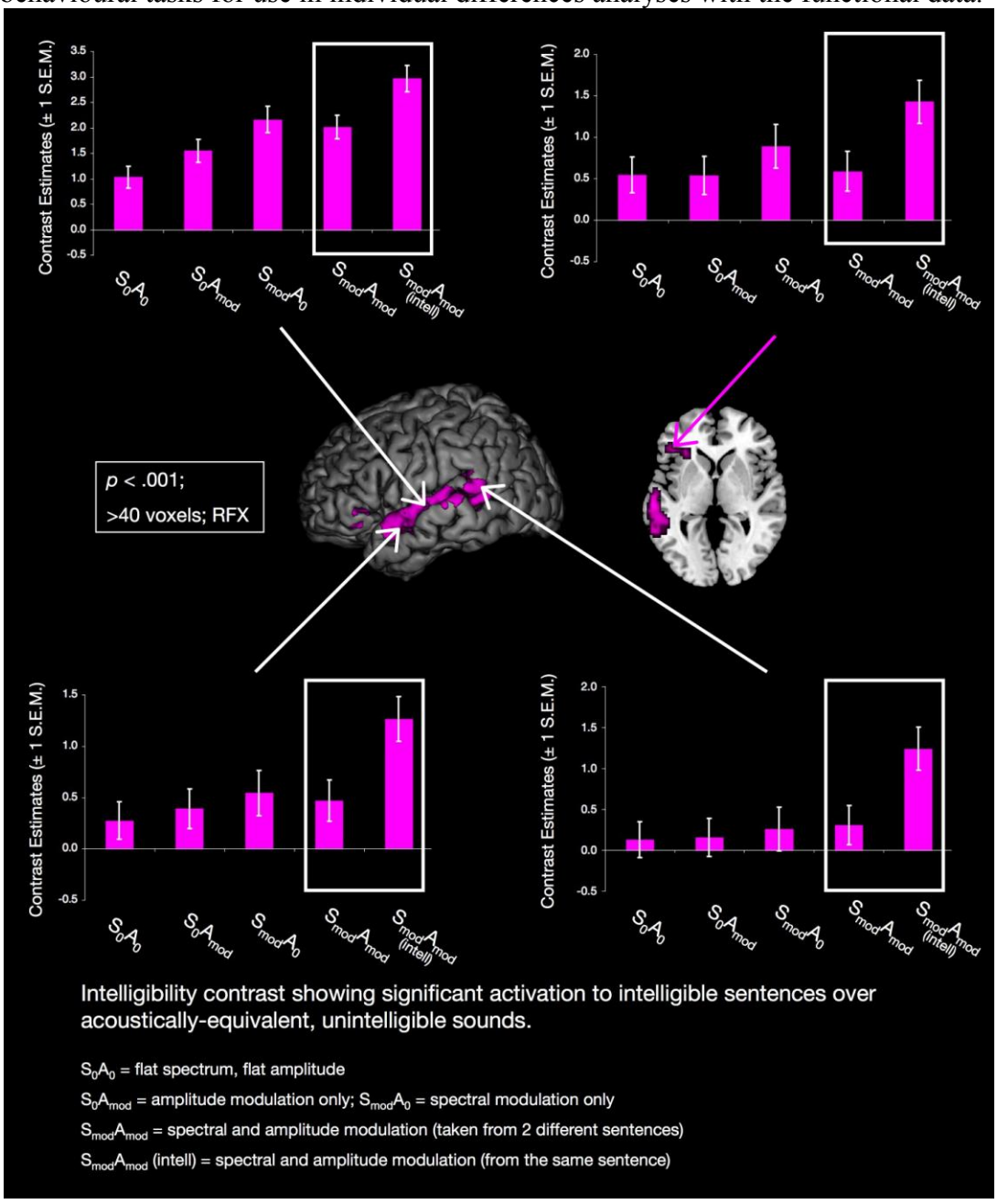
Support: Wellcome Trust Grant WT074414MA2

Title: The contributions of spectral and amplitude modulations to speech perception: Dissociating acoustic and linguistic responses with fMRI

Authors: *C. MCGETTIGAN, S. ROSEN, S. D. EVANS, Z. K. AGNEW, P. SHAH, S. K. SCOTT;
Univ. Col. London, London, United Kingdom

Abstract: We investigated the neural responses to amplitude and spectral modulations in speech, and how these interact with speech intelligibility, with the specific aim of addressing previous claims for hemispheric asymmetries in acoustic and linguistic processes in speech perception. Stimuli derived from the first two formants of simple

English sentences, in which modulations of spectrum and amplitude were either (i) absent (ii) applied singly or (iii) applied in combination, were presented to normal-hearing adults in a passive listening paradigm in fMRI. Additive responses to spectral and amplitude modulations for unintelligible stimuli were seen in bilateral superior temporal gyrus, with no clear indication of asymmetries in the response profiles. However, a comparison between an intelligible subset of the items with acoustically-equivalent unintelligible sounds gave a strongly left-lateralized pattern of activation along the extent of the superior temporal sulcus and in the left inferior frontal gyrus (LIFG), with indications of a selectivity for intelligible stimuli in both posterior and anterior sites in STS. Speech comprehension performance scores were collected from pre- and post-scan behavioural tasks for use in individual differences analyses with the functional data.



Disclosures: C. McGettigan, None; **S. Rosen**, None; **S.D. Evans**, None; **Z.K. Agnew**, None; **P. Shah**, None; **S.K. Scott**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.6

Topic: F.01. Human Cognition and Behavior

Support: Max Planck Society (J.O.)

German Science Foundation (N.W.)

Title: Differential influences of spectral and temporal features of speech on human oscillatory brain dynamics

Authors: *J. OBLESER[1], N. WEISZ[2];
[1]Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Univ. of Konstanz, Konstanz, Germany

Abstract: This study investigates the oscillatory brain dynamics in human EEG in response to acoustic features of speech. We specifically hypothesized that effortful comprehension under adverse listening conditions should affect time-course and extent of event-related desychronizations in the alpha (8--13 Hz) band.
Spectral and temporal features of acoustically degraded words were parametrically varied. We analysed evoked and induced (non-phase-locked) EEG responses in 24 healthy German participants, who listened and rated comprehensibility after each trial. Trials were grouped according to spectral detail (2-, 4-, 8-, 16-band vocoding) or temporal detail (2-, 4-, 8-, 16-Hz lowpass filtering of the vocoding envelopes). Permutation tests of a regression t-statistic for time-frequency-electrode clusters were run on wavelet transform data as well as conventional evoked potential data, followed by beamformer locations of effects of interest.
The most salient result was a first left-posterior, then broadening cluster of alpha desynchronization from 600 to 900 ms after stimulus onset: Alpha power appeared linearly suppressed for more spectral detail in a trial, and hence a more likely success of comprehension. Temporal detail had a very comparable effect in the same time/frequency range, but its effects were strongest over right-posterior channels. Beamformer localisations of the relevant time--frequency clusters added evidence for these

hemispheric differences in source space. Thus, comprehension-coupled alpha power behaves inversely and contralaterally to BOLD changes observed previously for these manipulations. When pooling across all spectral/temporal conditions in left and right mid-posterior channels, a significant negative correlation of Alpha power and comprehension ratings occured.
Generally, comprehension-rating scores confirmed the known predominance of spectral detail for word comprehensibility. In the same vein, parametric increases of spectral detail (i.e., increasing intelligibility) led to decreases of N100 and N400 amplitudes to word onsets, while left-anterior theta (4--6 Hz) synchronisation linearly increased.
Our results offer a new link between speech comprehension, processing of acoustic features, and oscillatory brain dynamics.

