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Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.01/A1

Topic: A.02. Neurogenesis and Gliogenesis

Support: University of Connecticut Research Advisory Committee

University of Connecticut Office of Undergraduate Research

Title: EphA4 signaling regulates tangential migration of neuronal precursors

Authors: M. B. EASTMAN, K. A. BAKER, N. B. GALLO, *J. C. CONOVER
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Abstract: Tangential migration of neuronal precursors from the subventricular zone (SVZ) to the rostral migratory stream (RMS) leading to the olfactory bulb is tightly regulated. Molecular guidance factors and physical barriers in the form of glial tubes contribute to neuroblast confinement along the SVZ and within the RMS. Disruption of glial tubes can lead to neuroblasts escaping from their prescribed migratory pathway; however, little is known regarding cell contact-mediated molecular interactions within this cell-dense region, particularly what governs the unique cytoarchitectural organization of glial tube astrocytes. Eph receptor tyrosine kinases and their membrane-bound ephrin ligands are critical in brain development and have been shown to have persistent roles in adult stem cell niches. Expression of EphA4 and its associated ligands have been detected in the postnatal and adult SVZ. Here we provide evidence for EphA4 kinase-mediated restriction of neuroblasts within SVZ and RMS boundaries in the developing, postnatal and adult brain, and propose an updated model for the regulation of tangential neuroblast migration via glial tubes within the anterior forebrain.

Disclosures: M.B. Eastman: None. **J.C. Conover:** None. **K.A. Baker:** None. **N.B. Gallo:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.02/A2

Topic: A.02. Neurogenesis and Gliogenesis

Support: MIUR PRIN 2010JFYFY2

EU FP7 n.: 602531

NIH Grant 8P20GM103449

Title: Synapsin III acts downstream Semaphorin 3A to regulate radial migration and orientation of pyramidal neurons *in vivo*

Authors: *L. CANCEDDA[1], L. PERLINI[1], J. SZCZURKOWSKA[1], B. A. BALLIF[2], A. PICCINI[3], S. GIOVEDÌ[3], F. BENFENATI[1,3]
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Abstract: Synapsins (Syns) are phosphoproteins abundantly expressed in the brain, where they associate with synaptic vesicles and the actin cytoskeleton. In contrast to Syn I and II,Syn III localizes at the neuronal cell body and axonal growth cone, is highly expressed at early stages of neuronal development and downregulated afterwards.While *in vitro* evidence suggests a role for Syn III in neurite extension, parallel *in vivo* evidence is missing. Here, we acutely modulated the expression levels of Syn III *in vivo* via*in utero* electroporation. Downregulation of Syn III expression by RNA interference caused defects in migration and orientation of layer II/III pyramidal neurons. On the other hand, overexpression, also affected neuronal migration and morphology, but not orientation. The expression of a shRNA-insensitive Syn III cDNA (rSyn III) together with the shRNA downregulating Syn III completely rescued the observed silencing phenotype. Interestingly,chronic downregulation of Syn III in Syn III KO mice also resulted in neuronal migration, morphology and orientation defects. In this work, we also identified a novel Cdk5 phosphorylation site on Syn III (Ser^{404}) and proved it is essential for the Syn III developmental functions in rescue experiments with phosphorylation site mutants. The S404A non-phosphorylatable rSyn III mutant did not rescue the defect in radial migration caused by Syn III downregulation, while the S404D pseudophosphorylated rSyn III mutant rescuedthe knockdown phenotype similar to wild type Syn III. Finally, we showed that Syn III phosphorylation at the CDK5 site is induced by activation of Sema3A pathway, which is also involved in migration, and orientation of cortical neurons, and is known to activate CDK5. First, Syn III phosphorylation on Ser^{404} was induced in cultured cortical neurons by Sema3A treatment. Second, S404D-rSyn III partially rescued the defect in radial migration caused by the *in vivo* downregulation of Sema3A receptor NP1. Thus, a fine-tuning of Syn III expression and

phosphorylation by Sema3A-activated CDK5 is essential for a proper migration and orientation of pyramidal neurons.

Disclosures: **L. Cancedda:** None. **J. Szczurkowska:** None. **B.A. Ballif:** None. **A. Piccini:** None. **S. Giovedì:** None. **F. Benfenati:** None. **L. Perlini:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.03/A3

Topic: A.02. Neurogenesis and Gliogenesis

Support: 1R15-NS060099-01

Title: Neural Crest Micro array after Slit2 gain of function

Authors: D. MARTINEZ, M. Y. REYES, N. ZUHDI, H. RA, *M. E. DE BELLARD
biology, Cal State Univ. Northridge, Northridge, CA

Abstract: Neural crest cells are a multipotent cell lineage that delaminates from the dorsal neural tube of developing vertebrate embryos and subsequently undergo an epithelial to mesenchymal transition enabling stationary cells to actively migrate to distant areas. Neural crest EMT is valuable to study since the same process occurs in cancer metastasis. Mechanisms controlling migration of neural crest cells are not fully understood. Slit2 is a chemorepellant guidance molecule that stimulates the motility of trunk neural crest cells and repels them from the developing gut. Slit2 is known to be a tumor suppressor molecule. The individual connections of neural crest cell EMT and Slit2 in cancer metastasis led us to believe that there may be a link between Slit expression and proper neural crest cell migration. Recently we found that Slit molecules are capable of impairing neural crest cell migration and suggested that it played a role in pre-migratory neural crest. The present study looked further into whether Slit/Robo interactions have a role in the process of neural crest delamination via gain-of-function (GOF) by electroporating Slit2 and loss-of-function (LOF) experiments by electroporating dominant negative Robo. We found that forced Slit or Robo expression in neural crest cells significantly impaired proper migration while LOF knockdown favored earlier migration of neural crest cells. Slit2 crest microarray showed that in addition to a large set of molecules known to be important in maintaining cells in a non-motile, epithelial phenotype: Rabl2B, Ankyrin D1, DSCAM-L1. A good number of these molecules are also known to be downstream of Slit-Robo signaling, but we

also found new molecules previously not associated with Robo signaling: Claudins, Shh, HoxA, ezrin, merlin, Delta, GDF5, glial genes (S100, FABP, gcm2), Bcl2-A1, DSCAML1, Nkx2.6, Otx2, NCAM2, and olfactory receptors. Also among the genes regulated by Slit2 GOF in our microarray were a wide range of cell division ones: Ezh1, Nothch1, cdk5, NEDD1, GAS proteins, and STAT4. Our findings reveal for the first time a new role for Slit2 in neural crest cell emigration and provide evidence for the ability of Slits to affect timely migration of neural crest cells in a Robo-dependent manner. Funding was by NIH/NINDS AREA grant 1R15-NS060099-01 and NIH-SCORE-5SC3GM096904-02 to MEdB.

Disclosures: D. Martinez: None. **M.Y. reyes:** None. **N. Zuhdi:** None. **H. Ra:** None. **M.E. De Bellard:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.04/A4

Topic: A.02. Neurogenesis and Gliogenesis

Title: Autism-related protein Negr1 is required for transition of migrating pyramidal neurons from layer V to layer II/III of the mouse cerebral cortex

Authors: *J. SZCZURKOWSKA[1], F. PISCHEDDA[2], F. MANAGO[1], C. HAAS[3], F. PAPALEO[1], M. SCHÄFER[4], G. PICCOLI*[2], L. CANCEDDA*[1]
[1]Neurosci. and Brain Technologies, Inst. Italiano Di Tecnologia, Genoa, Italy; [2]CNR Inst. of Neurosci. and Dept. of Pharmacol., Univ. of Milan, Milan, Italy; [3]Univ. of Freiburg, Freiburg, Germany; [4]Dept. of Anesthesiol., Univ. Med. Ctr. of Mainz, Mainz, Germany

Abstract: The mammalian cerebral cortex is a remarkably complex structure, and establishment of cortical neural circuitries requires its unique laminar organization. During perinatal development, newborn pyramidal neurons migrate along radial glia fibers, to create the six-layered structure of the neocortex. Disruption in neural migration can lead to brain malformations with functional consequences on proper wiring of the neuronal network, as already described in neurodevelopmental disorders such as Autism Spectrum Disorders (ASD). Common knowledge indicates cell-adhesion molecules (CAMs) as essential for proper neural migration. Neuronal growth regulator 1 (Negr1) is a CAM, and NEGR1 gene mutations have been recently associated to ASD. So far, nothing is known about Negr1 function in *in vivo*

neurodevelopment. By *in utero* electroporation coupled with RNA interference (siRNA), we downregulated Negr1 levels in late-born pyramidal neurons migrating to the superficial layers of the neocortex. We found that Negr1 siRNA caused ectopic positioning of neurons concentrated at the border between layer 5 and layer 4 of the somatosensory cortex. Downregulation of Negr1 did not cause migration defects in the motor or prefrontal cortices. We found that autism-related protein FGFR2 and its partner NCAM physically and functionally interact with Negr1 leading to activation of ERK signaling. Interestingly, downregulation of NCAM and FGFR2 *in utero* resulted in a strikingly similar phenotype on neuronal migration as found for Negr1, proving involvement of all three molecules in the same signaling pathway *in vivo*. Moreover, downregulation of Negr1 in the embryonic somatosensory cortex, but not in the prefrontal cortex resulted in decreased number of ultrasound vocalizations in pups. Finally, perinatal treatment of mouse pups with ERK signaling activator 7, 8-dihydroxyflavone (DHF) partially rescued both migration defect and vocalization impairment caused by Negr1 downregulation. These data suggest that Negr1/FGFR2/NCAM complex is necessary for proper neuronal migration of pyramidal neurons in the somatosensory cortex, indicating a possible role for this complex in autism. *: equal contribution

Disclosures: J. Szczurkowska: None. **F. Pischedda:** None. **F. Manago:** None. **C. Haas:** None. **F. Papaleo:** None. **M. Schäfer:** None. **G. Piccoli*:** None. **L. Cancedda*:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.05/A5

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH NS062849

NIH MH101268

Title: Co-regulated gene expression downstream of Fezf2 selects axon guidance choices of corticospinal motor neurons

Authors: *S. LODATO[1], B. MOLYNEAUX[1], E. ZUCCARO[1], L. GOFF[1], H.-H. CHEN[1], W. YUAN[1], S. MAHONY[2], J. L. RINN[1], D. GIFFORD[3], P. ARLOTTA[1]
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Abstract: The neocortex contains an unparalleled diversity of neuronal subtypes, each defined by distinct traits that are developmentally acquired under the control of subtype-specific and pan-neuronal genes. The regulatory logic that orchestrates the expression of these unique combinations of genes is unknown for any class of cortical projection neurons. Here, we report that Fezf2 is a selector gene able to regulate the expression of gene sets that collectively define CSMN. We find that Fezf2 directly induces the axon guidance molecule EphB1 *in vivo*, and demonstrate further that EphB1 is necessary to execute the ipsilateral extension of the corticospinal tract in the ventral forebrain. Our data indicate that co-regulated expression of neuron subtype-specific and pan-neuronal gene batteries by a single transcription factor is one component of the regulatory logic responsible for the establishment of CSMN identity.

Disclosures: S. Lodato: None. **B. Molyneaux:** None. **E. Zuccaro:** None. **L. Goff:** None. **H. Chen:** None. **W. Yuan:** None. **S. Mahony:** None. **J.L. Rinn:** None. **D. Gifford:** None. **P. Arlotta:** None.

Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant RO1NS039007

NIH Grant RO1MH071679

Title: Prox1 regulates the migration and maturation of caudal ganglionic eminence-derived cortical interneurons

Authors: *G. MIYOSHI[1], A. YOUNG[1], T. PETROS[1], T. KARAYANNIS[1], M. MCKENZIE CHANG[1], D. VAN VERSENDAAL[1], A. LAVADO[2], T. IWANO[3], M. NAKAJIMA[4], H. TANIGUCHI[5], J. Z. HUANG[5], N. HEINTZ[4], G. OLIVER[2], F. MATSUZAKI[3], R. P. MACHOLD[1], G. FISHELL[1]
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Abstract: Neurogliaform (RELN+) and bipolar (VIP+) GABAergic interneurons provide local inhibition within the superficial layers of the mammalian cerebral cortex. In contrast to the medial ganglionic eminence (MGE)-derived basket/chandelier (Pvalb+) and Martinotti (SST+) interneurons, these subtypes originate from the caudal ganglionic eminence (CGE), and as such, little is known about the specific genetic programs that direct their migration, differentiation and network integration into neocortical circuits. Here, we report that the transcription factor Prox1 is selectively expressed in postmitotic CGE-derived cortical interneuron precursors, and that loss of Prox1 impairs the integration of cells into superficial layers. Postnatally, Prox1 regulates the maturation of each specific subtype (RELN, Calb2/VIP and VIP) through intrinsic differentiation programs that operate in tandem with extrinsically driven neuronal-activity dependent pathways. Thus, Prox1 represents the first identified transcription factor specifically required for the acquisition of CGE-derived cortical interneuron properties.

Disclosures: G. Miyoshi: None. **A. Young:** None. **T. Petros:** None. **T. Karayannis:** None. **M. McKenzie Chang:** None. **D. Van Versendaal:** None. **A. Lavado:** None. **T. Iwano:** None. **M. Nakajima:** None. **H. Taniguchi:** None. **J.Z. Huang:** None. **N. Heintz:** None. **G. Oliver:** None. **F. Matsuzaki:** None. **R.P. Machold:** None. **G. Fishell:** None.

Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant HD067135

Title: The anti-epileptic drug valproic acid alters neuroprogenitor trajectories

Authors: *E. M. POWELL, R. F. MARTIN
Anat/Neurobio, Univ. Maryland, Baltimore, BALTIMORE, MD

Abstract: While it has been suspected for decades that prenatal exposure to antiepileptic drugs (AEDs) may cause birth defects, data gathered by pregnancy registries over the last decade show exposure to common AEDs increased the major malformations observed at birth by 2 - 10 fold

more than unexposed (control) children. In the case of valproic acid (VPA), nearly 10% of the children were born with major malformations. Verbal fluency and executive function, which are mediated by frontal cortical areas, were most affected. The primary mechanism of action of AEDs is to alter neurotransmitter systems. VPA increases inhibition by potentiating GABAergic transmission as a GABA transaminase inhibitor. VPA also acts as an inhibitor of histone deacetylase 1. Previous toxicity studies failed to show effects on neural stem cell viability or proliferation. Our *in vivo* data in embryonic mice exposed to a single dose of VPA during mid-gestation demonstrate increased exit from the forebrain proliferative zones and migration to final destinations. Adult mice that were prenatally exposed to VPA exhibit regional deficits in specific cortical GABAergic interneurons. The animal studies are supported by *in vitro* studies using human neural stem cells. Exposure to VPA alters the neuronal lineages and promotes the formation of glial populations. Our studies begin to reveal the molecular mechanisms and developmental perturbations that occur with AEDs. By understanding the critical pathways that lead to birth defects in the children of mothers who suffer from seizure and mood disorders, we can start to develop better therapies designed to effective treat women and protect their children.

Disclosures: E.M. Powell: None. **R.F. Martin:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.08/A8

Topic: A.02. Neurogenesis and Gliogenesis

Title: The miR379-410 cluster miRNA miR-543 regulates neurogenesis and neuronal migration *in vivo*

Authors: *J. WINTER[1], L. RAGO[2]
[1]Kemler, Univ. Med. Ctr. Mainz, Mainz, Germany; [2]Max Planck Inst. of Immunobiology and Epigenetics, Freiburg, Germany

Abstract: Several miRNAs of the miR379-410 cluster have important neuronal functions. Recently, we have shown that three miRNAs belonging to this cluster, namely miR-369-3p, -496 and -543, regulate the expression levels of N-cadherin in neural stem cells and neurons in the developing neocortex (Rago, Beattie et al. 2014). The overexpression of these three miRNAs in radial glial cells increased neural stem cell differentiation and neuronal migration - a phenotype which could be rescued when N-cadherin was expressed from a miRNA-insensitive construct. A

knockdown of the miRNAs reduced stem cell differentiation and increased cell proliferation. The overexpression of these miRNAs specifically in newborn neurons delayed migration into the cortical plate, whereas their simultaneous knockdown increased migration. Interestingly, migration already increased when only miR-543 was knocked down suggesting that this miRNA may have other targets in addition to N-cadherin that are involved in neuronal migration. We have now found that miR-543 directly regulates the expression of members of the semaphorin signaling pathway *in vitro* and *in vivo*. At the moment we are carrying out further studies to functionally evaluate this regulation. Rago, L., R. Beattie, et al. (2014). "miR379-410 cluster miRNAs regulate neurogenesis and neuronal migration by fine-tuning N-cadherin." EMBO J 2014 Apr 16;33(8):906-20.

Disclosures: J. Winter: None. **L. Rago:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

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Program#/Poster#: 208.09/A9

Topic: A.02. Neurogenesis and Gliogenesis

Support: Start-up Funds to E.S.T. from WVU

Title: Cortical interneurons require c-Jun N-terminal kinase (JNK) signaling to preserve migratory stream integrity during corticogenesis

Authors: A. K. MYERS[1], K. BAKER[2], J. P. SNOW[2], C. A. SMITH[2], *E. S. TUCKER[2]
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Abstract: Cortical interneurons travel in well-defined migratory streams as they enter the cerebral cortex during embryonic development. The formation and maintenance of migratory streams is essential, since perturbations to migratory streams may misroute migrating cortical interneurons and lead to cortical circuit malformations present in severe brain disorders such as epilepsy, schizophrenia, and autism. Although molecular mechanisms mediating the formation and maintenance of migratory streams have been well studied, molecular events controlling departure from migratory streams remain obscure. We previously demonstrated that initial entry of cortical interneurons into the cerebral cortex depends on JNK signaling, largely mediated by the cell autonomous activity of *Jnk1*, and now find cortical interneurons require JNK to travel within migratory streams at later periods of cortical development. Pharmacological blockade of

JNK signaling in *ex vivo* slice cultures leads to rapid departure of cortical interneurons from migratory streams; specifically, live-cell imaging reveals a prompt shift from tangential to radial modes of migration, and quantification of interneuron distribution in JNK-inhibited slices reveals dose-dependent dispersion from migratory streams. Analyses of interneuron distribution in the developing cortex of JNK-deficient embryos parallels data obtained through pharmacologic inhibition, indicating JNK activity preserves tangential progression of cortical interneurons within migratory streams *in vivo*. These data suggest JNK signaling may be developmentally regulated in migrating cortical interneurons to control the timing of migratory stream exit and subsequent entry into the cortical plate. Current efforts explore the spatial and temporal extent of interneuron displacement from migratory streams in JNK-deficient embryos and JNK-inhibited slice cultures, cellular and molecular mechanisms underlying JNK-dependent preservation of migratory streams, and analyses of interneuron distribution within the mature cerebral cortex of JNK-deficient mice. Completion of this work is expected to illuminate fundamental mechanisms of corticogenesis and offer insight into pathogenic mechanisms underlying neurodevelopmental diseases of cortical circuitry.

Disclosures: A.K. Myers: None. **J.P. Snow:** None. **C.A. Smith:** None. **E.S. Tucker:** None. **K. Baker:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.10/A10

Topic: A.02. Neurogenesis and Gliogenesis

Support: National Science Foundation (OTKA) No.: K 106 191

Title: Effects of optogenetic stimulation on motility of differentiating neural stem cells

Authors: *K. MARKO[1], N. PAPP[3], T. KOHIDI[3], A. JADY[3], Z. KORNYEI[3], T. ANDRASI[3], D. MARIC[2], E. MADARASZ[3]
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Abstract: Neural stem/progenitor cells (NSCs/NPCs) are exposed to bioelectric stimuli from their birth to tissue integration (Flavell, Greenberg 2008). Spontaneous Ca-oscillations are spreading in the early neural tube (Donovan 1999), and giant depolarizing potentials are

travelling in the developing brain (Ben-Ari 1989) before and during the formation of synaptically coupled neuronal networks. Ongoing potential changes influence the migration and integration of neuronal precursors in the adult hippocampus as well (Song et al, 2013). The responsiveness of various NSC/NPC populations to these stimuli or the necessity of such stimulation for survival, migration or adopting the right phenotype, however have not been explored in details. According to our previous data, NSCs/NPCs derived from the primary or secondary germinative zones display different electrophysiological properties (Jelitai et al., 2007; Marko et al., 2011). Novel optogenetic methods (Zhang et al., 2007) opened ways for controlled long-term, non-invasive stimulation of *in vitro*-maintained stem cell populations, and enabled us to follow the fate of stimulated cells in their later development. Preferential adhesion of neural stem cells to surfaces covered with a novel synthetic adhesive polypeptide (AK-cyclo[RGDfC]) provided a unique, rapid procedure for isolating a population of radial glia-like (RGl) cells from both fetal, perinatal or adult rodent brain (Marko et al., 2011). RGl cells displayed elongated cell shape, vivid nuclear translocation along the entire cell-axis, and migrated by sliding along neighbours, while in differentiating progenies, the motility decreased in parallel with the appearance of neuronal features. For non-invasive stimulation, channelrhodopsin- (ChR2)-expressing populations were established from existing RGl cell lines by transfecting with ChR2-coding constructs. RGl-type cell populations were isolated also from CAG^{loxp}Stop^{loxp}Chr2(H134)-EYFP transgenic mouse embryos and RG$^{loxp\text{Chr2}}$ cells were either transfected with Cre-recombinase coding constructs (RG$^{loxp\text{Chr2}+}$) or were used as ChR2-non-expressing (RG$^{loxp\text{Chr2}-}$) controls. Both, RG$^{loxp\text{Chr2}+}$ and RG$^{loxp\text{Chr2}-}$cells generated neurons in respone to EGF withdrawal and responded to illumination (488nm) with inward cation currents. Illumination modified the motility of differentiating RG$^{loxp\text{Chr2}+}$ cells by decreasing both nuclear translocations and migration. The findings indicate that optogenetic stimulation accelerates *in vitro* differentiation of radial glia-like neural stem cells.

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Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.11/A11

Topic: A.05. Axon and Dendrite Development

Support: ZIA NS002824-24

Title: Characterizing the role of VEGFR1 in GnRH neuronal migration

Authors: *E. FLANNERY[1], D. DONOHUE[2], S. WRAY[1]
[1]NINDS, NIH, Bethesda, MD; [2]Integrative Systems Biol., US Army Med. Res. and Material Command, USACEHR, Fort Detrick, MD

Abstract: Neuronal migration is essential during development for appropriate circuitry to form. Disruption of neuronal migration causes neurological disease states, leading to a number of devastating cognitive and reproductive problems. As such, it is critical to understand the mechanisms underlying normal neuronal migration to begin to address/treat the event(s) associated with a disease. In vertebrates, appropriate migration of gonadotropin-releasing hormone-1 (GnRH) neurons to their final location within the brain is necessary for hypothalamic control of sexual maturation and reproductive function. Failure of this process leads to hypogonadotropic hypogonadism (HH), resulting in delayed puberty and infertility. To discover ligand/receptor signaling impacting this migration, we compared the transcriptomes of migratory vs. post-migratory GnRH neurons. Microarray analysis revealed that the receptor tyrosine kinase Fms-like tyrosine kinase 1 (also known as vascular endothelial growth factor receptor-1, VEGFR1) was upregulated in migrating neurons as compared to post-migratory neurons. VEGFR1 is most commonly associated with the developing vasculature and has been shown to function in vasculogenesis by sequestering VEGFA from another receptor, VEGFR2. Notably, both VEGFA and a related VEGFR1 ligand, VEGFB, were expressed along the GnRH migratory route and in GnRH cells, respectively, lending credence to their potential role in regulating GnRH neuronal migration. Single cell PCR and immunocytochemistry confirmed expression of VEGFR1 in migrating GnRH neurons. Functional assays revealed chronic treatment with a blocking antibody specific for VEGFR1 decreased the distance GnRH cells migrated compared to controls. In addition, acute *in situ* assays using the blocking antibody revealed an immediate decrease in cell migration rate. Unexpectedly, blocking the ligand VEGFA revealed a significant increase in migration rate, suggesting a dynamic balance between VEGFA and VEGFB in modulating VEGFR1-mediated GnRH migration. These data demonstrate a novel role for this receptor tyrosine kinase and its ligands in neuronal migration and may provide new candidates for genetic screening in HH patients. Furthermore, this work sheds light on the complex, yet well-known players in vasculogenesis and may provide an unexplored link between two critical developmental processes—vasculogenesis and neuronal migration.

Disclosures: E. Flannery: None. **D. Donohue:** None. **S. Wray:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.12/A12

Topic: A.02. Neurogenesis and Gliogenesis

Support: Supported by grants from the NIH and Kavli Foundation

Title: A growth factor-dependent Ca2+ signaling network during stem cell proliferation and neuronal migration in the developing neocortex

Authors: *B. G. RASH[1], J. B. ACKMAN[1], P. RAKIC[2]
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Abstract: Development of the cerebral cortex depends on the sequential genesis of neuronal subtypes in the ventricular zone (VZ) followed by their active migration to proper areal and laminar positions in the embryonic cerebral cortex. Calcium flux is a potential regulatory factor for these complex processes, but spatio-temporal dynamics of Ca2+ activity remain poorly understood. Here we show that cortical stem cells generate non-synaptic Ca2+ transient activity from the earliest stages of corticogenesis *in vivo*--even before the onset of synaptogenesis. We demonstrate strong Ca2+ activity in response to growth factors like fibroblast growth factor (FGF) 2, which has been shown to promote radial glial cell (RGC) proliferation and can also induce the formation of artificial convolutions in the mouse cerebral cortex. In addition we found that this Ca2+ activity is carried long distances within the elongated RGC fibers and propagated to multiple adjacent cells, including other RGCs, intermediate neuronal progenitors and migrating neurons. Correlation analysis reveals that some of these, seemingly random, events, are highly correlated and that target cells responded to the activity by initiating their own Ca2+ transients, indicating that much of this activity is not intrinsically spontaneous, but triggered by the patterned network activity. Our results indicate that growth factor-dependent Ca2+ activity mediates a widespread electrical network spanning the cortical wall and is involved in the development of radial units. This coordinated activity cannot be suppressed by the gap junction blocker, octanol, or by the glutamate receptor antagonist, CNQX, suggesting that at early stages cortical radial units communicate by a ligand-receptor based mechanism. Finally, exposure to the therapeutic drugs, fluoxetine and valproic acid, interfered with calcium activity and disrupted the radial glial scaffold, leading to cortical abnormalities.

Disclosures: B.G. Rash: None. **P. Rakic:** None. **J.B. Ackman:** None.

Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

Support: JSPS Grant 23110006

JSPS Grant 252038

Title: AGO61, a causative gene for dystroglycanopathy, is required for the maintenance of the basement membrane integrity and neuronal migration

Authors: *N. NAKAGAWA[1], H. YAGI[2], K. KATO[2], S. OKA[1]
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Abstract: Dystroglycan (DG) is a cell-surface glycoprotein, which requires *O*-mannosyl glycosylation to interact with basement membrane (BM) components such as laminin, agrin, and perlecan. Mutations in the genes encoding enzymes responsible for DG glycosylation, including *AGO61*, cause dystroglycanopathy, a subclass of congenital muscular dystrophy with brain malformation. Recently, we generated AGO61-knockout mice and revealed that their brain exhibited the disrupted BM, disorganized radial glial fibers, and neuronal overmigration due to the lack of ligand binding glycans on DG. However, early pathological changes resulting from the DG hypoglycosylation that lead to such severe consequences remain unclear. In this study, to further characterize the cortical dysplasia of AGO61-deficient mice, we immunohistochemically analyzed the expression of several marker proteins in the developing cerebral cortex. In the AGO61-deficient brain, the pial BM was properly formed at the embryonic day 10.5 (E10.5), but was obviously discontinuous at E12.5. At E14.5, breaches of the BM were more severe than those at E12.5 and neuronal ectopias were found. These results indicate that the BM has been once formed without functional DG and then progressively disrupted along with cortical development. Moreover, the failure of preplate splitting, a characteristic phenotype of reeler mice, was observed in the AGO61-deficient brain. This finding suggests that neuronal migration defects have already begun in the migration of early-born neurons, providing an important insight into the understanding of the aberrant neuronal layer formation in dystroglycanopathy.

Disclosures: **N. Nakagawa:** None. **H. Yagi:** None. **S. Oka:** None. **K. Kato:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

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Program#/Poster#: 208.14/A14

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH R01 NS063959

NIH1R03NS077087-01

Title: Regulation of neuronal cell migration by sphingomyelin synthase 1 is mediated through modulation of the key cell cycle protein p27 and matrix metalloproteinase

Authors: U. V. WESLEY, J. HATCHER, *R. J. DEMPSEY
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Abstract: Sphingomyelin synthase (SMS) is a key enzyme involved in the generation of the lipid sphingomyelin (SM) and regulation of cell growth and survival. However, little is known about the effects of SMS on neuronal cell migration. In this study, we examined the direct effects of SMS1 in regulating the migratory properties of Neuro-2a cells that are a widely used *in vitro* cell culture model for neuronal studies. Neuro-2a cells transfected with SMS specific shRNA expressed significantly lower levels of SMS1. RNA interference-mediated depletion of SMS1 also caused a significant decrease in SM levels. Silencing of SMS1 inhibited the migratory potential of Neuro-2a cells as indicated by the scratch induced migration assay, and Boyden chamber 3D matrigel invasion assay. At the molecular level, these changes were accompanied by the up-regulation of cyclin-dependent kinase inhibitor p27, and decreased levels of cyclin D1 and phospho-Akt in SMS1 depleted cells. Interestingly, nuclear accumulation of p27 was evident in SMS1 deficient cells, as shown by immunofluorescence staining. Furthermore, screening by PCR array containing motility related genes revealed significant down regulation of matrix metalloproteinase family members including MMP-2, -14, and -9 in SMS1 silenced cells. These results indicate that SMS1 plays an important role in mediating the key signaling pathways that are involved in the tight coordination of neuronal cell migration, and therefore may have significant implications in neurodegenerative diseases, as neuronal cell migration is central to proper establishment of neural network, disruption of which can lead to neurological disorders.

Disclosures: U.V. Wesley: None. **J. Hatcher:** None. **R.J. Dempsey:** None.

Poster

208. Neuronal Migration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 208.15/A15

Topic: A.02. Neurogenesis and Gliogenesis

Title: Roles of MACF1 in pyramidal neuron migration during brain development

Authors: *M. KA, E.-M. JUNG, M. LATNER, W.-Y. KIM
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Abstract: Neuronal migration is an essential process for the development of the cerebral cortex. However, little is known about the regulatory mechanisms of neuronal migration in the developing brain. Here, we show that regulation of MACF1 activity is critical in radial neuronal migration and differentiation in the developing dorsal telencephalon. Using genetic manipulation *in utero*, we find that MACF1 deletion defects migration of cortical and hippocampal pyramidal neurons. Also, MACF1 floxed allele mice show aberrant neuronal positioning in the developing brain. We observed that MACF1 elimination suppressed elongation of the leading processes. Importantly, microtubule stability is severely damaged in neurons lacking MACF1, resulting in abnormal microtubule dynamics. Our findings demonstrate that MACF1 is equired for correct positioning of telencephalic pyramidal neurons via the regulation of microtubule dynamics.

Disclosures: M. Ka: None. **E. Jung:** None. **M. Latner:** None. **W. Kim:** None.

Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

Support: Manasaki Fellowship, Univ of Crete

Title: Impaired mitral cell migration due to TAG-1/CNTN2 deficiency leads to olfactory dysfunction

Authors: *D. KARAGOGEOS[1], G. G. BASTAKIS[2], M. SAVVAKI[2], A. STAMATAKIS[3], M. VIDAKI[2]

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Abstract: The olfactory system constitutes a sensory system of major importance for mammals as well as a valuable tool for studying neuronal connections in the central nervous system. Much knowledge has been obtained in recent years on the organization of the odorant receptor map, formed by the connections of olfactory receptor neurons with the dendrites of projection neurons of the olfactory bulb, in specialized structures, the glomeruli. On the other hand, little is known about the corresponding mitral cell map, located in the mitral cell layer, consisting of the main projection neurons of the olfactory bulb. Our work aims to shed light on the organization and function of the mitral cell layer. Our experimental approaches include immunohistochemistry and *in situ* hybridization, explant cultures, transplants *in vitro*, neuronal activation experiments, behavioral analysis. We show that the absence of Transient Axonal Glycoprotein-1/Contactin-2 (TAG-1/CNTN2), a cell adhesion molecule of the immunoglobulin superfamily, results in a significant reduction in the number of mitral cells inside the main olfactory bulb in mice. This reduction occurs as a consequence of impaired migration of a subpopulation of projection neurons born at embryonic day 11.5. We report on the developmental series of events that occur before the final positioning of projection neurons into the mature mitral cell layer. Our study reveals that the detected alterations in the number of mitral cells are reflected in an aberrant neuronal activation profile as well as disturbed olfactory behavior. Our results propose that TAG-1/CNTN2 function is crucial for the proper organization of projection neurons in the main olfactory bulb, a prerequisite for proper OB function. Moreover they suggest that disturbing this station of the odorant information route disrupts its integration into the olfactory circuitry.
#G.G.B. and M.S. contributed equally to this work

Disclosures: D. Karagogeos: None. **G.G. Bastakis:** None. **M. Savvaki:** None. **A. Stamatakis:** None. **M. Vidaki:** None.

Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant NINDS24014

USUHS RO7O9J

Title: Epigenetic modifications alter the expression pattern of KCC2 in a ferret model of cortical dysplasia

Authors: *F. T. DJANKPA[1], O. B. AKINOLA[2], S. POLUCH[3], D. BORST[3], C. DALGARD[3], S. L. JULIANO[3]
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Abstract: The pathogenesis of neurodevelopmental disorders such schizophrenia, autism spectrum disorders, and epilepsy are associated with cortical dysplasia. This migration disorder can result from defects in the movement of GABAergic interneurons during corticogenesis, but the specific underlying mechanisms remain elusive. We know that KCC2, NKCC1, and GABA are strongly involved in mediating migration into the neocortex. Using a model of cortical dysplasia in ferrets, we found that small unique doses of the methylating agent methylazoxy methanol acetate (MAM) results in disrupted migration during corticogenesis, specifically of interneurons, when administered to pregnant jills at E33. We recently found that an underlying cause of impaired migration may be a consequence of the precocious and increased expression of the Cl^- exporter, KCC2, as indicated by western blot analysis and electrophysiological recordings. These findings suggest that increased KCC2 may play a critical role in impaired migration leading to cortical dysplasia. As KCC2 normally increases during development, NKCC1 (a Cl~ importer) reduces; levels of NKCC1, however, did not differ from normal in MAM treated animals. Here we confirm that KCC2 expression is elevated in this model by measuring increased mRNA transcript levels using qPCR in the cortex of P0 MAM treated ferret kits compared to untreated controls. We also found the methyl CpG binding protein 2 (MeCP2) to be elevated in organotypic tissue cultures treated with MAM, but not in control tissue. These increases were further confirmed by finding an overall increase in methylation in the neocortex of animals treated with MAM. We also found a slight increase in methylation of the KCC2 gene using bisulfite sequencing. With this method we observed a small increase in methylation of 5 CpG islands targeted by our primers. These findings point to possible epigenetic modifications contributing to increased KCC2 expression after MAM treatment in ferrets, which ultimately leads to impaired migration.

Disclosures: F.T. Djankpa: None. **O.B. Akinola:** None. **S. Poluch:** None. **D. Borst:** None. **C. Dalgard:** None. **S.L. Juliano:** None.

Poster

208. Neuronal Migration

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Title: Cdk5-mediated phosphorylation of RapGEF2 is required for neuronal migration during cerebral cortex development

Authors: ***T. YE**[1,2,3], J. P. IP[1,2,3], A. K. FU[1,2,3], N. Y. IP[1,2,3]
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Abstract: The functional wiring of the cerebral cortex requires multiple coordinated steps of neuronal migration, including multipolar migration, locomotion and somal translocation. Although the small GTPase Rap1 regulates different steps of migration, how Rap1 is differentially activated during distinct migratory phases is not well understood. We found that activation of Rap1 by guanine nucleotide exchange factor RapGEF2 is specifically required for the initial phase of the migration process. RapGEF2 knockdown *in utero* arrests migrating neurons at the multipolar stage, leading to formation of subcortical band heterotopia in the postnatal mouse brain. The GEF activity of RapGEF2 towards Rap1 requires phosphorylation by the serine/threonine kinase Cdk5. In turn, Rap1 activation by RapGEF2 promotes the cell surface expression of N-cadherin, and subsequently enables the bipolar transition and radial neuronal migration. Our findings suggest that Cdk5-dependent phosphorylation of RapGEF2 plays an important role in multipolar-bipolar transition, and thus proper migration and wiring of the cerebral cortex.

Disclosures: **T. Ye:** None. **J.P. Ip:** None. **A.K. Fu:** None. **N.Y. Ip:** None.

Poster

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Topic: A.03. Postnatal Neurogenesis

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BBSRC Grant BB/J018635

Title: Galectin-3 decreases subventricular zone cell migration to the demyelinated corpus callosum

Authors: *J. M. HILLIS[1], J. SEVERS[1], R. E. JAMES[2], F. G. SZELE[1]
[1]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom; [2]Imperial Col. London, London, United Kingdom

Abstract: The glycoprotein binding protein Galectin-3 (Gal-3) has separately established roles in multiple sclerosis and subventricular zone (SVZ) cell migration. After demyelination SVZ cells migrate to the neighbouring corpus callosum, and we sought to determine if Gal-3 impacted this process. We treated Gal-3+/+ and Gal-3-/- mice with Theiler's murine encephalomyelitis virus (TMEV) or cuprizone demyelination models, and compared the SVZ responses using immunohistochemistry. Following TMEV Gal-3-/- mice contained more doublecortin-positive (Dcx+) neuroblasts in the corpus callosum. Following cuprizone treatment there were very few Dcx+ neuroblasts in the corpus callosum of either genotype. We had, however, administered bromodeoxyuridine (BrdU) at the beginning of cuprizone treatment to create label retaining SVZ cells, and after treatment there were more BrdU+ cells in the corpus callosum of Gal-3-/- mice. Preliminary results suggest these cells are Olig2+ oligodendrocytes. Interestingly Gal-3 co-labelled GFAP+ astrocytes and CD45+ immune cells, yet neither Dcx+ neuroblasts nor Olig2+ oligodendrocytes after cuprizone. Our working hypothesis is that Gal-3 secreted by resident astrocytes aids the migration of SVZ-originating neuroblasts and oligodendrocytes in a paracrine manner. When Gal-3 is absent the cells become 'uncoupled' from the rostral migratory stream and can more easily migrate to demyelinated regions such as the corpus callosum. Future short-term experiments will examine the molecular mechanisms behind these observations.

Disclosures: **J.M. Hillis:** None. **J. Severs:** None. **R.E. James:** None. **F.G. Szele:** None.

Poster

208. Neuronal Migration

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Program#/Poster#: 208.20/A20

Topic: A.02. Neurogenesis and Gliogenesis

Title: Identification of a functional role of Laminin alpha 1 in the developing brain

Authors: *C. HENG-KISSENBERGER[1], C. GAMPE[1], A. KLEIN[2], D. BAGNARD[2], J. BOLZ[1]
[1]Allgemeine Zoologie Und Tierphysiologie, Jena, Germany; [2]Inserm 1109 - MN3t Lab., Strasbourg, France

Abstract: The extracellular environment of the developing brain plays a crucial role in its organization and functionality. Here, we focused on the extracellular matrix glycoproteins of the Laminin family because of their well established function in cell migration and differentiation in various organs. The Laminin α1 chain (Lama1) gene has been reported in the list of the 50 genes important for recessive cognitive disorders in human (Najmabadi et al., 2011). Consistent with their critical implication in embryonic development, Lama1 deficiency causes early death of embryos due to a defect in Reichert's membrane (a protective extra-embryonic basement membrane) at E7.5 avoiding further developmental studies. To circumvent this issue we developed a conditional knockout (Lama1cKO) mouse of the gene coding for Lama1, overcoming early lethality. We already observed a strong defect in the organization of the adult cerebellum (Heng et al., 2011), demonstrating the significance of Lama1 in cerebellar development. Strikingly, we discovered cortical dysplasia in Lama1cKO brains linked to lateral ventricles dilatation. Histological and functional assays revealed that Lama1 absence leads to radial glia defects and abnormal migration of deep layer neurons. Moreover we observed a mispositioning of the Cajal-Retzius cells in the marginal zone, which could explain the cortical dysplasia by modifying the release of Reelin. This may have an effect on the brain mapping as Lama1cKO mice exhibit alterations of the cortical barrels. Furthermore we performed behavioral tests and revealed a less anxious behavior compared to control mice. Additionally, *in vitro* experiments demonstrated decreased axonal outgrowth but increased neuronal migration on Lama1 deficient substrates. Hence, we suppose that Lama1 has a key role in cortical development as „stop“ migration and „go“ differentiation factor. These data provide a starting point to dissect the molecular mechanism of Lama1 to identify the therapeutic potential of this secreted protein in central nervous system diseases.

Disclosures: C. Heng-Kissenberger: None. **C. Gampe:** None. **A. Klein:** None. **D. Bagnard:** None. **J. Bolz:** None.

Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

Support: KAKENHI24659036

Title: Specific proteolytic cleavage of Reelin regulates duration and range of its signaling

Authors: *K. OKUMURA, A. HISANAGA, M. KOIE, T. KOHNO, M. HATTORI
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Abstract: Reelin is a secreted protein that is essential for normal brain development and functions. Reelin is specifically cleaved around RR2 and RR3 (N-t site). We previously found that the N-t cleavage virtually abolishes Reelin signaling activity *in vitro*. As dysfunction of Reelin caused by N-t cleavage has been suggested to be involved in the pathogenesis of several neuronal diseases including Alzheimer's disease and schizophrenia, it is of great clinical importance to understand the N-t cleavage mechanism. In this study, We determined the exact N-t site catalyzed by a protease secreted by cerebral cortical neurons. N-t cleavage occurred between Pro1,244 and Ala1,245 within Reelin repeat 3. Reelin mutant in which Pro1,244 was replaced with aspartate (Reelin-PD) was resistant to a protease secreted by cerebral cortical neurons and its biological activity stayed longer than that of wild type Reelin. Interestingly, Reelin-PD remained in the intracellular region longer than wild type Reelin and persistently activated downstream signaling. We established a monoclonal antibody that recognizes uncleaved Reelin and found that it is localized in the vicinity of Reelin-producing cells while the N-terminal fragment diffuses, or is transported, to distant regions. These data demonstrate that N-t cleavage of Reelin plays critical roles in regulating the duration and range of Reelin functions. We also identified the protease in charge of N-t cleavage by purifying it from the culture supernatant of primary cortical neurons by a series of column chromatographies. Recombinant protein of this protease cleaved Reelin at N-t site and the cleavage of Reelin is markedly decreased in the embryonic cerebral cortex of its knockout mice. Importantly, the amount of Dab1 protein is decreased in the cerebral cortex of the knockout mice, indicating that Reelin signal is hyperactivated. Therefore, it is proven *in vivo* that N-t cleavage by a specific protease is the major inactivation pathway of Reelin in the developing cerebral cortex. The

understanding of its regulatory mechanism will help establish novel methods for prevention and treatment of neurological diseases.

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Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

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KAKENHI 22390016

KAKENHI 23123519

KAKENHI 24659036

Title: The novel function of Reelin in the dendrite development and layer formation in the postnatal brain

Authors: *T. KOHNO[1], T. HONDA[2], K.-I. KUBO[2], Y. NAKANO[1], A. TSUCHIYA[1], T. MURAKAMI[1], H. BANNO[1], K. NAKAJIMA[2], M. HATTORI[1]
[1]Biomed. science, Nagoya City Univ., Nagoya, Japan; [2]Dept. Anat., Keio Univ., Sch. of Med., Tokyo, Japan

Abstract: Reelin is a large secreted glycoprotein that regulates a variety of events in mammalian brain, including neuronal migration, neuronal dendritic formation, and synaptic plasticity. Reelin binds to apolipoprotein E receptor 2 (ApoER2) and very low-density lipoprotein receptor (VLDLR), and induces the tyrosine phosphorylation of an intracellular adaptor protein, Dab1. However, the primary role of Reelin in the developing brain and its underlying molecular

mechanisms remain largely unknown. Reelin protein is composed of the N-terminal domain, eight Reelin repeats, and the highly basic C-terminal region (CTR). The primary sequence of CTR is completely conserved in most vertebrates, suggesting its important function. Here we found that Reelin is proteolytically cleaved by furin-like proprotein convertases within CTR and a peptide of six amino acid residues is liberated. Reelin with an intact CTR, but not that without the last six residues, binds to a transmembrane protein that is different from the well-known Reelin receptors, ApoER2 and VLDLR. This interaction is blocked by the monoclonal antibody that specifically recognizes Reelin with an intact CTR. To clarify the significance of CTR *in vivo*, we generated a knock-in mouse lacking the CTR (ΔC-KI). We found that layer I of the cerebral cortex is narrower in postnatal ΔC-KI mice compared with wild-type mice. This phenotype is not observed in embryonic stage mice. Furthermore, we found that, in ΔC-KI mice, upper-layer neurons invade into layer I, and have misoriented and poorly branched apical dendrites. These results suggested that CTR is necessary for the correctly directed apical dendrites of migrated neurons in the cerebral cortex. Probably as a result of the failure in this process, the neuronal layers, especially layers II and III, become scattered and less packed. Thus, we propose that the interaction between Reelin and the transmembrane protein regulates the final stage of neuronal layer formation in the cerebral cortex and this event is regulated by specific proteolysis within the CTR.

Disclosures: **T. Kohno:** None. **T. Honda:** None. **K. Kubo:** None. **Y. Nakano:** None. **A. Tsuchiya:** None. **T. Murakami:** None. **H. Banno:** None. **K. Nakajima:** None. **M. Hattori:** None.

Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

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FAPERJ

CAPES

PROPPi-UFF

Title: Involvement of nucleotides in glial growth following scratch injury in avian retinal cell monolayer cultures

Authors: *A. VENTURA, T. M. SILVA, G. R. FRANÇA, I. M. ORNELAS
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Abstract: Müller cells are the main glial cell type in the retina that is capable of re-entering cell cycle in response to retinal damage or disease. When retinal cell cultures cultivated for 7 days were scratched with a pipette tip, a significant cell growth at the empty area was detected between the 1st and 3rd days after scratch and the area devoid of cells decreased by ~70% (from 22.3 ± 1.0 mm^2/10^{-2} to 7.7 ± 0.9 mm^2/10^{-2}). Three days after scratch, only glial cells 2M6 positive, β-tubulin III negative were detected in the scratched area. Both diving and migrating glia could be observed in the scratched area. Incubation of the cultures with 2.5 U/ml of apyrase (APY), 100 μM suramin or 40 μM Reactive Blue II, but not 30 μM MRS 2179, significantly attenuated the growth of glial cells in the scratched region after 3 days (scratched area without cells: control, 31%; APY, 79.1%; suramin, 69.1%; RBII, 52.8%; MRS2179, 24%), suggesting that activation of nucleotide receptors distinct from the P2Y1 receptor are involved in the growth of glial cells. Treatment of the scratched cultures with APY in the presence of the enzymatically stable agonists UTPγS (10 μM) or ADPβS (50 μM) revealed that only the P2Y2/4 agonist was able to significantly increase the growth of cells over the scratched area (area without cells: control, 24.1%; APY, 73.3%; APY + UTPγS, 31.5%; APY + ADPβS, 60.2%), suggesting the participation of UTP-sensitive receptor subtypes in the growth of glial cells. No significant decrease in the number of PCNA positive cells was observed at the border of the scratched area in apyrase-treated cultures, suggesting that nucleotides did not affect glial cell proliferation in the cultures. In apyrase-treated cultures, a lower phalloidin labeling could be noticed in glial cells at the edge of the scratch that were without protrusions and with less organized actin filaments or with stress fibers parallel to the border of the scratch. Significant increases in the phosphorylation of Akt and ERK were observed by the incubation of the cultures with 100 μM UTP, effects that were completely inhibited by SRC inhibitor 1 and LY 294002, two compounds that inhibit SRC and PI3K, respectively, Cell growth in the scratched area was also significantly attenuated by these inhibitors, suggesting the participation of SRC and PI3K/Akt signaling pathway in UTP-induced growth of glial cells. These results suggest that mechanical scratch of retinal cell monolayer cultures induces the growth of glial cells over the empty area through a mechanism that is dependent on activation of UTP sensitive nucleotide receptors, SRC and PI3K/Akt pathway.