Disclosures: J. Obleser, None; **N. Weisz**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.7

Topic: F.01. Human Cognition and Behavior

Title: Neural substrates of rhythm, timing, and speech comprehension

Authors: *S. A. KOTZ[1,1], M. SCHMIDT-KASSOW[2], K. ROTHERMICH[3];
[1]Neurocognition of Rhythm in Communication Group, Max-Planck Inst. Cog & Brain Sci., Leipzig, Germany; [2]Dept. of Med. Psychology, Johann Wolfgang-Goethe Univ., Frankfurt, Germany; [3]Neurocognition of Rhythm in Communication Group, MPI for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Cortical neural correlates of linguistic functions are well documented in the neuroscience and neuropsychological literature. However, the influence of non-linguistic functions such as rhythm and timing are still understudied in speech comprehension. This is surprising as rhythm and timing play a critical role in learning, can compensate acquired and developmental speech and language disorders, and further our understanding of subcortical contributions to linguistic and non-linguistic functions. For example, recent neuroimaging and clinical evidence has confirmed the contributions of classical motor control areas (cerebellum (CE), basal ganglia (BG), supplementary motor area (SMA)) in rhythm, timing, music, and speech perception (Chen et al., 2008; Grahn et al., 2007; Geiser et al., 2009; Kotz et al., 2005; 2009). We consider serial order and

temporal precision to be the mechanisms that are shared in simple and complex motor behaviour (e.g. Salinas, 2009) and speech comprehension (Kotz et al., 2009). Here we investigate with event-related brain potentials (ERPs) and functional magnetic resonance imaging (fMRI) (1) how syntax, adhering to serial and hierarchical order, and rhythm, organizing the temporal unfolding of utterances in speech, interact, and (2) how classical motor areas interface with supposed specialized areas in the perisylvian speech comprehension network. Our results reveal an interaction of syntax and rhythm in the P600 ERP component that is linked to sentential integration processes (Schmidt-Kassow & Kotz, 2009),a facilitatory effect of rhythmic regularity in classical perisylvian speech areas such as the superior temporal gyrus/sulcus (STG/STS), and the recruitment of classical motor areas (preSMA, lateral premotor cortex, BG, and CE) highlighting the impact of rhythm on syntax in speech comprehension.

Disclosures: S.A. Kotz, None; **M. Schmidt-Kassow**, None; **K. Rothermich**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.8

Topic: F.01. Human Cognition and Behavior

Support: MRC U.1055.04.013.01.01

Title: Semantic ambiguity resolution is impaired in the absence of directed attention: fMRI studies

Authors: *M. DAVIS[1], J. E. PEELLE[1], S. S. VITELLO[3], R. J. EASON[2], J. M. RODD[3]; [2]Cognition and Brain Sci. Unit, [1]Med. Res. Council, Cambridge, United Kingdom; [3]Div. of Psychology and Language Sci., Univ. Col. London, London, United Kingdom

Abstract: When comprehending sentences containing words with multiple meanings (e.g., “bark”), listeners must select the appropriate meaning for the current linguistic context (e.g., dog or tree). These disambiguation processes are associated with increased activity in a fronto-temporal network for activating, maintaining and selecting between competing meanings. Ambiguity-associated activation in patients with disorders of consciousness and disruption by sedation allow ambiguity resolution to be used as a marker for intact comprehension. However, the degree to which ambiguity resolution relies on directed attention in awake participants is unclear.

We report two fMRI studies in which listeners heard sentences containing ambiguous words ("the shell was fired towards the tank") along with matched control sentences. Attention was manipulated by presenting multiple concurrent stimuli and instructing participants to respond to sentences or distractors. In Experiment 1, the concurrent task was a visual, non-linguistic target detection. Results showed that left inferior frontal ambiguity effects were diminished but not obliterated when participants attended to the distractor task while left anterior fusiform ambiguity responses were less affected by distraction (Figure 1a).
In Experiment 2, participants performed one of two concurrent tasks: auditory target detection or visual lexical decision. Inferior frontal ambiguity responses were absent when the sentences were not attended to (Figure 1b) whereas anterior fusiform activation remained when participants attended to auditory distractors, but not when they attended to linguistic visual distractors. Hence we see that inferior frontal responses are susceptible to disruption by distractor tasks that load on overlapping sensory or linguistic processes whereas inferior temporal responses are preserved for all but the most severe disruption. This graded degradation of neural responses associated with comprehension suggests differing levels of automaticity within frontal and temporal language regions.
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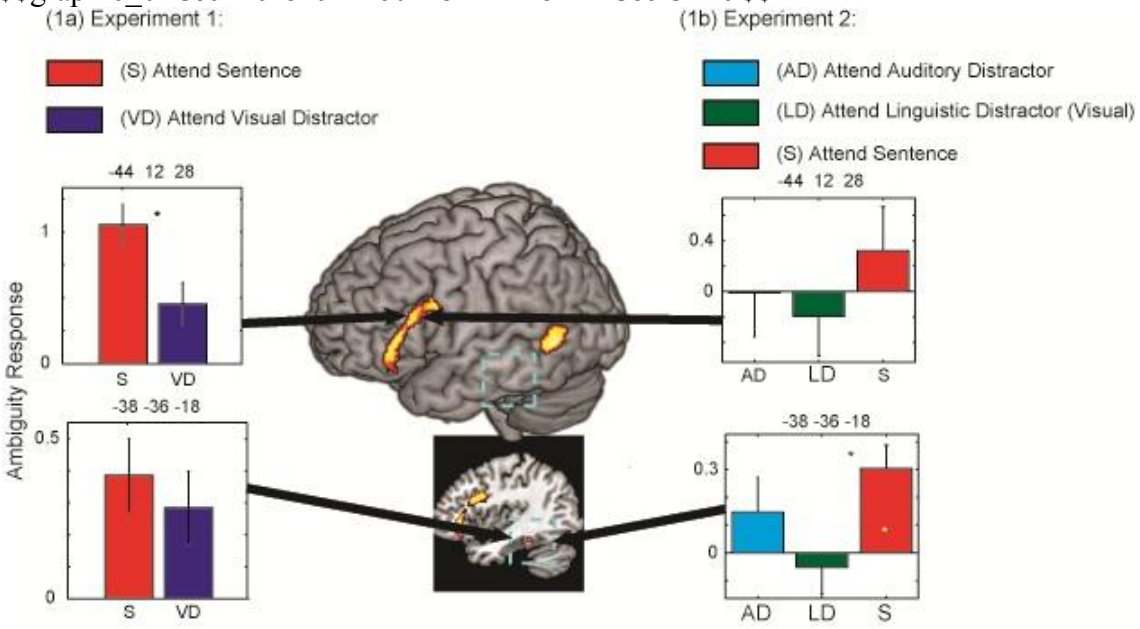

Disclosures: M. Davis, None; **J.E. Peelle**, None; **S.S. Vitello**, None; **R.J. Eason**, None; **J.M. Rodd**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.9

Topic: F.01. Human Cognition and Behavior

Support: NSERC

CIHR

Government of Ontario (ERA)

Title: Attention enhances the processing of degraded speech: Evidence from fMRI implicates left temporal and inferior frontal cortex

Authors: *C. J. WILD[1], A. YUSUF[2], D. WILSON[2], J. E. PEELLE[3], M. H. DAVIS[3], I. S. JOHNSRUDE[2];
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Abstract: Functional magnetic resonance imaging (fMRI) studies of speech perception, in which spoken sentences are presented without any competing stimulus, reveal characteristic intelligibility-dependent patterns of activity in frontal and temporal cortex. However, most real-world communication involves conversing in the presence of competing stimuli, and hearing speech when attention is elsewhere. We used fMRI to examine the degree to which unattended speech is processed, and whether such processing depends on the clarity of the speech. Twenty-one volunteers (age 19-27 years) participated in this study. On every trial, subjects attended to one of three concurrently-presented stimuli: a sentence, an auditory distracter, or a visual distracter. Speech stimuli consisted of English sentences presented at one of four intelligibility levels: clear speech (CL), six-band noise-vocoded speech (NVHI, highly intelligible), compressed six-band NVS (NVLO, marginally intelligible), and spectrally rotated NVS (rNV, always unintelligible). Thus, we had a 4 (distortion type) x 3 (attention) factorial design. Following the scanning session, participants performed an old/new discrimination task, indicating whether they had heard sentence items in the scanner. Analysis of d' scores based on these behavioural data revealed that recognition of NVHI and NVLO items was improved when subjects were attending to these items, compared to when they were attending to distracters. In contrast, recognition was similar for CL items regardless of the focus of attention. These results suggest that whereas clear speech can be processed even when it is not attended, the processing of degraded speech is enhanced by attention, allowing it to be better remembered later. Our imaging results replicated previous studies: when attention was directed towards speech, activity in regions of bilateral superior temporal cortex correlated positively with the intelligibility of spoken sentences, and activity in left inferior frontal cortex was elevated for degraded, compared to clear, speech. Finally, we observed Distortion x Attention interactions, such that activity elicited by degraded speech in left temporal and inferior frontal cortex was enhanced in

the presence of attention. In contrast, activity in speech-sensitive cortex around Heschl's gyrus, the approximate location of primary auditory cortex, did not depend on attention. These results suggest that temporal-lobe responses to speech reflect largely automatic processing that can nonetheless be enhanced by attention, whereas the processing of degraded speech by inferior frontal regions is less automatic and requires attention.

Disclosures: C.J. Wild, None; **A. Yusuf**, None; **I.S. Johnsrude**, None; **D. Wilson**, None; **J.E. Peelle**, None; **M.H. Davis**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.10

Topic: F.01. Human Cognition and Behavior

Support: Medical Research Council support U.1055.04.013.01.01

Title: Perceptual learning in speech comprehension governed by power law dynamics

Authors: *J. E. PEELLE[1], T. OLAFSEN[2], M. H. DAVIS[1], A. WINGFIELD[2];
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Abstract: Power law dynamics are ubiquitous in biological systems, and have proven particularly useful in describing learning and adaptation in cellular and systems neuroscience. We therefore hypothesized that perceptual learning in speech comprehension would be governed by such processes. We tested this prediction by studying listeners' adaptation to speech manipulated using two orthogonal types of acoustic degradation (Fig 1a). In a behavioral study we presented 75 listeners with 30 time-compressed and 30 frequency-compressed sentences, using word report as a trial-by-trial measure of intelligibility. Across subjects, perceptual learning was indeed well characterized by a power function (Fig 1b, blue). We then conducted an fMRI experiment using analogous methods, taking advantage of the assumption that adaptation effects are driven by underlying power law processes. Under this assumption, our behavioral measure of word report reflects both perceptual learning (which we assume to occur smoothly over time), and attentional, executive, and linguistic processes (which we expect to cause intertrial variability). To dissociate these two contributions, we fit a power law function to each subject's recall data, which we entered into our statistical

model separately from recall scores. We first looked for neural activation associated with listening to both kinds of speech and word report (i.e. intelligibility) (Fig 1c). Next, for each type of acoustic degradation we used the individually-fit power functions to search for regions of the brain that showed a profile associated with perceptual learning for time- or frequency-compressed speech (Fig 1d). Anatomically distinct regions showed associations with learning across the two manipulations. These results demonstrate for the first time that listeners' ongoing adjustments to the speech stream obey power law dynamics, and suggest that the networks subserving these learning processes may depend on the acoustic characteristics of the signal.$$graphic_{4E32090D-05E3-46AE-96B9-6FB3B144E7B6}$$

Disclosures: J.E. Peelle, None; **T. Olafsen**, None; **M.H. Davis**, None; **A. Wingfield**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.11

Topic: F.01. Human Cognition and Behavior

Support: ARO 54228_LS-MURI

Title: Auditory efference copies in speech: MEG evidence from an adaptation design

Authors: *X. TIAN[1], D. POEPPEL[2];
[1]New York Univ., NEW YORK, NY; [2]New York Univ., New York, NY

Abstract: Previous research supports the concept of an internal forward model in speech production. A core component of such a model is the sensory prediction generated during output planning. Consistent with this view, recent magnetoencephalography (MEG) data from mental imagery of speech provide direct and temporally constrained evidence for auditory cortex activation consequent to imagined articulation. Here we performed an MEG study using an adaptation/repetition design to investigate the characteristics of an auditory efference copy in speech. We tested whether the response to an overt auditory probe was modulated by overt and covert (mental imagery) adaptors. Four adaptors (all consonant-vowel syllables) were presented in four different conditions. Participants were required to passively listen (hearing), overtly pronounce (articulation), imagine articulating (articulation imagery), and imagine hearing (hearing imagery). An auditory

probe (syllable) always followed the adaptor. Neuromagnetic recordings were performed throughout (KIT System, Kanazawa, Japan). The neurophysiological adaptation effects were quantified by comparing the responses to the auditory probes as a function of the adaptors. Typical repetition suppression was found around 200 ms after probe onset (M200) in the control hearing condition (two identical auditory stimuli presented sequentially). Similar response amplitude decreases were also observed in the M200 component for hearing imagery. Interestingly, repetition enhancement was obtained with similar timing in the articulation imagery condition, when participants were required to imagine pronouncing the syllables preceding the probe. No effect was found in the articulation condition. The common timing of the adaptation results in the hearing, hearing imagery, and articulation imagery conditions implicates overlapping neural systems. However, the different direction of the adaptation effects (repetition enhancement in articulation imagery vs. repetition suppression in hearing imagery) demonstrates distinctions in the top-down processes generating auditory representations and, specifically, highlights that planned speech activates auditory cortex in absence of any external stimulation, supporting the idea of an auditory efference copy.