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Poster

208. Neuronal Migration

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Topic: A.02. Neurogenesis and Gliogenesis

Title: Cellular dynamics underlying the generation of linear arrays of oligodendrocytes in CNS white matter

Authors: *T. D. MERSON[1,2], B. H. A. CHUANG[1], T. J. KILPATRICK[1,2], P. T. RÖTH[1]
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Abstract: Linear arrays of glial cell somata, enriched with interfascicular oligodendrocytes, align the longitudinal axes of white matter tracts throughout the central nervous system (CNS). These structures consist of repeating units of cell bodies of glial cell bodies in tandem alignment with the principal axonal axis. The mechanisms responsible for linear array formation are poorly understood. We sought to investigate the cellular dynamics underlying the formation of linear arrays during early postnatal development and during the course of CNS remyelination. To address this question, sections of corpus callosi from C57BL/6 mice were labelled with cell-type specific markers to define the cellular composition and differentiation events during linear array formation. Here we demonstrate that linear arrays of cells in the corpus callosum first appear before the onset of postnatal myelination and their generation and complexity increases throughout ontogeny. Between 60-70% of cells within linear arrays belong to the oligogendroglial lineage, irrespective of postnatal age, with the proportion that adopt a mature myelinating phenotype progressively increasing with age. Analysis of hemizygous female H253 transgenic mice that carry an X-linked β-galactosidase gene on their X chromosome revealed evidence that both clonal expansion of sentinel cells and random reorganization of non-clonally related cells contribute to the formation of linear arrays. To assess the clonal relationship between cells within linear arrays during remyelination, H253 mice were submitted to the cuprizone model of de- and remyelination. Cuprizone challenge in adult mice abolished linear arrays but these were largely regenerated within 7 weeks after cuprizone withdrawal. Comparable patterns of cellular X-gal labelling within linear arrays were evident in control and cuprizone-challenged mice at recovery. These data demonstrate that the mechanisms underlying linear array formation and oligodendroglial differentiation during remyelination closely mirror the mechanisms that occur during postnatal myelination of the corpus callosum. Collectively our data suggest a model of linear array formation in which both *in situ* proliferation and random alignment of oligodendroglial cells contribute, a developmental mechanism that is reinstated during remyelination.

Disclosures: **T.D. Merson:** None. **P.T. Röth:** None. **B.H.A. Chuang:** None. **T.J. Kilpatrick:** None.

Poster

208. Neuronal Migration

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Title: Label-free quantification of the emergent behavior of human neuron networks

Authors: ***T. KIM**[1], M. MIR[2], A. MAJUMDER[3], M. XIANG[2], R. WANG[2], C. LIU[2], M. U. GILLETTE[2], S. STICE[3], G. POPESCU[2]
[1]Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [3]Univ. of Georgia, Athens, GA

Abstract: The emergent self-organization of neuronal networks is a complex process, which involves a variety of cues, both internal and external. The spatial organization of a system of neurons, or a neural circuit, is known to be closely related to the functional activity of the system. Since the functional activities of the nervous system are based on the interactions among individual neurons within the neural circuit, the formation and behavior of neural networks deserves further study. Various models have been suggested in order to properly explain the neuronal network using modern microscopy imaging techniques, including phase contrast and fluorescence confocal microscopy. While these techniques provide the morphological structure and specificity at high resolution, they are limited by effects of phototoxicity. Further, adding fluorescence markers for live imaging perturbs the cells from their natural state. Here we report a new approach for label-free imaging of live neurons. Spatial light interference microscopy (SLIM) is a recently developed quantitative phase imaging technique based on a commercial

phase contrast microscope (Wang et al, Opt Express, 19, 2011). Therefore, SLIM provides a proper environment for label-free *in vitro* studies of live cells utilizing the atmospheric and temperature control peripherals. Due to its quantitative phase measurement, SLIM ultimately provides the dry mass density at each point in space and time. Also, SLIM provides a very high temporal and spatial sensitivity, which is capable of measuring the dry mass change at a femtogram level. Using this technique, a stem cell differentiated human neuron culture is imaged over 24 hours. Hundreds of neurons are captured under the field of view and are imaged every 10 minutes over 24 hours to provide the dry mass map of the entire field of view as they form a network. Increase in dry mass for the first 10 hours is detected, suggesting that the network stabilizes after the first 10 hours of growth. Further investigation of the spatial organization is done at scales corresponding to the cluster size, inter-cluster distance, inter-cellular distance and single-cell size. The result shows a gradual change in the signal corresponding to these scales, and shows the migration, aggregation and network formation among the cells over 24 hours. This result is compared with a different culture treated to suppress neurite growth, which does not show significant change in signals over time. Thus we show that SLIM is a label-free live imaging system that can be used to probe the dynamics of cell migration over long time intervals.

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Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.01/A26

Topic: A.08. Transplantation and Regeneration

Title: Antrodia cinnamomea promotes neuritogenesis in mouse primary dorsal root ganglia neurons

Authors: *C. RAZA[1], K. S. Y. LEUNG[3], C. H. E. MA[1,2]
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Abstract: Axonal regeneration after peripheral nerve injury is crucial for functional recovery but the intrinsic axonal growth rate of peripheral neuron is extremely slow. Traditional Chinese

medicines (TCMs) have been used over the century for their diverse therapeutic potentials such as medicinal mushroom *Antrodia cinnamomea* (*Niu-chang-chih* in Chinese) has shown promising neuroprotective effects against amyloid β-protein-induced neurotoxicity in PC-12 cells and memory impairment in animal model of Alzheimer's disease. However, it's potential for repairing peripheral nerve after injury remains unclear. Current study aims to investigate promoting effects of *A. cinnamomea* extracts on axonal regeneration in mouse dorsal root ganglia (DRG) neurons. DRG neurons were treated with *A. cinnamomea* extracts (M8 or M13), incubated for 17 hours. Neurons were then fixed and immunostained with neuronal marker (anti-β-tubulin III) and visualized under fluorescent microscope. Neurons treated with the mushroom extract showed a significant increase (25-35%) in neurite outgrowth, as compared to control. Our results revealed that *A. cinnamomea* extract enhances the intrinsic growth capacity of peripheral neurons significantly. Further studies will be required to investigate the promoting effect of *A. cinnamomea* in animal model of peripheral nerve injury.

Disclosures: C. Raza: None. **K.S.Y. Leung:** None. **C.H.E. Ma:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.02/A27

Topic: A.08. Transplantation and Regeneration

Title: Effects of extra-cellular matrix proteins on neurite extension in isolated neuronal cultures of dorsal root ganglia

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Abstract: Extracellular matrix provides structural support to the cells and widely used in preparation of neuronal cultures to promote attachment of the neurons and neurite extension. To study their roles in mature peripheral nervous system regeneration, the effects of laminin, fibronectin, collagen type 1 and 4 on neurite extension were investigated in isolated dorsal root ganglia neuronal cultures. Dorsal root ganglia were removed from Balb/C mice sacrificed under ketamin anesthesia and isolated neuronal cultures were prepared using routine methods. Neuronal cultures were visualized at 24th and 48th hours using time lapse microscopy. The number of neurons that extend neurites and the number and length of neurites were measured in

ImageJ program. Laminin, fibronectin and collagen 4 significantly increased the number of neurons extending neurites at 24th hour (66%, 56% and 52%, respectively vs. 45% control) while the effects of only laminin and fibronectin were significant at 48th hour (85% and 76%, respectively vs. 70% control). Laminin and collagen 4 significantly increased the neurite number each neuron extended at 48th hour (6.5 and 6.0, respectively vs. 5.7 control). Laminin and fibronectin increased the total length of neuritis extended by each neuron both at 24th (136.0 μm and 88.6 μm, respectively vs. 61.2 μm control) and 48th hours (362.9 μm and 218.1 μm, respectively vs. 118.9 μm control) while collagen type 1 and 4 had significant effects only at 48th hour (147.1 μm and173.4 μm, respectively). The findings compares the effectiveness of the extracellular matrix proteins in promoting neurite growth in cultures and suggest that laminin and fibronectin promotes neurite extension more effectively than collagen type 1 and 2 in isolated neuronal culture of dorsal root ganglia. Laminin and fibronectin could be better candidates to encourage axonal elongation in the studies of neuroregeneration.

Disclosures: **A. Him:** None. **Y. Uren:** None. **G. Ozturk:** None. **E.K. Oguz:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

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Topic: A.08. Transplantation and Regeneration

Support: NIH RO1 NS034484-15

Adelson Foundation

Title: Regenerating sensory and motor neurons preferentially reinnervate modality-specific trophic environments

Authors: N. RAHBIN[1], C. LI[1], A. VYAS[1], A. O'DALY[1], R. SKOLASKY[1], F. NEAF[1], R. WOLINSKY[1], A. HOKE[2], *T. M. BRUSHART[1]
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Abstract: Hypothesis: Growth factors produced by grafts of ventral root and cutaneous nerve promote modality-specific regeneration of their native axon population (Hoke et al., 2006). These factors are upregulated by graft predegeneration (Brushart et al., 2013). We now ask

whether similar specificity can be generated within grafts of mixed nerve by selectively predegenerating the axons of one modality. **Methods**: Experiments were performed on 250gm Lewis rats and were approved by the Johns Hopkins Animal Care and Use Committee. Grafts of the femoral nerve and its sensory and motor branches were predegenerated by excising the L2, L3, and L4 DRGs and/or transecting the L2, L3, and L4 ventral roots, or by transecting the entire femoral nerve. Grafts are designated by the duration of motor (M) and sensory (S) predegeneration in weeks. Five groups of 12 grafts were prepared (I- M3,S3; II- M3,S12; III- M3,S0; IV-M12,S3; V-M12,S12) and transferred to fresh recipient animals. Regeneration was evaluated 8 weeks later by counting sensory and motor neurons retrogradely labeled from femoral sensory and muscle branches; DRG sections from groups II and IV were further processed with antibodies to heavy neurofilament (SMI-32) to identify large light neurons. **Results**: When motor pathways were predegenerated for 3 weeks, preferential reinnervation of the motor branch by motoneurons (PMR) was dramatic if sensory pathways were predegenerated for 3 weeks (I-M3,S3) or 12 weeks (II-M3,S12), but not when they were freshly axotomized (IV-M3,S0). Reinnervation of sensory and motor branches by motoneurons were equal when motor pathways were predegenerated for 12 weeks (IV-M12,S3 and V-M12,S12). Significantly more small DRG neurons reinnervated the sensory branch in group IV (M12,S3) than in group II (M3,S12). **Summary**: Motor axon regeneration is most effective through motor pathways predegenerated for 3 weeks except when acute Wallerian degeneration impedes pathfinding. The converse is true for sensory axons. This evidence confirms that sensory and motor pathways in adult animals differ in fundamental ways that can be sensed and responded to by both sensory and motor axons. Furthermore, acute nerve repair, the clinical standard, may degrade the potential for regeneration specificity.

Disclosures: **N. Rahbin:** None. **T.M. Brushart:** None. **A. O'Daly:** None. **R. Skolasky:** None. **C. Li:** None. **F. Neaf:** None. **A. Vyas:** None. **R. Wolinsky:** None. **A. Hoke:** None.

Poster

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Topic: A.08. Transplantation and Regeneration

Support: NIH Grant P20-GM10431801

Title: Injury-induced sensory axon regeneration is regulated by oxidative changes in the skin microenvironment

Authors: *S. RIEGER, T. LISSE
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Abstract: Acute injury stimulates cutaneous axon regeneration and sprouting of nerve fibers into the wound. The molecular mechanisms underlying this process are however not well understood. We previously demonstrated that hydrogen peroxide (H2O2) production upon skin injury in larval zebrafish is essential for cutaneous axon regeneration. Because H2O2 is diffusible and secreted into the skin microenvironment, we hypothesized that keratinocytes play a role in axon regeneration. H2O2 can act as a second messenger by oxidizing reactive cysteine residues in membrane-bound enzymes, leading to conformational changes due to disulfide bond formation and altered enzymatic activity. To test for the role of cysteine oxidation, we performed immunohistochemistry and assessed axon regeneration with time-lapse imaging in animals where oxidized thiol reactivity was inhibited with the chemical dimedone. We further expressed a transgene for glutathione peroxidase 1a (gpx1a) in keratinocytes to scavenge H2O2. Finally, we analyzed the role of the candidate Epidermal growth factor receptor (EGFR) in this process, as human EGFR was shown to be activated by H2O2-dependent oxidation. Our analyses revealed that cysteine oxidation occurs selectively at the wound margin. Accordingly, treatment of zebrafish larvae with dimedone significantly reduced axon regeneration. Mosaic expression of gpx1a in keratinocytes further revealed that axons are repelled from skin patches where H2O2 is scavenged. These results suggest that thiol oxidation in keratinocytes is essential for axon regeneration. To analyze the role of EGFR, we used a pharmacological approach to inhibit this receptor tyrosine kinase during injury. This showed a significant reduction in axon regeneration. In contrast, EGFR inhibition during development significantly enhanced cutaneous axon growth. Thus, EGFR is essential for injury-induced sensory axon regeneration but has an inhibitory function during development. The latter is similar to EGFR knockout mice, which show a higher axon density in the epidermis. To test for EGFR oxidation, we expressed a zebrafish EGFR variant with a mutated cysteine residue (C796A) in keratinocytes. Time-lapse imaging showed that keratinocytes expressing this mutant variant either repelled regenerating axons upon contact or induced axon branch degeneration. In conclusion, our results demonstrate that injury-induced sensory axon regeneration is dependent on H2O2-mediated oxidation of EGFR in keratinocytes.

Disclosures: S. Rieger: None. **T. Lisse:** None.

Poster

209. Environmental Regulation of PNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: NS 017512

NS 067431

DK 097223

P30 Y11373

OSA 125390

Title: Macrophages may not be necessary for axonal and myelin degeneration after peripheral nerve injury in the CCR2-/- mouse

Authors: *J. LINDBORG, J. P. NIEMI, A. DEFRANCESCO-LISOWITZ, R. E. ZIGMOND
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Abstract: Wallerian degeneration is the process by which transection or crushing of the axons of peripheral neurons leads to degeneration and clearance of the distal axonal segment. Studies on Wallerian degeneration widely report that monocyte entry into the degenerating distal nerve is necessary for phagocytosis and is required to promote regeneration by the proximal nerve segment. Macrophages, which differentiate from blood monocytes upon entering tissues, are purported to be the key player in this clearance process. Using a CCR2-/- mutant mouse model, in which the infiltration of monocytes is inhibited, we found that macrophages may not play as pivotal a role in axonal degeneration as previously believed. Specifically, the disappearances in the distal nerve of myelin and the axonal light neurofilament protein in CCR2 -/- mice were similar to that in wild type mice 7 days after a sciatic nerve transection, in spite of a lack of macrophage accumulation. We hypothesize that in the absence of infiltrating monocytes in the CCR2-/- mouse, a different phagocyte plays a compensatory role in the clearance of degenerating nerve debris. Flow cytometry, electron microscopy, immunohistochemistry, and a time course after injury are used to identify the fluctuating patterns of phagocytosis by leukocytes and Schwann cells during Wallerian degeneration of the sciatic nerve in wild type and CCR2-/- animals.

Disclosures: J. Lindborg: None. **J.P. Niemi:** None. **A. DeFrancesco-Lisowitz:** None. **R.E. Zigmond:** None.