Disclosures: X. Tian, None; **D. Poeppel**, None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.12

Topic: F.01. Human Cognition and Behavior

Support: WT074414MA

Title: Neural responses to mouth sounds reveals no enhanced role for motor cortex in speech perception

Authors: *S. K. SCOTT[1], Z. AGNEW[2], C. MCGETTIGAN[2];
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Abstract: Are both sensory and motor cortices involved in speech perception? Clinically, speech perception has been associated with the left dorsolateral temporal cortex. Motor activity has also been reported during speech perception, and a number of functional imaging and transcranial magnetic stimulation studies have claimed to find a central role for motor cortices in speech processing. We used functional neuroimaging to investigate

the response profiles of motor and auditory cortex to naturally produced examples of speech and non-speech mouth sounds, as a direct test of the extent to which motor and premotor cortices show an enhanced response to speech. The speech sounds used were British English unvoiced consonants, and the non-speech sounds were ingressive 'click' sounds, which are consonants in some African Languages (e.g. Xhosa), and which cannot be assimilated into British English phonemic categories. Relative to a signal correlated noise baseline, the two sound categories produced comparable activity in right bilateral posterior temporal regions. Speech sounds produced greater activation than non-speech mouth sounds in mid superior temporal gyri/sulci, with a greater response on the left extending into the anterior superior temporal gyrus. Non-speech mouth sounds produced greater activation in posterior medial planum temporale. In anterior cortical fields, region of interest analyses demonstrated no significant difference in mouth motor cortex during perception of speech and non-speech mouth sounds. Therefore, in direct contrast to theories that posit a central role for motor cortices in speech perception, our study reveals no preference for speech sounds in mouth motor areas. Furthermore, this study suggests qualitative differences in the kinds of auditory processing seen in temporal and motor areas, consistent with different roles for these cortical structures in human communication.

Disclosures: **S.K. Scott:** Research Grant; WT074414MA. **Z. Agnew:** None. **C. Mcgettigan:** None.

Nanosymposium

837. Auditory Language Studies

Location: Room 25A

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 837.13

Topic: F.01. Human Cognition and Behavior

Support: VACSR&D Career Development Award (Turken)

VA CSR&D Merit Award (Dronkers)

Title: White matter pathways subserving the language comprehension network

Authors: *A. U. TURKEN[1], N. F. DRONKERS[1,2];
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Abstract: Language comprehension is subserved by a left peri-slyvian network. Lesion (Dronkers et al., 2004) and functional imaging (Binder et al., 2009) studies have identified the middle temporal gyrus (MTG), anterior superior temporal gyrus (STG), posterior superior temporal sulcus (STS) and the angular gyrus (AG), orbital part of inferior frontal gyrus (BA 47) and the middle frontal gyrus (BA 46) as key nodes in this network. Here, we assessed the structural and functional connectivity of the regions that were to be found to be critical for sentence comprehension in a voxel-based lesion-symptom mapping analysis (Dronkers et al., 2004). We used diffusion imaging and resting state functional MRI data from healthy subjects as well as lesion data from aphasic patients. Fiber tractography and functional connectivity analyses indicated that MTG, anterior BA 22, STS/AG and BA 47 comprise a richly interconnected network. The inferior fronto-occipital fasciculus, inferior longitudinal fasc., superior longitudinal fasc. and middle longitudinal fasc. were found to be the white matter pathways that are critical for language comprehension (Fig 1). These findings highlight the importance of long association fiber systems for the integrated functioning of the cortical regions which together support comprehension (Duffau, 2008, Saur et al., 2009), and have implications for the diagnosis and recovery of aphasic patients with comprehension deficits.

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Duffau H. (2008). The anatomo-functional connectivity of language revisited. New insights provided by electrostimulation and tractography. Neuropsychologia, 46(4):927-934.

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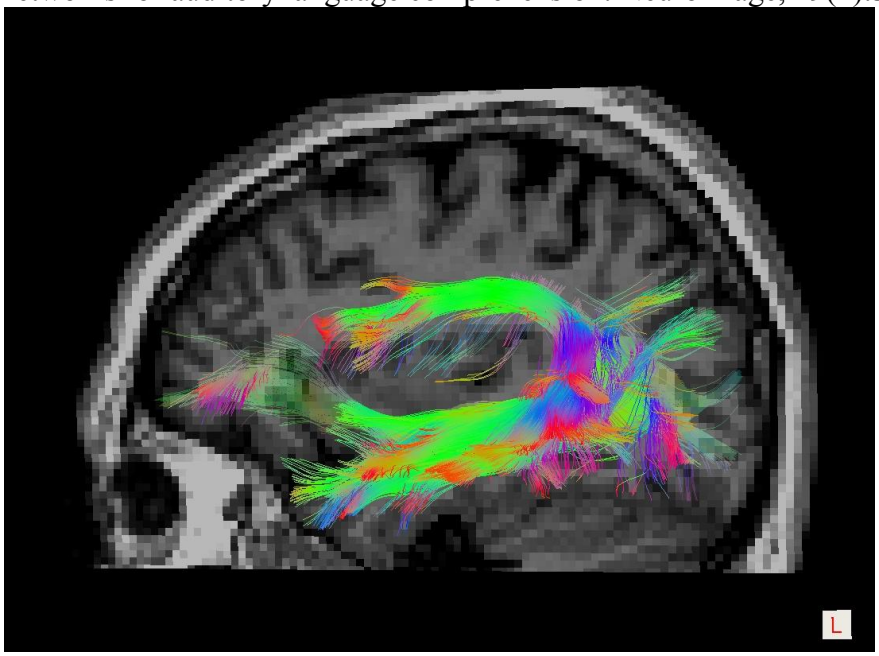


Disclosures: A.U. Turken: None. **N.F. Dronkers:** None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.1

Topic: G.07. Data Analysis and Statistics

Support: This research was supported by a grant (M103KV010016-08K2201-01610) from Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology

This works was supported by a grant (NRF-2009-351- D00026) by the National Research Foundation of the Republic of Korea

Title: Connectivities between spectral and spatial co-clusters of MEG signal using nonnegative matrix tri-factorization

Authors: *H. LEE[1,2,4], H. PARK[5,4,3], H. KANG[5,4], M. K. CHUNG[2,6,7], E. KANG[8], J. KIM[9], C. CHUNG[9], D. LEE[5,4,3];
[1]Nuclear Med., Jongno-Gu, Seoul, Korea, Republic of; [2]Brain and Cognitive Sci., [3]Interdisciplinary Program in Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [4]Inst. of Radiation Medicine, Med. Res. Center, Seoul Natl. Univ., Seoul, Korea, Republic of; [5]Nuclear Med., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [6]Biostatistics and Med. Informatics, [7]Waisman Lab. for Brain Imaging and Behavior, Univ. of Wisconsin, Madison, WI; [8]Psychology, Kangwon Natl. Univ., Chuncheon, Korea, Republic of; [9]Neurosurg., MEG Center, Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of

Abstract: **Background**:
Various spectral and spatial properties of MEG signal help us to understand of new aspects of complex rhythmical brain activities, however, its complexity makes hard to analyze MEG data. In this study, *we simplified the spectral and spatial MEG components by simultaneous clustering, a.k.a., co-clustering and observed their connectivities based on nonnegative matrix tri-factorization (NMTF)*. Cognitive processes involved in visuospatial working memory versus non-memory perceptual processing were analyzed using NMTF.
Methods:
The MEG data (Elekta-Neuromag, 306 sensors) were observed during visuospatial

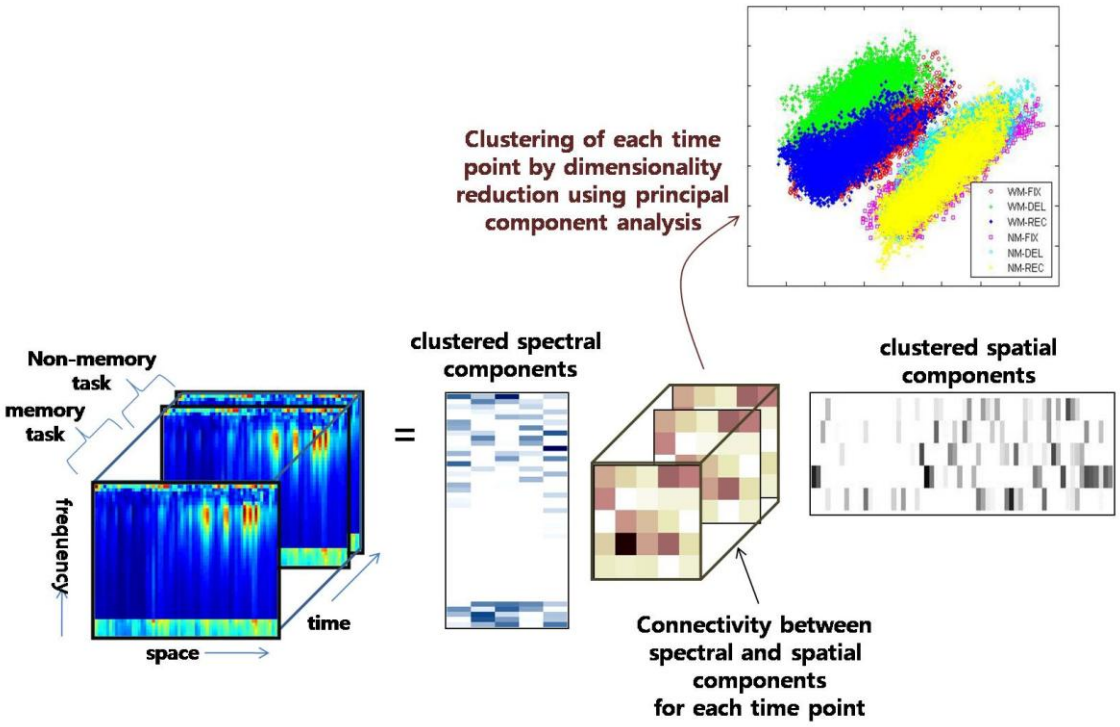
delayed matching-to-sample task (DMST) vs. non-memory task. After transforming the MEG data to the time-frequency representation using complex Morlet wavelets, we obtained the spectro-spatial matrix for each time point, which used for examining the complex interconnectivity between spectro-spatial co-clusters of MEG signals. NMTF simultaneously clusters the spectral and spatial components which were activated for the memory or no memory task and also finds their interconnectivities.

Results:

We visualized their interconnectivities using principal component analysis (PCA). It shows that the working memory (WM) task, including fixation (FIX), delay (DEL) and recognition (REC), and the no memory (NM) task, including FIX, DEL and REC are clearly separated. However, the variability of FIX, DEL and REC in WM is slightly larger than one in NM task. Each time point is correctly clustered into true label, WM or NM, with classification accuracy 95 %.

Discussion:

We showed interconnectivities between spectrally and spatially co-clustered components with complex MEG data using NMTF. Though we did not assume fixed frequency bands or spatial constraint, this method demonstrated that it extracted automatically the hidden network structures during each phase of working memory vs. no memory task. Thus, we suggest that the NMTF could contribute to our growing knowledge of functional connectivity, especially of data with complex structures such as EEG and MEG.



Disclosures: H. Lee, None; **H. Park**, None; **H. Kang**, None; **D. Lee**, None; **E. Kang**, None; **M.K. Chung**, None; **J. Kim**, None; **C. Chung**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.2

Topic: G.07. Data Analysis and Statistics

Title: Improvements to time-frequency analysis of event related EEG and MEG

Authors: *T. CARNEY, S. KLEIN, D. KIM;
Sch. Optometry, Univ. California-Berkeley, BERKELEY, CA

Abstract: Time-frequency (spectrogram) analysis of EEG and MEG is gaining in popularity because it captures not only the fixed response to events, but also responses that are not precisely time-locked. In addition Fourier based coherence analysis is claimed to be informative about connectivity between brain regions. The usual approaches to time-frequency analysis have two problems for which we propose solutions:
1) Achieving fine time resolution in 1/f noise. It has been recently shown that cortical gamma oscillations have a broader bandwidth than previously realized, implying that wavelet analysis requires finer time resolution (say 1 cycle) and broader frequency tuning than previously used. Given the 1/f nature of the EEG and MEG signals in the frequency domain, the standard filters have an insufficient falloff on the low frequency side. We propose using Cauchy wavelets specified by $C(t,n) =(t + i)^{n+1}$, where i=sqrt(-1), t is time and n controls the bandwidth in frequency or number of cycles in time. A scale parameter, not shown, multiplies t to adjust the temporal frequency. The real and imaginary parts of C(t, n) are Hilbert pairs with identical Fourier transforms. The Fourier transform of C(t,n) is $FC(f) = f^n \exp(-f)$. FC(f) has a peak at n, a mean of n+1 and a variance of n+1. The factor f^n produces a low frequency attenuation that is especially important for 1/f noise. Beta oscillations at 20 Hz would be less sensitive to alpha oscillations at 10 Hz.
2) Phasor spectrograms rather than power spectrograms. The quadratic nature of spectrogram power greatly limits their usefulness for source separation. By using the topography of the dominant ERP source as a reference one can identify a likely reference phase for the phase variable activity. Simulations show that this procedure is effective and efficient in isolating phase coherence. It is able to avoid problems such as "volume conduction coherence" that cause difficulties for the standard methods of coherence analysis. These methods can be applied to search tasks where time-locking onto saccadic landings are not sufficiently precise. The novel phase locking approach that we have developed enables the use of saccades as time-locking events almost as flexibly as one can time-lock onto stimulus events.

Disclosures: T. Carney, None; **S. Klein**, None; **D. Kim**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.3

Topic: G.07. Data Analysis and Statistics

Support: NSF-0238442

NIH-HD53727

NIH RO1 DC006287

Falk Family Medical Research Trust and the Ralph and Marion Birnschein Foundation.

Title: Integration of EEG-fMRI in an auditory oddball paradigm using joint-independent component analysis

Authors: *J. MANGALATHU ARUMANA[1,2], E. LIEBENTHAL[1,3], S. A. BEARDSLEY[2,4];
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Abstract: A major challenge in cognitive neuroscience is to understand how different brain regions interact and process information over time. The combination of simultaneous EEG and fMRI with joint spatiotemporal analysis allows characterization of the neural dynamics with high temporal and spatial resolution. This research aimed to determine the spatiotemporal dynamics of neural sources in the brain using joint independent component analysis (joint-ICA).
A tone auditory oddball paradigm was used, in which sequences of 4 tones composed of 3 standard 1000 Hz tones and 1 deviant tone were presented binaurally. Subjects were tested with deviant tones at 5 levels of frequency separation (2-28 ± 2 Hz), corresponding to 50, 65, 75, 85, and 90% correct performance. The subjects' task was to indicate whether the deviant tone was higher or lower in frequency than the standard tones.