Poster

209. Environmental Regulation of PNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: NS 017512

NS 067431

DK 097223

P30 Y11373

Title: Neuroinflammation near injured neuronal cell bodies is necessary for peripheral nerve regeneration

Authors: *J. P. NIEMI, J. LINDBORG, A. DEFRANCESCO-LISOWITZ, R. E. ZIGMOND
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Abstract: Macrophages play a significant role in the regeneration of peripheral nerves following axotomy. After transection of a peripheral nerve, the axons distal to the lesion degenerate and the axons proximal regenerate. It is known that macrophages respond to nerve injury by accumulating in the distal nerve, as well as, around axotomized cell bodies which are located in ganglia (e.g., Schreiber et al., 1995). Utilizing various methods to block macrophage accumulation, previous studies have shown the necessary role macrophages play in the degenerative process of the distal nerve. In the nerve, macrophages clear debris, which is considered a necessary prerequisite for successful regeneration. The function of macrophage accumulation around axotomized cell bodies is less understood, but has recently been shown to be necessary for a maximal regenerative response following injury. Using the CCR2 -/- and slow Wallerian degeneration (Wld^S) mice as models, we showed that when macrophages did not accumulate in dorsal root ganglia, following sciatic nerve injury, and regeneration was significantly reduced. How macrophages, surrounding injured neuronal cell bodies, influence regeneration is not well understood. The present study further examines the role monocyte-derived macrophages play in stimulating regeneration by providing more direct evidence of this neuroimmune interaction, as well as examining the significance of neuron-derived CCL2. A bone marrow rescue of the CCR2 -/- macrophage accumulation deficiency was carried out by irradiating CCR2 -/- mice and transplanting WT bone marrow. The converse experiment was also done by irradiating WT mice and transplanting CCR2 -/- bone marrow to achieve a significant deficit in macrophage accumulation in dorsal root ganglia (DRGs) following injury. Both groups of animals were then given a conditioning lesion and regeneration was measured in

an *in vitro* assay. To probe the importance of CCL2 expression in neurons following injury, CCL2 was overexpressed specifically in DRG neurons. The effect of this overexpression was assessed alone and in the context of injury. Furthermore, chemokine and cytokine arrays were performed on WT DRGs and superior cervical ganglia (SCGs) to identify candidate molecules involved in macrophage-neuron signaling. Together, these experiments should provide direct evidence that inflammation around axotomized neuronal cell bodies, specifically macrophage accumulation, is necessary for a maximal regenerative response following peripheral nerve injury.

Disclosures: J.P. Niemi: None. **J. Lindborg:** None. **A. DeFrancesco-Lisowitz:** None. **R.E. Zigmond:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.07/A32

Topic: A.08. Transplantation and Regeneration

Title: The ErbB2 receptor pathway regulates regeneration following the repair of acute peripheral nerve transection injuries in a rat model

Authors: *M. HENDRY[1], E. PLACHETA[2], M. ALVAREZ-VERONESI[1], T. GORDON[1], G. H. BORSCHEL[1]
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Med. Univ. of Vienna, Vienna, Austria, Austria

Abstract: Objective: Chronic denervation, the harmful consequence of long regeneration times and distance, profoundly inhibits functional recovery following nerve injuries. Surgical strategies that demonstrate the ability to protect against chronic denervation may do so by supplementing the growth supportive environment within the denervated nerve stump. Neuregulin, a potent Schwann cell mitogen that signals through its endogenous ErbB2 receptor is among the candidate regulators of these effects. Neuregulin regulates several aspects of peripheral nerve regeneration, however its exact role in regulating nerve regeneration and Schwann cell proliferation is unclear. In this study we selectively inhibit the receptor for neuregulin, ErbB2, with the high affinity monoclonal antibody Herceptin to examine its effect on nerve regeneration in a rat model. **Methods**: The common peroneal nerves of Sprague-Dawley rats were surgically transected and repaired with and without the systemic administration of Herceptin. Nerve repair was performed immediately or after 3 months of chronic denervation. The extent of nerve

regeneration was examined 1, 2 or 4 weeks after repair using retrograde labeling techniques where regenerated motoneurons were counted in the ventral spinal cord. Histomorphometry quantified myelinated fiber number and structural dimensions in the regenerated nerve. ErbB2 receptor activation and cellular proliferation were examined. **Results**: Significantly greater numbers of motoneurons regenerated in rats treated with Herceptin (169 ± 31) compared with rats receiving saline (62 ± 15) when assessed 1 week following immediate repair ($p < 0.05$). Total myelinated fiber counts were significantly increased in rats receiving Herceptin (2488 ± 154) compared to rats that received saline (1896 ± 251) ($p < 0.05$). When delayed repair was performed after a 3-month period of chronic denervation, Herceptin increased the number of acutely, but not chronically axotomized motoneurons after two weeks. Interestingly, Western blot analysis revealed no change in ErbB2 activation despite increased numbers of proliferating Schwann cells. **Conclusions**: Inhibition of the ErbB2 receptor with Herceptin paradoxically enhances nerve regeneration following acute and delayed nerve repair independent of neuregulin signaling. A novel inhibitory association between ErbB2 and EGFR may explain this effect. This raises the exciting possibility of using targeted molecular therapy to improve outcomes following surgical repair of nerve injuries.

Disclosures: M. Hendry: None. **E. Placheta:** None. **M. Alvarez-Veronesi:** None. **G.H. Borschel:** None. **T. Gordon:** None.

Poster

209. Environmental Regulation of PNS Regeneration

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Program#/Poster#: 209.08/A33

Topic: A.08. Transplantation and Regeneration

Title: Neuromuscular electrical stimulation following nerve transection and immediate repair enhances reinnervation and functional recovery

Authors: *M. WILLAND, C. D. CHIANG, J. J. ZHANG, S. W. P. KEMP, T. GORDON, G. BORSCHEL
Div. of Plastic Reconstructive Surgery, The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Introduction: The use of chronic electrical muscle stimulation for treating partially or completely denervated muscle has been met with much controversy. Our previous work has shown that a moderate stimulation paradigm can significantly improve the numbers of motor units following long term muscle denervation and subsequent nerve repair. However, the use of

chronic electrical muscle stimulation using a clinically translatable stimulation paradigm immediately following nerve repair has not been thoroughly investigated. Research question: Does a moderate electrical muscle stimulation paradigm delivered chronically over 3 months improve reinnervation and functional outcome measures following nerve transection and immediate repair? Methods: Six groups of Thy1-GFP transgenic male rats were subjected to tibial nerve transection and immediate repair using two epineurial sutures. One group of rats underwent daily electrical muscle stimulation of the gastrocnemius with a paradigm comprising of 600 equally separated contractions throughout one hour, delivered 5 days per week. Rat gastrocnemius muscles were electrically stimulated for either 1, 2, or 3 months and then underwent terminal assessments which included evaluating muscle force, contractile properties, motor unit numbers, and wet weight. Rats in the 3 month group performed a tapered beam test weekly which assessed skilled location. Muscles were then harvested for immunohistological examination of motor end plate reinnervation. Results: Muscles that received daily electrical stimulation had a significantly greater number of motor units for all three time points (1, 2, and 3 months) as characterized using electromyographic methods. Mean motor unit sizes were significantly smaller in stimulated muscles suggesting that muscle stimulation may inhibit terminal sprouting as reported by others. This may allow for a more natural course of reinnervation resulting in improved functional recovery. Indeed, skilled loco-motor tests showed that stimulated muscles enhanced and maintained recovery at levels no different than normal functioning rats whereas non-stimulated controls became progressively worse and did not recover to baseline. Significance: Chronic treatment of denervated muscle using electrical stimulation can significantly enhance muscle reinnervation and functional recovery. As the muscle continues to become reinnervated, tailoring the stimulation paradigm to improve muscle force and fatigability may lead to shorter recovery times and reduce extensive physiotherapy and rehabilitation requirements.

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Poster

209. Environmental Regulation of PNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: Physician Services Incorporated (PSI)

Canadian Institute of Health Research (CIHR)

Title: Enhancement of nerve regeneration with N-acetyl cysteine and acetyl-L-carnitine following neonatal nerve injury

Authors: *J. CATAPANO, C. CHIANG, M. C. ALVAREZ-VERONESI, T. GORDON, G. H. BORSCHEL
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Abstract: Objective: An overlooked component of disability following neonatal peripheral nerve injury is the death of central motor and sensory neurons crucial for repair and regeneration. Our lab has previously demonstrated that a novel aminopropyl carbazole, P7C3, protects neurons from retrograde death following neonatal peripheral nerve injury and improves functional outcomes. However, P7C3 is not yet approved for clinical use. N-acetyl cysteine (NAC) and acetyl-L-carnitine (ALC) are approved for use clinically and have demonstrated sensory neuron protection following adult peripheral nerve injury. This study investigates the efficacy of NAC and ALC in protecting motor and sensory neurons from cell death in a rat model of neonatal nerve injury. Method: Neonatal Lewis rats were used and a blinded observer completed all analysis. Animals were injured 3 days after birth with either a crush or transection injury of the sciatic nerve. Animals were then treated with i.p injections of NAC or ALC for 2 weeks at a dose of 750mg/kg/day and 300mg/kg/day respectively. One month after injury, surviving neurons were labeled proximal to the site of injury with a silicone well containing 4% Fluorogold solution for one hour. Animals were sacrificed 1 week after labeling. Samples distal to the site of injury were harvested for nerve histomorphometry and dorsal root ganglia and spinal cords were harvested for neuronal counts. Results: NAC significantly enhanced motor neuron survival following sciatic nerve crush injury (505.4 ± 39) compared to vehicle (403.7 ± 57) ($p<0.05$). Uninjured animals demonstrated significantly higher axon counts (7155 ± 198) than vehicle (5508 ± 908) and ALC treated animals (5915 ± 718) but were not significantly different from NAC treated (6224 ± 620). NAC and ALC did not protect motor or sensory neurons following a neonatal transection injury without nerve repair. Conclusions: NAC improved motor neuron survival and may improve axon regeneration following neonatal crush injury. NAC and ALC may not have demonstrated a similar benefit for motor and sensory neurons following transection injury because longer treatment periods may be required following more severe injuries where axon regrowth is delayed or inhibited.

Disclosures: J. Catapano: None. **C. Chiang:** None. **M.C. Alvarez-Veronesi:** None. **G.H. Borschel:** None. **T. Gordon:** None.

Poster

209. Environmental Regulation of PNS Regeneration

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Program#/Poster#: 209.10/A35

Topic: A.08. Transplantation and Regeneration

Support: CIHR

Title: Improved peripheral motor and sensory nerve regeneration after delayed repair of transected common peroneal (CP) nerve is promoted by prior side-to-side bridging from a donor intact tibial nerve (TIBdonor) to the 3m chronically denervated common peroneal nerve stump (CPden) in an experimental Sprague Dawley rat model

Authors: *T. GORDON[1,2], J. J. ZHANG[1], A. LADAK[3], A. SNYDER WARWICK[2], J. HENDRY[4], G. H. BORSCHEL[5]
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Abstract: Success of peripheral nerve regeneration declines with time and distance: injured neurons lose regenerative capacity with time and/or distance and denervated Schwann cells (SC) lose their ability to support regenerating axons, progressively reducing regenerative capacity to <5-10%. We asked "Can denervated SCs be 'protected' and, in turn, improve nerve regeneration through chronically denervated nerve stumps?" In Experiment#1 TIB motor and sensory neurons that grew axons into a recipient 3m CPden nerve stump were counted after aseptic surgeries to insert and secure 1) 1-3 autologous 3.2mm CP nerve grafts as side-to-side 'bridges' between corresponding numbers of ~0.5 mm epineurial windows opened in 10 mm parallel intact TIBdonor nerve and CPden distal nerve stump, and 2) 3-9 autologous 3.2mm long CP bridges between TIBdonor nerve and 3m CPden nerve stump, both of which had been stripped of epineurium over a 10mm length. TIB axons crossed 1 bridge in 10% of rats but crossed ≥3 bridges in all rats; very few TIB neurons sprouted axons into CPden stump. Motor and sensory neurons regenerated axons equally into the CPden stump either side of 3-9 bridges with very few neurons sending axons in both directions. A total of 25-35% neurons regenerated their axons through 3 bridges to occupy and become myelinated within endoneurial tubes in the CPden stump. Most axons regenerated through the first bridge with progressively fewer through the next bridges. Numbers of regenerating neurons and axons declined when >3 bridges were placed through the stripped epineurium, with many regenerated axons growing outside of the

endoneurial tubes. In Experiment #2, 5m after delayed CP nerve coaptation, a 3-bridge 'protection' of the 3m CPden nerve stump resulted in a significant 3-fold increase in the CP motor and sensory neurons that regenerated axons as compared to the numbers of CP neurons that regenerated axons without bridge 'protection'. Failure of >3 bridges to promote nerve regeneration concurred with regeneration of ~50% of the TIB axons outside of the endoneurial tubes of the CPden distal stumps. Measurement of evoked muscle and motor unit contractile forces in reinnervated extensor digitorum longus (EDL) muscles demonstrated a 2-fold increase in muscle twitch and tetanic contractile forces and increase in numbers of motor axons that reinnervated muscles when CP motoneurons regenerated their axons through a 3 bridge-protected 3m CPden nerve stump. Candidate molecules responsible for the "protection" of chronically denervated SCs may include neuregulin that is released from regenerating axons and would promote SC proliferation and expression of the growth permissive SC phenotype.

Disclosures: T. Gordon: None. **J.J. Zhang:** None. **A. Ladak:** None. **A. Snyder Warwick:** None. **J. Hendry:** None. **G.H. Borschel:** None.

Poster

209. Environmental Regulation of PNS Regeneration

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Topic: A.08. Transplantation and Regeneration

Support: Create Fertility Centre

Title: Human umbilical cord perivascular cells (hUC-PVCs) rescue central nervous system and peripheral nervous system axonal degeneration *in vitro*

Authors: T. A. BARRETTO[1], K. PARK[1], J. FISH[1], *A. S. GAUTHIER[2], C. LIBRACH[1]
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Abstract: Human umbilical cord tissue-derived perivascular cells (PVCs) and other MSCs are putative cell therapy candidates for regenerative medicine applications including neural injuries and degeneration. Here, we used *in vitro* models of neuronal injury to test our hypothesis that PVCs can prevent axonal degeneration following neuronal injury via paracrine or cell-cell contact mechanisms. Axonal degeneration induced by withdrawal of nerve growth factor (NGF) of cultured rat sympathetic cervical ganglia (SCG) was used as a PNS injury model1. Axon compartments were washed to remove NGF 7 days after neuronal cultures were established, and

treated with ultraculture media, or with established lines of fluorophore-labeled PVCs, BMSCs (Lonza) or neonatal fibroblasts (ATCC) for 3 days. Alternatively, compartments were treated with cell conditioned media (CM). Axons were immunostained for βIII tubulin, and the number of degenerating axons were counted. Similar treatments were used to test the ability of PVCs to protect rat cortical neurons from axonal degeneration following oxygen-glucose deprivation (OGD). When compared to control conditions (injury alone), treatment with PVCs reduced the number of degenerating SCG axons by 60± 13% ($n \geq 5$), BMSCs by 44± 6 % ($n=3$), and fibroblasts by 35± 18 % ($n=3$). PVCs dynamically extended processes and made localized contacts with axons after injury. PVC-CM treatment did not rescue axonal degeneration, and paradoxically, in most experiments exacerbated the injury level. PVCs, but not PVC-CM, were able to rescue axon degeneration in the OGD cortical neuron injury model. In summary, PVCs had the most profound effect on rescue of SCG axonal degeneration ($p<0.05$). Our data suggest that PVC-mediated protection against axonal degeneration is dependent on direct PVC-axon interactions and that, at least under the conditions tested, factors secreted by PVCs are not sufficient to rescue axonal degeneration. This suggests that local delivery of PVCs to injured nervous system would be a more effective approach for repairing certain types of neuronal injuries, for example in the PNS (peripheral neuropathy) or CNS (traumatic brain injury, spinal cord injury). References: 1 Bamji SX et al. J Cell Biol.,1998. 2 Park KJ, et al. J Cereb Blood Flow Metab 2013.

Disclosures: T.A. Barretto: None. **K. Park:** None. **J. Fish:** None. **A.S. Gauthier:** None. **C. Librach:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.12/A37

Topic: A.08. Transplantation and Regeneration

Support: Smoking Research Foundation

Title: Neurotrophic effects of nicotine on perivascular adrenergic nerves in rat mesenteric arteries

Authors: *S. TAKATORI[1], H. HINO[2], F. TAKAYAMA[2], N. ONO[3], H. KAWASAKI[1]
[1]Matsuyama Univ., Matsuyama, Japan; [2]Okayama Univ., Okayama, Japan; [3]Fukuoka Univ., Fukuoka, Japan

Abstract: We reported that a topical phenol application on the rat superior mesenteric artery pronouncedly decreased a distribution of perivascular sympathetic adrenergic nerves and calcitonin gene-related peptide (CGRP)-containing nerves in small mesenteric arteries, and that nerve growth factor (NGF) reinnervated both nerves and nicotine regenerated only adrenergic nerves. To reveal possible mechanisms, the present study was investigated whether nicotine affects NGF contents of superior cervical ganglia (SCG), dorsal root ganglia (DRG) and mesenteric arteries and NGF receptor (TrkA) expression in SCG after topical phenol treatment *in vivo*, and neurite outgrowth of primary cultured SCG and PC12 cells *in vitro*. 10-week old Wistar rats underwent a topical application of 10% phenol to the superior mesenteric artery proximal to the bifurcation of the abdominal aorta. 7 days after the phenol treatment, animals were subjected to ELISA measurement of NGF contents in SCG, DRG and small mesenteric arteries, and Western blot analysis for TrkA expression in SCG. Nicotine at doses of 3 mg/kg/day (1.5 mg/kg/injection, twice a day) was subcutaneously (s.c.) administered for 7 days immediately after phenol treatment. The nicotinic acetylcholine receptor antagonist hexamethonium (5 mg/kg/day s.c.) was pretreated 15 min before nicotine administration. In *in vitro* studies, SCG cells isolated from rats and PC12 cells purchased were primarily cultured and neurite outgrowth from cell body was measured in the presence of nicotine, NGF or nicotine + NGF. Results: In *in vivo* study, nicotine markedly increased levels of NGF contents in SCG and mesenteric arteries, but not DRG. Nicotine also produced increased levels of TrkA expression in SCG. Nicotine-induced increases in NGF contents and TrkA expression were inhibited by hexamethonium pretreatment. In *in vitro* study using primary cultured SCG and PC12 cells, NGF (100 ng/ml) and nicotine (100 μM) induced a neurite outgrowth of tyrosine hydroxylase-immunopositive SCG cells and PC12 cells. Combination of NGF and nicotine further increased a neurite outgrowth of SCG and PC12 cells. These results suggest that nicotine has a neurotrophic effect on perivascular adrenergic nerves through activation of α7nAChR, which results in increase in NGF levels and TrkA expression.

Disclosures: **S. Takatori:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Smoking Research Foundation. **H. Kawasaki:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Smoking Research Foundation. **H. Hino:** None. **F. Takayama:** None. **N. Ono:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.13/A38

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: R21

Title: Synergistic effects of neurotrophins and pleiotrophins in stimulating nerve regeneration across long gap peripheral nerve defects

Authors: *M. I. ROMERO-ORTEGA[1,2], N. Z. ALSMADI[1,2], R. GRANJA[1,2], B. JOHNSTON[1,2], A. KANNEGANTI[1,2], G. BENDALE[1,2], E. HOR[1], H. SUMDANI[1], S. TRINH[1], M. LE[1]
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Abstract: Peripheral nerve injuries resulting in the extensive loss of nerve continuity pose a challenge in reconstructive surgery. Current treatments, such as autograft, isografts and simple tubularization, are limited by the need for donor nerve harvest, minimal functional recovery, and gap restrictions(< 3cm). These limitations can be attributed to the lack of appropriate growth substrate and trophic support. Growth factors such as brain-derived growth factor (BDNF), glial-derived nerve factor (GDNF) and pleiotrophin (PTN) have been demonstrated to induce axonal growth of spinal cord motor neurons, as well as the proliferation and migration of Schwann cells, fibroblasts and endothelial cells. In this current study, a systematic approach to find a suitable combination of growth factors was tested *in vitro* to provide growth factor specific regenerative potency. Further, we tested whether multiluminal biosynthetic nerve implants (BNI) loaded with a combination of PTN and GDNF in the microchannels provide a synergistic effect to promote regenration across a critical long gap (4 cm) in a rabbit model. The toe spread index, measured at 19 weeks post implantation, increased by 25% with PTN and GDNF treatment compared to 15% for the BSA group when compared to those at 4 weeks post injury. As expected, the autograft group shows maximal functional recovery, with a 38% increase between the two time points. (Similarly, the ankle angle revealed that, from 4 weeks to 20 weeks post injury, the GDNF group (14° decrease) had the highest recovery when compared to BSA (2.5°), PTN (0°), and PTN+GDNF (3°), while the autograft treatment (24°) still remained the best . In addition, immunohistochemistry of the distal tissue sections showed that the total area containing regenerated axons was higher in the animals treated with PTN+GDNF than BSA, PTN, and GDNF. Our results demonstrated that PTN and GDNF act synergistically to bridge a long gap. The functional recovery assessed by toe spread index and ankle angle measurement indicate successful motor neuron regeneration. In addition, immunohistochemistry showed positive staining for β-tubulin and P0 indicating neural regeneration across the 4 cm gap. This approach

improves the current nerve repair constructs to bridge the transected neuron across critical peripheral gaps and improves the recovery of functions.

Disclosures: **M.I. Romero-Ortega:** None. **N.Z. Alsmadi:** None. **R. Granja:** None. **B. Johnston:** None. **A. Kanneganti:** None. **G. Bendale:** None. **E. Hor:** None. **H. Sumdani:** None. **S. Trinh:** None. **M. Le:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.14/A39

Topic: A.08. Transplantation and Regeneration

Support: The US Army Medical Research and Materiel Command #W81XWH-11-1-0700

Title: A peripheral nerve regeneration strategy using a combination of bone marrow-derived stem cells transdifferentiated into Schwann-like cells and micropatterned biodegradable polymer conduits

Authors: ***S. ZBARSKA**[1], A. D. SHARMA[2], S. K. MALLAPRAGADA[2], D. S. SAKAGUCHI[3]
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Abstract: Peripheral nerve injuries (PNI) can lead to serious neurological deficits resulting in sensory/motor dysfunctions, including paralysis. Autologous nerve grafts including Schwann cells (SCs) are considered the “gold standard” for treatment of severe PNI. SCs are peripheral glia forming the myelin sheath around axons of motor and sensory neurons. SCs also secrete trophic and growth factors which promote neural regeneration. Previously we have demonstrated that SCs implanted inside of micropatterned nerve regeneration conduits (NRC) enhanced peripheral nerve regeneration (Rutkowski et. al, 2004). However, SC harvesting requires an additional surgery and sacrifice of a donor nerve that results in donor site morbidity. Due to these limitations, alternative cell sources and strategies are being investigated. Recently we have shown that rat bone marrow-derived mesenchymal stem cells (MSCs) can be transdifferentiated into SC-like phenotypes on micropatterned biodegradable poly-lactic acid (PLA) substrates. These results revealed that substrate topography strongly influenced the morphology and growth of the MSCs. Cells aligned in the direction of the grooves when grown on micropatterned PLA substrates, and micropatterning did not impact the level of transdifferentiation making

micropatterned PLA films an ideal base-component for fabricating NRCs. In our ongoing project we are using a synergistic approach combining micropatterned topographical cues in a NRC and biological cues in the form of transdifferentiated rat bone marrow-derived cells. Three groups of Brown Norway rats were implanted with micropatterned PLA NRCs to bridge a 10 mm sciatic nerve transection gap. Group 1 was implanted with control, empty conduits; Group 2 was implanted with conduits pre-seeded with tMSCs (Schwann-like cells); and Group 3 was implanted with conduits pre-seeded with undifferentiated MSCs (uMSCs). Both populations of MSCs express green fluorescent protein (GFP). After implantation animals are assessed for possible recovery of motor and sensory function. In the future, in addition to pre-seeded tMSC-NRCs, we plan to incorporate neurotrophic factor encapsulated nanoparticles to enhance peripheral nerve regeneration.

Disclosures: S. Zbarska: None. **A.D. Sharma:** None. **S.K. Mallapragada:** None. **D.S. Sakaguchi:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.15/A40

Topic: A.08. Transplantation and Regeneration

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No.: 01122016)

Title: Pacific ciguatoxin-1 reduces regenerative capacity of peripheral neuron *in vitro*

Authors: *N. P. B. AU[1], Y. L. MAK[2], L. L. CHAN[2], P. K. S. LAM[2], C. H. E. MA[1,2]
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Abstract: Ciguatoxins (CTXs) are the most potent heat-stable neurotoxins acting on voltage-sensitive sodium channels (VSSCs). CTXs are commonly found in reef fish and consumption of these contaminated fish in human results in gastrointestinal and neurological disorders known as ciguatera fish poisoning (CFP). Due to the increase in international trading of tropical fish species, CFP has drawn significant attention as a global public health issue and increasing incidence of CFP has been reported around the world not limited to tropical regions. An estimated of 50000 victims all over the world are affected by CFP annually. CFP patients exhibit

a range of peripheral neurological and gastrointestinal symptoms such as muscle weakness, and sensory disturbance. Gastrointestinal symptoms usually last for about 2 weeks but severe neurological deficiencies involving the peripheral nervous system (PNS) can last for months to years in 20% of patients. However, little is known about the direct effect of CTXs on peripheral neurons and peripheral nerve regeneration. In the present study, we examined the effect of Pacific ciguatoxin-1 (P-CTX-1), which is described as the most abundant and potent form of CTXs, on the regenerative capacity in PNS using *in vitro* culture of dissociated dorsal root ganglion (DRG) neurons prepared from adult C57BL/6 mice. Dissociated DRG culture is widely used as an *in vitro* model to study PNS regeneration allowing assessment of neurite outgrowth in primary neurons individually. P-CTX-1 significantly attenuated the neurite outgrowth of DRG neurons at concentrations of 1 and 3ng/ml ($p<0.05$) without any adverse effect on the cell viability. These data suggest that P-CTX-1 reduces the regenerative capacity of DRG neurons by limiting neurite extension and thus, this may somewhat explain the phenomenon that damages of nerve were still observed several months after CFP due to the delayed or failure of axonal regeneration.

Disclosures: **N.P.B. Au:** None. **Y.L. Mak:** None. **L.L. Chan:** None. **P.K.S. Lam:** None. **C.H.E. Ma:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.16/A41

Topic: A.08. Transplantation and Regeneration

Support: NIH Grant NRSA F31 NS076197-03

Title: Extracellular Matrix modifications guide pathway selection in peripheral nerve regeneration

Authors: *J. ISAACMAN-BECK, M. GRANATO
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Abstract: Following injury, peripheral nerves maintain the remarkable ability to reestablish functional connections. Trophic factors such as BDNF and NGF provide critical and well-defined signals to spur regrowth, but the molecular cues that return these nerves to the correct pathway at choice points remain largely unknown. We use the larval zebrafish peripheral motor

nervous system to determine these regenerative guidance signals. In these larvae, all motor nerves exit the spinal cord together and initially fasciculate along a common ventral path. Just prior to the horizontal myoseptum, a subpopulation of axons turns acutely from this shared corridor and extends to innervate the dorsal myotome. After we transect both dorsally and ventrally projecting nerves, regenerating axons from both nerves navigate to their original synaptic targets, suggesting that cues exist to guide this binary pathway selection. Here, we provide real-time *in vivo* evidence that constituents of the extracellular matrix (ECM) govern pathway selection for peripheral motor nerve regrowth. In mutants lacking the ECM glycosyltransferase *lysyl hydroxylase 3 (lh3)*, ventral motor nerves regrow robustly to the ventral myotome, but dorsal nerves fail to stabilize growth to their dorsal targets. Interestingly, larvae deficient in the ECM collagen *collagen4a5* (*col4a5,* a known *lh3* substrate) and the canonical axon guidance receptor *robo2* show similar pathway selection defects in nerve regrowth. In mammalian models, *col4a5*, *robo2* and its ligand *slit* are upregulated after peripheral nerve transection, and *col4a5* is known to bind *slit* with high affinity to facilitate *slit-robo2* guidance of axons. We will link these findings with data that suggest that after injury, *lh3* acts in nerve associated glia, modifying the ECM to provide *robo2*-mediated guidance for dorsal pathway selection. To our knowledge, this is the first direct evidence defining a functional mechanism for ECM cues in establishing pathway selection in peripheral nerve regrowth *in vivo*.

Disclosures: J. Isaacman-Beck: None. **M. Granato:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.17/A42

Topic: A.08. Transplantation and Regeneration

Support: Med EL

Title: An implantable electrical interface for *in vivo*, chronic studies of electromyography in canine laryngeal muscles

Authors: *Y. LI[1], S. HUANG[2], D. ZEALEAR[2]
[1]Hearing and Speech Sci., Vanderbilt Univ. Med. Center/Oto, Nashville, TN; [2]Otolaryngology, Vanderbilt Univ., Nashville, TN

Abstract: It has been demonstrated that electrical stimulation of a denervated laryngeal muscle preferentially repressed reconnection by foreign motoneurons, thereby promoting correct reinnervation. However, its mechanism is unknown. Electromyography plays an important role in exploring the underlying mechanisms of selective reinnervation. Objective: The purpose of this study was to develop and test a simple, inexpensive, implantable system that could be used for repeated recordings of spontaneous and evoked EMG from the larynx over a long period of study. Methods: This system consisted of four bipolar nerve stimulus cuffs and four EMG recording electrodes, as well as an interface plug. During a sterile surgery, nerve stimulus cuffs were placed on the recurrent laryngeal nerves (RLNs) and the internal branches of superior laryngeal nerves (SLNs) bilaterally. The recording electrodes were put into the vocal fold adductor (thyroarytenoid, TA) and abductor (posterior cricoarytenoid, PCA) muscles bilaterally. EMG recordings from the PCA muscles during RLN stimulation gave an index of the overall magnitude of its reinnervation. EMG activity during SLN stimulation showed amount of incorrect reinnervation of the PCA muscle by foreign reflex glottic closure motoneurons. The spontaneous EMG activity during hypercapnic breathing provided a good estimate of the magnitude of appropriate PCA muscle reinnervation by inspiratory motoneurons. Small metal female pins were attached at the other end of coiled electrode leads and inserted into the holes of the skin plug. The plug was sutured to the anterior neck and served as an interface for connection between all implanted electrodes and the external equipment. This system was implanted and tested in 3 short-term and 2 long-term canines for as long as 6 months. Results: Device showed good compatibility in all animals. All the lead wires and recording electrodes remained intact and functional. Consistent EMG signals were recorded from the PCA and TA muscles in all animals. The shape and amplitude of the potentials were comparable to recordings from previous studies. Stimulus artifacts were minimal and did not impact the biological signals. The average rectified and integrated EMG potentials recorded from PCA muscle during RLN stimulation, SLN stimulation and spontaneous hypercapnic breathing were 12.2±4.1 μVs, 1.87±0.95 μVs and 0.73±0.33 mVs, respectively. Conclusion: We presented a chronic EMG implant method that was simple and capable of obtaining stable EMG recordings from both abductor and adductor muscles in canine's larynx for periods up to 6 months with minimal risk of device breakage, trauma or infection.

Disclosures: **Y. Li:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Med EL. **D. Zealear:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Med EL. **S. Huang:** A. Employment/Salary (full or part-time):; Vanderbilt University.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.18/A43

Topic: A.08. Transplantation and Regeneration

Support: Med-El, Inc

NIH grant RO1-DC008429

Title: Stimulation of a denervated laryngeal muscle promotes selective reinnervation, reduces synkinesis and restores function

Authors: *D. L. ZEALEAR, Y. LI, S. HUANG
Vanderbilt Univ. Med. Ctr., NASHVILLE, TN

Abstract: Previously, electrical stimulation of a denervated canine laryngeal muscle was shown to promote reinnervation by original over foreign motoneurons. An implantable nerve stimulation-EMG system was used to index the appropriateness of reinnervation of the vocal fold abductor (posterior cricoarytenoid, PCA) muscle by inspiratory versus foreign reflex glottis closure (RGC) motoneurons following recurrent laryngeal nerve section and repair. In the present study in nine canines, a clinical model was used, where both nerves were sectioned and ventilation compromised due to loss of abduction. The EMG system and a pulse generator were implanted, the latter for electrical conditioning of PCA muscles. After nerve section, animals were randomly assigned to four groups to assess the effect of different muscle stimulus paradigms on reinnervation quality and degree of functional recovery: 1)40 pps train, 2)20 pps train 3)10 pps train and 4)control-no stimulation. One msec pulses were applied with 4 sec on/4 sec off duty cycle during the post neurorrhaphy regeneration period. In bimonthly sessions, spontaneous vocal fold movement was measured endoscopically during induced hypercapnea in the anesthetized animal. Exercise tolerance was measured on a treadmill in the awake animal using pulse oximetry. Rectified integrated EMG potentials were recorded from abductor muscles and adductor (thyroarytenoid, TA) muscles in the anesthetized animal. Recordings were obtained during hypercapnic respiration to index reinnervation by inspiratory motoneurons, and during superior laryngeal nerve stimulation to index reinnervation by RGC motoneurons. Results demonstrated that nonstimulated controls, 40 pps stimulated and 20 pps stimulated animals had faulty reinnervation (EMG), severe paradoxical closure of the glottis during hypercapnea, and poor tolerance to exercise. In contrast, stimulated 10 pps animals showed a near normal pattern of low PCA and high TA reinnervation by RGC motoneurons, minimal paradoxical glottis closure, and normal exercise tolerance (12 minutes up to 8 mph). It would appear that low-frequency stimulation of a denervated PCA muscle simulating endogenous activity inhibits

reinnervation by foreign RGC motoneurons, leaving receptor sites available for native inspiratory motoneurons. As a consequence, reinnervation of adductor muscles by inspiratory neurons is depressed; paradoxical glottis closure is reduced and exercise tolerance restored to normal.