Clustered fMRI and continuous EEG were acquired simultaneously as subjects performed the task. Joint-ICA was applied to compute spatio-temporally independent EEG/fMRI components by identifying a common set of unmixing coefficients across deviant levels. The input to the joint-ICA combined the spatiotemporal array of ERPs across electrodes together with the fMRI spatial maps, to incorporate full spatial and temporal information of both modalities across trials.
ERP results showed an increase in peak amplitude with frequency difference ranging from 1-9 μV across the P300 component (400-600ms). FMRI analysis using the generalized linear model (GLM) with deviant level as a regressor, showed activation in a large network of regions including the left pre and post central gyri, superior and middle temporal gyri bilaterally, and right temporo-parietal junction. Using the P300 peak amplitude in electrode PZ as a regressor, activation was present in a subset of the same network, including left precentral gyrus and left superior temporal gyrus. Joint-ICA results revealed sources in the left pre and post central gyri, left middle frontal gyrus, right superior temporal gyrus, and right temporo-parietal junction.
In the joint-ICA results, the left fronto-parietal activation may reflect right-hand motor planning and motor response, whereas the right temporo-parietal activation may reflect auditory sensory and attentional processes. Compared to the P300 amplitude regression analysis, the joint-ICA approach detected more regions associated with the sources of the P300 as described in prior reports. The results suggest that with the incorporation of spatial and temporal information from both imaging modalities, joint-ICA may provide a more sensitive method to extract common sources between EEG and fMRI.

Disclosures: J. Mangalathu Arumana, None; **E. Liebenthal**, None; **S.A. Beardsley**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.4

Topic: G.07. Data Analysis and Statistics

Title: Estimating and compensating systematic artifacts in fMRI time series by multivariate analysis of synthetic controls

Authors: *M. REIMERS;
Virginia Commonwealth Univ., RICHMOND, VA

Abstract: The effective signal to noise ratio of fMRI data is still far above the random noise level. It seems likely that much of this excess variation between time images is due to systematic sources such as head motion, changing susceptibility due to breathing, and other factors. We still do not have effective and simple means to capture and compensate these artifacts, and we are not entirely sure what the causes are. It would be easier if we had controls that captured these effects.
Here I introduce a generic method of 'synthetic controls' for identifying artifacts in fMRI and other high-throughput time-series data. The key idea is to compare measures that should record essentially the same biological signal, but should be affected differentially by technical artifacts. For most fMRI data, scanned in an interleaved fashion, differences between neighboring voxels in the same brain region but in adjacent scanning planes will work. I find that 70% of the variation over a large number of such voxel pairs can be explained by a few factors. By regressing the signal from individual voxels on these factors putative systematic artifacts of various sorts may be identified.
This approach isolates a large fraction of the systematic variance and leaves the unexplained variance closer to that of random noise, thereby greatly increasing the power of statistical tests.

Disclosures:

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.5

Topic: G.07. Data Analysis and Statistics

Support: NIA/NIH grant RO1 AG029523

Title: Increasing measurement accuracy of age-related BOLD signal change: Minimizing vascular contributions by resting-state-fluctuation-of-amplitude scaling

Authors: *B. BISWAL[1], S. S. KANNURPATTI[1], M. A. MOTES[2], B. RYPMA[2]; [1]Radiology, UMDNJ - NJMS, Newark, NJ; [2]Brain Sci., Univ. of Texas at Dallas, Dallas, TX

Abstract: Earlier work from our laboratory has demonstrated the validity, feasibility and reliability of a hemodynamic scaling method that relies upon resting-state fMRI signal fluctuations, or "resting-state fluctuation of amplitude" (RSFA; Biswal & Kannurpatti,

2008). In this study we applied RSFA scaling to fMRI data collected in healthy younger and older subjects performing motor and cognitive tasks. RSFA correlated with breath hold (BH) responses throughout the brain in both subject groups. RSFA and BH scaling both reduced the skew of the BOLD response amplitude distribution in each subject in addition to reducing mean BOLD amplitudes and its variability in both subject groups. Statistically significant differences in intra-subject and inter-subject BOLD response variation were observed in both subject groups. Intra- and inter-subject variability differences between the younger and older groups were mitigated after scaling. RSFA, though similar to BH in eliminating the skew in the un-scaled BOLD amplitude distribution, attenuated neural activity related BOLD responses significantly less than BH. The amplitude and spatial extent of group activation were lower in older than in younger subjects prior to and after scaling. After accounting for vascular variability differences with RSFA scaling, age-related BOLD response differences persisted during the motor and cognitive tasks, suggesting genuine age-related neural activity differences during task performance. Thus RSFA scaling, accounted for known age-related hemodynamic coupling changes, and yielded more veridical estimates of age-related differences in task-related neural activity.

Disclosures: B. Biswal, None; **S.S. Kannurpatti**, None; **M.A. Motes**, None; **B. Rypma**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.6

Topic: G.07. Data Analysis and Statistics

Support: NIH R01 AG029523

Title: Reliability of resting state fluctuation amplitude as a hemodynamic response scaling factor

Authors: *B. P. RYPMA[1], S. KANNURPATTI[2], M. MOTES[1], B. BISWAL[2];
[1]Behavioral & Brain Sci., Univ. of Texas at Dallas, RICHARDSON, TX; [2]Radiology, Univ. of Med. and Dent. of NJ, Newark, NJ

Abstract: High correlations have been observed between the resting state fluctuation of amplitude (RSFA; Biswal & Kannurpatti, 2008), breath hold (BH) fluctuation of

amplitude and CO2 fluctuation of amplitude. The high correlation between RSFA and hypercapnia (BH and CO2) response fluctuations arises from arterial CO2 variation but other factors, including large BOLD signal variation at the edges of the brain and CSF compartments can also contribute to the correlation. In this study, we tested the reliability of correlations between RSFA and BH response fluctuations three ways. First, we removed highly variable voxels from the brain edges and CSF compartments and observed that this manipulation did not affect RSFA-hypercapnia correlations. Second, we used bootstrap resampling both spatially and temporally to validate the reliability of RSFA and to account for unknown factors that may influence the correlation. The spatial bootstrap analysis indicated that RSFA over all regions of the brain is a reliable hemodynamic response-scaling factor. Temporal bootstrapping analysis indicated that RSFA could be reliably estimated using a time series of greater than or equal to 30 time-points (1 minute). Finally to test the reliability and feasibility of RSFA scaling we compared the performance of scaling parameters obtained using RSFA and BH on fMRI-BOLD data acquired while participants performed blocked sensorimotor and event-related cognitive tasks. The results indicated that variability was significantly and reliably reduced in both design types when the data were scaled by an RSFA parameter estimated from at least 30 2-sec time points of resting-state data.

Disclosures: B.P. Rypma, None; **S. Kannurpatti**, None; **M. Motes**, None; **B. Biswal**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.7

Topic: G.07. Data Analysis and Statistics

Support: NIH R21DA026109

Title: Evaluation of independent components as a dimension reduction technique for classification of fMRI scans

Authors: *A. ANDERSON[1], J. BRAMEN[2], C. CULBERTSON[3], A. BRODY[4], M. S. COHEN[2];
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Abstract: Independent Components Analysis (ICA) is a popular dimension reduction method used in fMRI to extract spatial activity patterns. Here, we investigate the ability of ICA to generalize across patients, incorporating it into a classifier that uses weightings of ICs at a individual time points to predict whether a patient is viewing a smoking related video or an auditory task with smoking-related cues. We developed a group IC dictionary by automatic clustering of ICs from 52 tobacco dependent subjects. We compared the effectiveness of group ICA methods against single-subject ICA to predict outcomes across subjects and runs. Finally, we evaluated the effects of different spatial dimension reduction methods within the creation of independent components within and across groups, comparing the signal lost when dimension reduction is performed with a blind mathematical approach defined by functional activity vs. an atlas space specified by anatomical features. Collectively, we present an fMRI classification method to evaluate the process of generating spatial components, and the ability of these components to predict a task condition within and across subjects during real time.

Disclosures: **A. Anderson:** Research Grant; R21DA026109. **J. Bramen:** R21DA026109. **C. Culbertson:** None. **A. Brody:** None. **M.S. Cohen:** NIH R21DA026109.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.8

Topic: G.07. Data Analysis and Statistics

Support: University of Pittsburgh Medical Center

Title: High-definition fiber tracking of human cortical eye fields

Authors: *J. S. PHILLIPS[1,3,4], S. K. PATHAK[2,3,4], T. VERSTYNEN[1,3,4], W. W. SCHNEIDER[1,3,4];
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Abstract: High Definition Fiber Tracking (HDFT) using Diffusion Weighted Imaging (DWI) allows non-invasive mapping of human white-matter tracts with sub-millimeter resolution. We have demonstrated the ability to replicate known truths, such as mapping

and quantifying the motor system cortico-spinal tracts (Verstynen, OHBM 2010). Here we map out the human eye movement control network and relate it to known mappings in non-human primates. A chief node in this network, the frontal eye field (FEF), is located near the junction of the superior frontal and pre-central sulci (SFs and PrCs) and is connected to eye movement areas in posterior parietal and dorsolateral prefrontal cortices (Lynch & Tian, 2006). We localized the FEF using this connection profile. Three human subjects underwent diffusion spectrum imaging (DSI; 257 directions, bmax=7000) on a Siemens 3T Trio with a 32-channel coil. Orientation distribution functions were calculated using generalized q-sampling imaging reconstruction (GQI; Yeh, 2010). Seed and target regions were created for 3 subjects in bilateral DLPFC, PrCs, and PPC. Fiber tracking was performed between each seed/target pair, and the FEF was defined by voxels which received fiber projections from both DLPFC and PPC. Overlap masks of fiber endpoints in a standard reference space indicated greatest convergence in the anterior bank of the PrCs, slightly lateral and inferior to the SFs. The FEF formed a circuit with 1) an area of the middle frontal gyrus (MFg) superior to pars triangularis and 2) an area spanning the intraparietal sulcus. These findings correspond to expectations derived from invasive studies in non-human animals. Furthermore, both manual segmentation and k-means clustering revealed multiple, discrete u-shaped bundles between the FEF and MFg, which may relate to saccadic vs. smooth pursuit subdivisions of FEF. HDFT imaging of functional networks such as the eye fields provide a valuable complement to functional neuroimaging of behavior, and in some cases may provide greater precision in localizing and segmenting functional areas. In particular, these results may help resolve competing claims about the location of areas such as the human FEF. For additional information see http://www.lrdc.pitt.edu/schneider/SFN/.

Disclosures: J.S. Phillips, None; **S.K. Pathak**, None; **T. Verstynen**, None; **W.W. Schneider**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.9

Topic: G.07. Data Analysis and Statistics

Support: UPMC

Title: High definition fiber tracking in neurosurgery & traumatic brain injury

Authors: *W. SCHNEIDER[1,2,3], S. PATHAK[1], J. FERNANDEZ-MIRANDA[4], D. OKONKWO[4], K. JARBO[1], J. ENGH[4], A. MINTZ[4], F. BOADA[5];
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Abstract: High Density Fiber Tracking (HDFT) uses MRI based Diffusion Weighted Imaging (DWI) to produce high resolution images of fiber tracts to reliably follow the tracts through crossings to the cortex. Most previous techniques have poor ability (<50%) to track fibers to cortex or subcortical structures. By enforcing termination of the fibers on functionally consistent endpoints, accuracy improved. Our HDFT can reliably track fibers to cortex, show the shape morphology of the contact surface of the cortex, show clear gyral patterns, and allow quantification of the tract volume and integrity. We utilize the techniques in an advisory capacity to evaluate neurosurgical and traumatic brain injury cases. In neurosurgery, the methods have been examined in over fifteen patients and, in the majority of cases, the information was viewed as valuable to plan the approach and in a third influenced the operative procedure. HDFT methods provided a historic first real-time use of HDFT to visualize fiber tracts in the operating room, guide surgery during tumor removal, and enable quantification of surgery-induced fiber damage and projection of fiber fields**.** Our methods provide far better resolution of fiber tracts than previous HARDI, DSI or DTI-based methods. Utilization of HDFT in planning tumor resection and importing this data into the image guidance systems may enable greater resection of tumor tissue while preserving function. In planning tumor resections we can pre-operatively assess if there are axonal fibers within the planned trajectory or within the tumor itself which should be avoided in order to prevent post-operative deficits. In Traumatic Brain Injury (TBI) cases, we detected and quantified substantial fiber breakage using HDFT finding breakage that could not/was not detected by current CT or MRI methods. We have detected very high breakage rates (>50%) in TBI patients in specific fiber tracks where normal control subjects typically showed low breakage rates (<5%) when analyzed utilizing the same procedures. HDFT can trace the projection field of the broken fibers to identify which cortical areas are potentially deteriorated due to the loss of fibers. This identification of disconnections may help guide rehabilitation training that activates the input/output fields to regrow fibers. For additional information see http://www.lrdc.pitt.edu/schneider/SFN/

Disclosures: W. Schneider, None; **S. Pathak**, None; **J. Fernandez-Miranda**, None; **D. Okonkwo**, None; **K. Jarbo**, None; **J. Engh**, None; **A. Mintz**, None; **F. Boada**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.10

Topic: G.07. Data Analysis and Statistics

Support: UPMC

Title: HD fiber tracking: Non-invasive quantification of fiber tract volume

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Abstract: Non-invasive mapping of brain connectivity would benefit from having a meaningfully metric of fiber volume of fiber bundles as they cross and change shape. High Definition Fiber Tracking (HDFT) Diffusion Weighted Imaging (DWI) allows mapping of axon tracts but popular methods do not provide interpretable metrics. (e.g., count streamlines with no scale related to the physical volume). We provide a quantitative metric of the volume of the fiber tract going in a specific direction within a voxel we call Directional Quantitative Anisotropy (DQA). DQA quantifies the spin detected in a given voxel quantifying the volume and cross sectional area of a tract of a given diffusion tensor (Yeh, 2010). The DQA calculation yields the amount of diffusion volume in each direction within a given diffusion Orientation Distribution Function (e.g., 30% of the volume is moves in the horizontal axis and 20% in the vertical axis defined by the spin distribution function value at the resolved fiber orientation minus the background isotropic diffusion component. In this study we are estimating variation of DQA along a fiber tract. With this metric we can quantify the volume of directional flow of water that is related to the diffusion within axons and the myelin sheath. This enables quantification of fiber tract volume that can be compared across scanning pipelines and across individuals. It allows quantification of tract volume as tracts cross, compress/expand, and morph (e.g., optic radiations) to assess the degree to which the volume is preserved along their length. It can be used to quantify disease or genetic factors alter fiber tract volume. These connectivity measures can quantify the amount of fiber damage in neurosurgery, Traumatic Brain Injury (TBI) or learning related changes in the brain. Three human subjects underwent Diffusion Spectrum Imaging (DSI) scan on a Siemens 3T Trio with a 32 channel coil with 257 gradient direction, bmax = 7000. Orientation Distribution Function is calculated using generalized q-sampling imaging (GQI) (Yeh 2010) reconstruction method.250,000 fibers are generated using streamline fiber tracking algorithm. Five major tracks are segmented manually and DQA is calculated along those tracts. These directional diffusion estimates are then used to test out hypothesis that track volume is preserved along the fiber tracts.
Fang-Cheng Yeh, Van J. Wedeen, Wen-Yih Isaac Tseng. “Generalized Q-Sampling Imaging." IEEE TMI, 2010