Disclosures: D.L. Zealear: A. Employment/Salary (full or part-time):; Med-El, Inc. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Med-El, Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Med-El, Inc. **Y. Li:** None. **S. Huang:** None.

Poster

209. Environmental Regulation of PNS Regeneration

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 209.19/A44

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: R21

Title: Computational modeling of drug release from nerve guide conduit

Authors: *N. Z. ALSMADI[1,2], L. PATIL[1,2], C.-J. CHUONG[1,2], M. ROMERO-ORTEGA[1,2]
[1]Bioengineering, Univ. of Texas at Arlington, Arlington, TX; [2]Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: In the injured adult nervous system, re-establishment of growth-promoting molecular gradients is known to entice and guide nerve repair. However, incorporation of three-dimensional chemotactic gradients in nerve repair scaffolds, particularly in those with multi-luminal (ML) architectures, remains extremely challenging. To address this limitation, we have developed a method that establishes highly tunable, three-dimensional molecular gradients in ML nerve guides (NG) by anchoring nerve growth-factor (NGF) releasing coiled microfibers onto the walls of collagen-filled hydrogel microchannels. The gradient is achieved by differential pitch in the coiling of neurotrophin-eluting fibers, and *in vitro* studies demonstated that axonal growth from dorsal root ganglia (DRG) is 60% longer and more linear as indicated by a reduced turning angle ratio, compared to those exposed to uniform growth factor concentration. Here, we developed a computer model to estimate the dynamics of growth factor release and the diffusion

into the luminal collagen, in six different designs of drug release conduits: a) collagen filled NG, b) NGF-Microparticle release in NG, c) NGF-coil release in NG, d) collagen filled ML-NG, e) NGF-Microparticle release in ML-NG, f) NGF-coil release in ML-NG. Finite element computational was used to calculate the spatiotemporal distributions of NGF in the six types of conduits over time, and to compare of growth factor diffusion over time in each of these devices. We further assessed the effect of geometrical parameters on the efficacy of drug release in NGs. Our models provides quantitative insights with time-varying NGF distribution in the microenvironment of the nerve guide conduits. The model could assist in enhancing the design of growth factor secreting nerve conduits, and improve the current nerve repair strategies to optimize the regeneration of injured neurons and the recovery of function.

Disclosures: **N.Z. Alsmadi:** None. **L. Patil:** None. **C. Chuong:** None. **M. Romero-Ortega:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.01/A45

Topic: A.10. Adolescent Development

Support: NARSAD Young Investigator Grant

NIH Grant DA-015403

NIH Grant DA-026485

Title: The role of the D1 receptor in the prefrontal cortex in the emergence and reduction of depressive-like behavior

Authors: *N. B. FREUND[1,2], R. A. NEVE[3], S. L. ANDERSEN[2]
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Abstract: The adolescent prefrontal cortex undergoes major structural and neurochemical changes during maturation. For example, the density of dopamine D1 receptor (D1R) positive cells projecting from the prelimbic prefrontal cortex (plPFC) to the nucleus accumbens is transiently increased during this maturational stage. This peak in D1R elevates the motivational

salience of environmental cues, allowing the individual to process adverse situations. Disruptions in the maturation of this D1R population might decrease motivational salience sufficiently to induce depression. Here, we investigated the role of developmental plPFC D1R changes in learned helplessness in juvenile (21 days), adolescent (40 days), and adult (70 days) Sprague-Dawley rats of both sexes. Rats were conditioned on Day 1 with a tone and a light predicting the onset of a shock that they could not escape. On Day 2 subjects were placed in the same shuttle box where tone and light signaled shock, but subjects had the opportunity to escape. The latency to escape and escape failures decreased with age (main age effect: $F_{2,51}$=13.63; p<0.01 and $F_{2,51}$=17.84; p<0.01). While adolescents' escape latencies decreased relative to juveniles', significant differences were only observed between juveniles and adults (p<0.008, Bonferroni corrected). Sex differences were found for escape failures with more failures in males compared to females in adulthood (main sex effect: $F_{1,51}$=5.37; p=0.02). The role of high D1R density in learned helplessness was investigated by over-expressing D1R in glutamate neurons in the plPFC of juvenile animals with an adenovirus. In females, D1R over-expression decreased escape latencies and failures compared with females that expressed a control protein (latency: $F_{1,8}$=10.18; p=0.013; failures: $F_{1,8}$=12.35; p=0.008, Bonferroni corrected); no differences between virus groups were found in males. Immunohistochemical staining for *c-Fos* in animals sacrificed 90 minutes after behavioral testing revealed that the D1R over-expression increased neuronal activity in the nucleus accumbens shell of juvenile females ($F_{1,8}$=6.82; p=0.035). Furthermore the number of *c-Fos* positive cells in the nucleus accumbens shell positively correlates with escape behavior (latency: r=-0.671; p=0.048 and failures r=-0.763; p=0.017). Taken together, learned helplessness decreases with maturation, an effect that may be partially due to increased motivational salience mediated by plPFC D1R density. Failure or disruption in the maturation of prefrontal D1R expression may be responsible for the onset of depression during adolescence.

Disclosures: **N.B. Freund:** None. **R.A. Neve:** None. **S.L. Andersen:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.02/A46

Topic: A.10. Adolescent Development

Support: NSERC

CIHR

FRSQ

Title: The dynamic changes in adolescent dopamine receptor expression are altered in a model of dcc-haploinsufficiency

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Abstract: Netrins are guidance cues involved in the proper organization of neuronal connectivity. We have shown that variations in the function of the netrin-1 receptor, DCC, result in *selective* changes to the development and organization of medial prefrontal cortex (mPFC) dopamine (DA) circuitry. In comparison to their wild-type littermates, adult *dcc*-haploinsufficient mice demonstrate increased DA innervation and exaggerated DA function in the mPFC in adulthood. These changes, in turn, lead to structural and functional alterations in mPFC Layer V pyramidal neurons and to changes in cognitive flexibility. Interestingly, none of the anatomical, neurochemical, and behavioural phenotypic traits of adult *dcc*-haploinsufficient mice are present prior to adolescence; a period during which mPFC DA circuitry undergoes substantial reorganization and functional refinement including overproduction and elimination of DA receptors. To begin to investigate whether *dcc* also plays a role in DA receptor regulation during adolescence we conducted quantitative receptor autoradiography experiments. We characterized the expression of D1 and D2 receptors in pregenual mPFC and striatal regions in male *dcc*-haploinsufficient and wild-type mice across three postnatal ages: postnatal (PND) 21±1, PND33±2, and adulthood (PND 85±15). Autoradiograms were generated by apposing serial brain sections incubated with either [^{3}H]SCH-23390 or [^{3}H]raclopride alongside tritium standards. Receptor binding was quantified using microdensitometric image analysis system (MCID System). We observed age- and region-specific differences in D1 and D2 receptor density between *dcc*-haploinsufficient and wild-type mice. Notably, dcc-haploinsufficent mice do not show the typical pattern of D2 receptor pruning observed in the mPFC of wild-type mice and, consequently, have an increased density of mPFC D2 receptor in adulthood. Conversely, we observed a trend toward decreased expression of D1 receptors in the mPFC of adult dcc-haploinsufficient mice relative to wild-type littermates. Furthermore, *dcc*-haploinsufficient animals show reduced receptor density in striatal regions at younger ages; this effect is transient. These findings suggest a role for DCC-mediated netrin-1 signaling in directing the dynamic refinement of DA receptor expression over the course of adolescence.

Disclosures: A.D. Grant: None. **F. Shahabi:** None. **Y. Dumont:** None. **C. Flores:** None. **C. Manitt:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

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Program#/Poster#: 210.03/A47

Topic: A.10. Adolescent Development

Support: R01 MH090091

Title: A subset of pubertally born cells differentiate into astrocytes within the posterior-dorsal medial amygdala

Authors: *N. STAFFEND[1], F. GARCIA[2], M. M. MOHR[1], L. DONCARLOS[2], C. L. SISK[1,2]
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Abstract: The posterior-dorsal medial amygdala (MePD) evaluates and assigns valence to social stimuli from conspecifics and is sexually dimorphic, such that it is larger in adult males than females. We have established that cell addition is a general mechanism for structural remodeling of the brain during puberty in rats, including sexually dimorphic structures like the MePD [Ahmed et al., 2008]. The present study aimed to identify the cellular phenotype of the pubertally born cells within the MePD, specifically whether they differentiate into mature neurons or astrocytes. Three injections of the thymidine analogue, bromo-deoxyuridine (BrdU; 200 mg/kg ip), were given at 8 hr intervals to 6 male and 6 female rats during early puberty, on P30; rats were sacrificed 21 days post-BrdU injection. Tissue was processed for BrdU, the mature neuronal marker, NeuN, and the mature astrocytic marker, GFAP. Using triple-label immunofluorescence and confocal microscopy, analyses conducted thus far of 3 males and 3 females revealed that the majority (86% in males, 85% in females) of BrdU labeled cells were not labeled with either NeuN or GFAP. However, a small proportion of BrdU-positive cells in both males (10%) and females (12%) did co-label with GFAP, indicating these pubertally-born cells mature into astrocytes. Even fewer BrdU-positive cells co-labeled with the mature neuronal maker, NeuN (4% males, 3% females). Although data exist demonstrating greater numbers of astrocytes in the adult male MePD compared to the adult female [Johnson et al., 2008], the current analysis of 3 animals per sex detected no significant sex difference in the number of pubertally born cells that differentiated into mature astrocytes ($p = 0.294$); analysis of remaining animals is ongoing. In addition, this experiment examined cells born on a single day during early puberty (P30), which may or may not be representative of the fate of all MePD cells born throughout puberty. Although we cannot yet determine whether there are sex differences in the phenotypes of pubertally born MePD cells, these data extend our previous findings by

demonstrating that a subset of pubertally born cells in both sexes differentiate into mature astrocytes, positioning these cells to contribute both structurally and functionally to pubertal maturation of the MePD and the behaviors it governs.

Disclosures: **N. Staffend:** None. **M.M. Mohr:** None. **C.L. Sisk:** None. **L. DonCarlos:** None. **F. Garcia:** None.

Poster

210. Adolescent Vulnerability: Animal Models

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Topic: A.10. Adolescent Development

Support: Canadian Institutes for Health Research

Natural Sciences and Engineering Research Council of Canada

Fonds de Recherche en Sante du Quebec

Title: The enduring effects of amphetamine on baseline dopamine function vary by age of drug administration

Authors: *C. FLORES[1,3], L. YETNIKOFF[4], M. POKINKO[2,3], M.-P. COSSETTE[5], A. ARVANITOGIANNIS[5]
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Abstract: Adolescence is a vulnerable period in the lifespan, wherein individuals are prone towards perilous and impulsive behavior. The choices and decisions made during this vulnerable period can have long-term consequences on well being during adulthood. For example, in humans, experimentation with drug use during adolescence leads to a higher risk of developing drug abuse and addiction later in life than does initiation of drug use during adulthood. While the precise mechanisms underlying this enhanced risk are not clear, it is well-established that the behavioral vulnerabilities of adolescence are associated with numerous progressive and regressive changes in brain structure and function, particularly within the mesocorticolimbic dopamine system. Exposure to drugs of abuse during adolescence may therefore impinge on the

normal development of mesocorticolimbic circuitry, leading to long-lasting alterations in its function. Here we examined how repeated exposure to amphetamine at three distinct periods of the lifespan affects the functioning of the mesocorticolimbic dopamine system as assessed by baseline measures of dopamine and its metabolites. Specifically, early adolescent (P21-31), mid adolescent (P35-45) and adult (> P60) wild-type mice were administered 5 injections of amphetamine (4 mg/kg) or saline on alternating days. One month after drug pretreatment all animals were killed and bilateral punches of the nucleus accumbens, dorsal striatum and medial prefrontal cortex were obtained. Baseline levels of dopamine content and turnover were assessed by high performance liquid chromatography. Remarkably, repeated exposure to amphetamine during early and mid adolescence, but not during adulthood, induced long-lasting changes in baseline function of dopamine and/or its metabolites, with the direction of the observed effects varying by region examined. These findings suggest that enduring, age-specific changes in the function of dopamine circuitry by stimulant drugs of abuse underlie the increased vulnerabilities of early onset, as compared to adult onset, drug use.

Disclosures: C. Flores: None. **A. Arvanitogiannis:** None. **L. Yetnikoff:** None. **M. Cossette:** None. **M. Pokinko:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

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Program#/Poster#: 210.05/A49

Topic: A.10. Adolescent Development

Support: NSF Grant IOS 1253188

Title: Pubertal expression of PSA-NCAM in the medial amygdala of the male Syrian hamster

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Abstract: Male Syrian hamsters enter puberty around postnatal day 28 (P28), coinciding with an increase in serum androgen levels. In addition, male Syrian hamsters start displaying significant sexual motivation by P40 - a time when puberty is thought to have ended. Previous work has shown that pubertal androgens remodel the posterodorsal medial amygdala (MePD), a brain region that participates in socio-sexual behaviors, many of which emerge at puberty. The adult MePD contains high levels of polysialylated neural cell adhesion molecule (PSA-NCAM), which

plays a permissive role in synaptogenesis and plasticity throughout the brain during development, and in a few regions of the adult brain. In male hamsters ranging in age from P27 - P145 (n = 4 - 8 animals per age), we measured body, brain, and seminal vesicle weights, and serum testosterone and cortisol levels. In addition, we visualized NCAM and PSA-NCAM in the MePD of these same hamsters. As expected, there was a surge in testosterone from P27 to P40, and we observed strong positive correlations between age and body weights, age and seminal tissue weights, and age and brain weights. Interestingly, PSA-NCAM immunoreactivity also surged during pubertal development, reaching an apex around P32, and dropping to its nadir by P145. The increase in MePD PSA-NCAM occurred at the same time that there was a surge in serum testosterone levels, suggesting that androgens regulate MePD PSA-NCAM expression. Future studies will test this hypothesis, and will identify the necessity of PSA-NCAM for MePD neuronal remodeling and the emergence of MePD-dependent behavior.

Disclosures: **M.O. Job:** A. Employment/Salary (full or part-time):; The Neuroscience Institute of Georgia State University, Atlanta, GA.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

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Program#/Poster#: 210.06/A50

Topic: A.10. Adolescent Development

Support: 1IK2BX001562-01A1

Title: Exposure to prenatal, adolescent, or combined prenatal and adolescent stress increases contextual fear conditioning in male but not female Sprague-Dawley rats

Authors: *K. M. SCHULZ[1,2], M. B. BURKE[2], M. ZAJKOWSKI[2], I. RAMOS[2]
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Abstract: Numerous rodent studies demonstrate that stress exposure during prenatal and adolescent development alters sexually-dimorphic cognitive and affective behaviors in adulthood. However, fewer studies have investigated the effects of prenatal and adolescent stress on fear-related learning, and most have focused only on males. Therefore, we investigated the effects of prenatal stress, adolescent stress, or combined prenatal and adolescent stress on adult contextual fear conditioning in both sexes. Male and female Sprague Dawley rats experienced

one week of chronic variable stressors prenatally (gestational days 14-21), during adolescence (postnatal days 23-30), a combination of these stressors during both prenatal and adolescent periods, or were nonstressed controls. In adulthood (~100 days old), animals were exposed to a series of foot shocks within a testing chamber, and contextual freezing behavior was assessed 24 hours later when subjects were returned to the same testing chamber for five minutes (no shocks administered). A significant sex by treatment interaction was observed in which stress increased contextual freezing durations in male but not in female rats [$F(3,124)=1.47$, $p=0.034$]. Specifically, males that had experienced prenatal ($p=0.03$), adolescent ($p=0.09$), or combined prenatal and adolescent stress ($p=0.01$) exhibited greater freezing durations than nonstressed males. In contrast, prenatal, adolescent, or combined stress exposures did not significantly impact contextual freezing in females. These data suggest that developmental stress exposure facilitates contextual fear learning in a sex-dependent manner, and highlight the importance of including both sexes in experimental designs.