Disclosures: S.K. Pathak, None; **F. Yeh**, None; **W. Schneider**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.11

Topic: G.07. Data Analysis and Statistics

Support: NIH Grant DA023700

Title: Novel use of matched filtering for synaptic event detection and extraction

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Abstract: Efficient and dependable methods for detection and measurement of synaptic events are important for studies of synaptic physiology and neuronal circuit connectivity. As previous methods with detection algorithms based upon amplitude thresholding, and fixed or scaled template comparisons are of limited utility for detection of signals with variable amplitudes and superimposed events that have complex waveforms, the published techniques are not optimal for detection of evoked synaptic events in photostimulation and other similar experimental situations. Here we report on a novel technique that combines the design of a bank of matched filters with the detection and estimation theory for automatic detection and extraction of photostimluation-evoked postsynaptic currents (PSCs) from individually recorded neurons in cortical circuit mapping experiments. In our technique, a range of synaptic responses with different amplitudes and shapes from typical experimental data are first selected and fitted by polynomial models to create a bank of approximate matched filters (templates) in the training stage. In the fully automated detection stage, experimental data traces are convolved with the polynomial templates, with candidate PSCs matching templates better, and thus yielding larger convolution amplitudes. Given that the identified synaptic responses and the synthesized templates provide rich waveform information, our method also utilizes statistical parameters derived from the initial template fitting for PSC detection, extraction and characterization. The sensitivity and specificity of the method were evaluated on both simulated and experimental data, with its performance comparable to or better than that obtained by visual event detection by a human operator. In the present study, this new technique was applied to quantify and compare

photostimulation-evoked EPSCs obtained from excitatory pyramidal cells and fast-spiking interneurons. Given the nature of general-purpose signal recognition algorithms of template fitting, our technique can be further applied to detect and extract other electrophysiological signals such as extracellular responses, local field potentials and EEG events, as well as optical imaging responses.

Disclosures: Y. Shi, None; **Z. Nenadic**, None; **X. Xu**, None.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.12

Topic: G.07. Data Analysis and Statistics

Support: BMBF Grant 01GQ0410

Title: A maximum entropy mutual information test for spike counts of V1

Authors: *A. ONKEN[1], S. GRÜNEWÄLDER[2], V. DRAGOI[3], K. OBERMAYER[1]; [1]Technische Univ. Berlin, Berlin, Germany; [2]Univ. Col. London, London, United Kingdom; [3]Univ. of Texas - Houston Med. Sch., Houston, TX

Abstract: Previously, the importance of modeling detailed spike count dependencies beyond linear correlation has been demonstrated in macaque prefrontal cortex and premotor cortex only. In these studies the underlying data set was very rich in the number of samples. The great number of samples was also required for the copula analysis which was applied. In many electrophysiology data sets, however, the number of samples in each experimental condition is rather small. This shortcoming makes it necessary to develop other methods that can deal with less samples.
We developed a method that allows to investigate the importance of taking the detailed dependency structure into account even when the number of samples is very small. For this purpose, the maximum entropy distribution with marginal distributions and the correlation coefficient as constraints is used as a reference distribution. We devised a Monte Carlo goodness-of-fit test based on this distribution and the mutual information as the divergence measure. This test is applicable even when the number of samples in each condition is on the order of 50. The method is applied to spike counts recorded simultaneously in anesthetized cat V1 during an adaptation experiment.
We find that the maximum entropy hypothesis can be rejected for a substantial number of

pairs in V1, thus showing that more detailed dependency structures can be important when estimating information theoretic quantities in V1.

Disclosures: **A. Onken**, Technische Universität Berlin, Employment; BMBF grant 01GQ0410, Research Grant; **K. Obermayer**, Technische Universität Berlin, Employment; BMBF grant 01GQ0410, Research Grant; **S. Grünewälder**, University College London, Employment; **V. Dragoi**, University of Texas - Houston Medical School, Employment.

Nanosymposium

838. Novel Methods on Data Analysis

Location: Room 10

Time: Wednesday, November 17, 2010, 1:00 pm - 4:15 pm

Program Number: 838.13

Topic: G.07. Data Analysis and Statistics

Support: Human Brain Project/Neuroinformatics MH057153

Human Brain Project/Neuroinformatics MH068012

Title: Integrated spike train analyses via neuroanalysis.org

Authors: ***D. GARDNER**, E. CHAN, A. JAGDALE, M. A. REPUCCI, D. H. GOLDBERG, J. D. VICTOR;
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Abstract: Analyses of neural coding—the representation and processing of information with spike trains—require multiple analytic methods. This is because specific neural systems are likely to use different models or representations, and also because different methods require specific types and amounts of data, and so particular analytic approaches. Toward increasing availability and applicability of an informative suite of information theoretic algorithms, we have developed and released open source via neuroanalysis.org the Spike Train Analysis Toolkit, now in version 1.5 and downloaded over 1,300 times. Aiding selection and application of its five information-theoretic and eight entropy estimators, STAToolkit offers informative guides and demonstrations (Goldberg et al. Neuroinformatics 7, 165-178, 2009).
Complementing the STAToolkit, and supporting adoption and use of these algorithms beyond the computational neuroscience community, we report enhanced development of our parallel AnalysisServer, an open-access dedicated large-scale 32-node computational

array available to neurophysiologist users. Newly-live ‘Analyze’ links enable dataset preparation and entropy and information determination from data at neurodatabase.org. Client, server, and scheduler components aid and guide use:

• Within the neuroanalysis.org framework, neurodatabase.org users can access the Spike Train Analysis interface to concatenate, segment, group, and submit data to the server for analysis.

• A user submissions page shows submission history and status, including expected completion for in-progress jobs. Completed jobs report results of the analyses and allied metadata.

• The AnalysisServer estimates the computational time for the analysis and places the datasets into a short term, mid-term (exceeds 5 sec), or long-term queue (exceeds 30 min). For analysis jobs in the long-term queue require administrator submission. Other administrator functions allow starting or stopping analyses and viewing submissions by user information, submission ID, or submission status

• General guidelines are provided for users. For example, using either binless or direct-method categorical methods to analyze multi-neuron datasets of up to 5 neurons, computation times are 50 ms or less. Using metric space methods to analyze single-neuron datasets, typical computation times for modest datasets are 100 ms or less. Using metric space methods to analyze multi-neuron datasets of up to 5 neurons, computation times can range up to 20 hours.

Like the downloadable STAToolkit, the AnalysisServer is made available open source and open access, toward open discovery for neuroscience.

Disclosures: D. Gardner: None. **E. Chan:** None. **A. Jagdale:** None. **M.A. Repucci:** None. **D.H. Goldberg:** None. **J.D. Victor:** None.