Disclosures: **K.M. Schulz:** None. **M.B. Burke:** None. **M. Zajkowski:** None. **I. Ramos:** None.

Poster

210. Adolescent Vulnerability: Animal Models

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Topic: A.10. Adolescent Development

Support: Canadian Institutes for Health Research

Natural Sciences and Engineering Research Council of Canada

Fonds de Recherche en Sante du Quebec

Title: Mesocortical dopamine depletion reverses the protective phenotype of netrin-1 receptor deficient mice

Authors: *M. POKINKO[1,3], L. MOQUIN[3], A. GRATTON[2,3], C. FLORES[2,3]
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Abstract: The netrin-1 receptor, DCC, which is highly expressed by ventral tegmental area dopamine (DA) neurons, establishes the extent of their innervation to the medial prefrontal cortex (mPFC) during adolescence. In turn DCC signaling determines vulnerability to the

behavioral effects of stimulant drugs of abuse in adulthood. Interestingly, DCC-positive DA neurons in the ventral tegmental area co-express another netrin-1 receptor, UNC5C, from adolescence onwards. Furthermore, both adult dcc and unc5c haploinsufficient mice have increased DA innervation to the mPFC and exhibit blunted behavioral responses to amphetamine. This protective phenotype only emerges after adolescence. Because DA transmission in the mPFC can negatively regulate behavioral responses to drugs of abuse, we hypothesized that reduced sensitivity to the effects of amphetamine in dcc and unc5c haploinsufficient mice results from increased mPFC DA innervation. We therefore examined whether selective mPFC DA depletion in adulthood reverses the protective phenotype of these mice. To this end, adult wild-type, dcc, and unc5c haploinsufficient mice, bred in our animal facility, received bilateral injections of 6-hydroxydopamine (lesion) or vehicle (sham) into the mPFC. Ten days later, we challenged these mice with an i.p. injection of 2.5 mg/kg amphetamine and measured their locomotor activity for 90 min. As expected, dcc and unc5c haploinsufficient mice that received sham lesions showed significantly reduced amphetamine-induced locomotion compared to wild-type groups. Remarkably, dcc and unc5c haploinsufficient mice that underwent mPFC DA lesions no longer showed this blunted response to amphetamine challenge; their locomotor activity was similar to that observed in wild-type controls. There was no difference in amphetamine-induced locomotion between lesion and sham wild-type groups. These findings demonstrate that the protective phenotype of adult dcc and unc5c haploinsufficient mice results from the effects of DCC and UNC5C receptors on the development of the DA input to the mPFC. These finding raise the possibility that signaling mediated by DCC/UNC5C receptor complexes may be at play.

Disclosures: **M. Pokinko:** None. **L. Moquin:** None. **A. Gratton:** None. **C. Flores:** None.

Poster

210. Adolescent Vulnerability: Animal Models

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Topic: A.10. Adolescent Development

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Canadian Institutes of Health Research

Natural Science and Engineering Research Council of Canada

Title: Impact of dietary omega-3 fatty acid deficiency on DCC and other dopamine related measures

Authors: *S. LOTFI[1], C. MANITT[5], J. J. BALCITA-PEDICINO[2], C. O. BONDI[3], S. R. SESACK[4], C. FLORES[5], B. MOGHADDAM[4]
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Abstract: Identifying mechanisms by which environmental factors affect the onset and course of psychiatric disorders is critical for their prevention and treatment. Nutritional factors, in particular dietary deficiency of omega-3 polyunsaturated fatty acids (n-3 PUFAs), have been implicated in the onset of schizophrenia and mood disorders in young individuals who are at high risk to develop these conditions. Animal models of this dietary deficiency in adolescents and adults are critical for understanding how n-3 PUFA deficiency influences overall behavioral health and symptoms of these illnesses. We recently developed such a model involving consecutive generations of n-3 PUFA deficiency based on the assumption that dietary trends toward decreased consumption of these fatty acids began four-five decades ago when the parents of current adolescents were born (Bondi et al., Biological Psychiatry, 2014). In addition to behavioral disruptions that were augmented in the second generation (G2) of n-3 PUFA deficient adolescent animals, we observed changes in the expression of dopamine-related proteins that were different in adolescents as compared to adults. To expand on these findings, we have focused on assessing DCC and TH expression, as well as dopamine cell number and structural characteristics in these diet groups. DCC is a receptor for the axonal guidance cue netrin-1, and is involved critically in the adolescent developmental organization of dopamine connectivity. Previous work shows that changes in DCC expression lead to altered DA connectivity and function in a manner that is dependent on age and region (Manitt et al., Translational Psychiatry, 2013). We find that DCC expression in G2 n-3 PUFA deficient adolescents is lower in the nucleus accumbens (NAc) and higher in the dorsal striatum (DS) compared to adequately fed counterparts. In G2 adult animals with n-3 PUFA deficiency, DCC expression in NAc and DS also is affected, but in an opposite direction from what is seen in adolescents: DCC expression is higher in NAc but lower in DS, as compared to adequately fed animals. Neuroanatomical analyses to determine potential alterations in dopamine cell number, somal size, and ultrastructural features are ongoing.

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Poster

210. Adolescent Vulnerability: Animal Models

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Topic: A.10. Adolescent Development

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NIH NS045195

NSF IOS-0956831

Title: Sex difference in pubertal cytogenesis in the posterodorsal medial amygdala of mice

Authors: *J. L. KIM[1], M. A. MOHR[2], N. A. STAFFEND[2], L. L. DONCARLOS[3], S. M. BREEDLOVE[2], C. L. SISK[1,2], C. L. JORDAN[2]
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Abstract: The posterodorsal medial amygdala (MePD) is a sexually dimorphic brain region involved in social and sexual behaviors, many of which are sexually differentiated and emerge during puberty. Previous studies in rats and hamsters have demonstrated that new cells are added in sexually dimorphic regions of the brain including the MePD, that pubertal hormones promote their addition, and that some of these pubertally born cells differentiate into mature neurons or glia that are functionally incorporated into neural circuits [Nature Neuroscience (2008). 11(9):995-7; PNAS (2013). 110(12):4792-7]. Given the wide array of genetic tools available in mice to dissect and understand mechanisms of hormone action on pubertal cytogenesis in the brain, we now ask whether new cells are added during puberty in the mouse brain. Male and female juvenile mice were injected daily with bromodeoxyuridine (BrdU), a cell birth date marker, for 21 days (postnatal day 28-49). Animals were sacrificed as adults on postnatal day 60; BrdU-labeled cells could range from 11-32 days of age at the time of tissue collection. Using immunohistochemistry, we find evidence of BrdU+ cell labeling in many regions of the mouse brain including the dentate gyrus and MePD. Quantitative analysis using Neurolucida software revealed comparable numbers of BrdU labeled cells (# BrdU-labeled cells/mm2) in the dentate gyrus ($F=0.03$, $p=0.86$) of males and females but a significant sex difference in their number in the MePD ($F=12.19$, $p<0.02$), consistent with previous findings in rats, with males having nearly 1.5 times more BrdU-labeled cells than females. These results show for the first time that pubertal cytogenesis occurs in the mouse brain and that these newly born cells survive into adulthood with more in the MePD of males than females, suggesting they may contribute to the emergence of sexually differentiated social-sexual behaviors in adulthood. Critical questions to address include answering what the phenotype of these newly generated cells and determining

whether genesis or survival of BrdU labeled cells underlies the adult sex difference in their number.

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Poster

210. Adolescent Vulnerability: Animal Models

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Title: Adolescent Bisphenol A exposure leads to enduring alterations in cognition and dendritic spine density in adult rats

Authors: *S. C. DEWOLF[1], M. FRANKFURT[2], V. LUINE[3], H. KHANDAKER[3], R. BOWMAN[1]
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Abstract: Bisphenol-A (BPA) is an endocrine disrupter that exerts effects on a variety of neural, physiological, and behavioral measures. BPA effects during early developmental periods are well established and we have recently shown that adolescent exposure increases anxiety, impairs spatial memory, and decreases neuronal spine density. This study examined whether or not BPA exposure during adolescence (postnatal days [PND] 42-49) leads to behavioral and neural alterations in adulthood. Male and female, adolescent, 6 week old, rats received BPA, 40 µg/kg/bodyweight, or control treatments for one week. At 11 weeks of age, subjects were tested for anxiety (elevated plus maze), spatial memory (object placement), non-spatial visual memory (object recognition), and sucrose preference. In addition, we examined whether adolescent BPA exposure altered serum corticosterone levels in response to a restraint stress challenge immediately prior to sacrifice (13 weeks of age) and measured dendritic spine density in the medial prefrontal cortex (mPFC) and CA1 region of the hippocampus (CA1). BPA-treated males were more anxious as indicated by fewer total visits on elevated plus maze. While adolescent BPA exposure did not alter spatial memory, it did lead to long-term alterations in exploration and

BPA treated males had decreased exploration compared to other groups. On the object recognition task, the ability to discriminate between the old and new objects was decreased in BPA males, but not females. There were no significant group differences in sucrose preference or serum corticosterone levels in response to a stress challenge. However, BPA exposure, regardless of sex, significantly decreased CA1 spinal density on both apical (21% decrease) and basal dendrites (19% decrease) but led to a small, but significant, 5% increase in spine density of basal dendrites in the mPFC. The current data shows that adolescent BPA exposure, at a level below the current reference safe daily limit set by the U.S.E.P.A., leads to enduring alterations in behavior and neuronal morphology.

Disclosures: S.C. Dewolf: None. **M. Frankfurt:** None. **V. Luine:** None. **H. Khandaker:** None. **R. Bowman:** None.

Poster

210. Adolescent Vulnerability: Animal Models

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EPA RD 83459301

EPA RD 835401

NIEHS T32 ES007326

Title: Long-term effects of adolescent exposure to bisphenol A on neuron and glia number in the rat prefrontal cortex differs between the sexes

Authors: *L. M. WISE[1], R. N. SADOWSKI[2], T. KIM[1], S. L. SCHANTZ[3], J. M. JURASKA[1]
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Abstract: Bisphenol A (BPA), an endocrine disruptor used in a variety of consumer products, has been found to alter neuroanatomical structure in multiple brain areas. However, few studies

have examined long-term effects on the prefrontal cortex, an area where both the number of neurons and glia change during adolescence. In the current study, Long-Evans male and female rats were administered 0, 4, 40, or 400 µg/kg/day BPA during adolescent development (postnatal days 27-46). All other sources of BPA exposure were eliminated for the lifespan of the subjects. In adulthood (postnatal day 150), the number of neurons and glia in the prefrontal cortex were stereologically assessed. There were no changes in the number of neurons, but there was a significant dose x sex interaction in number of glia in the prefrontal cortex ($p=.05$). Pairwise comparisons between controls and each dose show a significant increase in the number of glia between 0 and 40 µg/kg/day in females ($p=.04$), and a trend towards a significant decrease in the number of glia between 0 and 4 µg/kg/day in males ($p=.06$). In order to determine the type of glia cells that are changing in these groups in response to adolescent BPA administration, immunohistochemistry was conducted to assess astrocyte number in the prefrontal cortex. There is a sex difference between male and female control animals with females having fewer astrocytes in layer 5/6 than the males ($p=.04$). There is also a trend towards an increase in astrocytes between 0 and 40 µg/kg/day in females ($p=.08$). There was no change in astrocyte number between 0 and 4 µg/kg/day in males. Ongoing research includes immunohistochemistry to assess the number of microglia to obtain a more complete picture of how BPA exposure during adolescence alters the prefrontal cortex.

Disclosures: L.M. Wise: None. **R.N. Sadowski:** None. **T. Kim:** None. **S.L. Schantz:** None. **J.M. Juraska:** None.

Poster

210. Adolescent Vulnerability: Animal Models

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Program#/Poster#: 210.12/A56

Topic: A.10. Adolescent Development

Title: Isolation stress during adolescence in mice: Effects on adult behavior and hippocampal glutamate/GABA

Authors: *S. LANDER[1], I. GAISLER-SALOMON[2]
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Abstract: The age of onset for many psychiatric disorders is adolescence, a time period characterized by enhanced sensitivity to environmental factors and by accelerated maturation of cortical and hippocampal circuitry. Human and animal studies show that the characteristics of the

social environment during adolescence are particularly crucial to adult brain function and behavior. In this study, we exposed adolescent and adult C57bl/6 male mice to a 3-week social isolation procedure and compared behavioral and gene expression patterns to those exhibited by control group-housed adult mice. Our behavioral battery included several assays of schizophrenia-like behavioral abnormalities: baseline and amphetamine (2 mg/kg)-induced locomotor activity, novel object recognition, and social preference. In addition, we tested reversal learning and extradimensional set-shifting in a modified wet T-maze with spatial and visual cues. In light of recent evidence supporting the involvement of hippocampal glutamate and GABA in susceptibility to schizophrenia, we also tested the effects of adolescent social isolation on gene expression of glutamatergic and GABAergic markers in hippocampal subregions. Results showed anxiety-like behavior and increased object exploration in all isolated mice. Interestingly, impairments in reversal learning and extradimensional set shifting were observed only in mice exposed to isolation stress during adolescence. Also, increased social preference was observed in this group alone. In the amphetamine challenge test, mice exposed to isolation stress during adulthood showed a greater response compared to mice isolated in adolescence. Taken together, these findings indicate that social isolation in adolescence impairs cognitive set-shifting in adulthood but does not disrupt behavior in all tests used to assess schizophrenia-like behavior. Further analysis of gene expression patterns may reveal loci of vulnerability to environmental insult. Combined with predisposing factors that affect hippocampal glutamate levels at early stages of development, adolescent social isolation may act as an environmental “trigger” that sets the stage for the onset of florid schizophrenia symptoms in early adulthood.

Disclosures: **S. Lander:** None. **I. Gaisler-Salomon:** None.

Poster

210. Adolescent Vulnerability: Animal Models

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Topic: A.10. Adolescent Development

Title: The involvement of NMDA receptors in the consolidation and reconsolidation of cocaine place preferences across development

Authors: *A. R. URUENA[1], C. L. KIRSTEIN[2]
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Abstract: Drug-associated memories are created and stabilized following repeated drug use and have been shown to stimulate drug craving, motivate drug-seeking behavior and thereby increase the likelihood of drug relapse. Similarly, in animal models, repeated cocaine exposure in a specific environment results in a conditioned place preference (CPP) for the drug-paired environment. Glutamate-dependent neuroplasticity may mediate preference expression, as findings have shown pretreatment with the NMDA receptor antagonist, MK-801 disrupts the formation of cocaine CPP. Given that drug use before the age of 18 impacts maturation of neural systems implicated in neuroplasticity, it is critical to investigate the role of NMDA receptors in the acquisition and expression of cocaine-associated memories that underlie place preferences across developmental time periods. To determine if age differentially impacted the effects of routine MK-801 pretreatment on the acquisition of a cocaine conditioned place preference, adolescent (postnatal day (PND) 30) and young adult (PND 60) male Sprague-Dawley rats were pretreated with MK-801 (0.2 mg/kg, i.p.) 30 minutes before each cocaine CPP session (20.0 mg/kg, i.p.; 15 minutes). The effectiveness of acute and routine MK-801 treatment in blocking cocaine CPP expression was also assessed. Regardless of age, MK-801 pretreatment blocked the acquisition of a cocaine CPP, as well as the expression of place preferences following a cocaine challenge. Acute administration of MK-801 attenuated but did not completely block cocaine CPP in both ages examined. Findings suggest that similar mechanisms mediate the acquisition and expression of cocaine CPP across developmental time periods. Place preference attenuation following acute MK-801 exposure suggests that routine MK-801 treatment may be effective in blocking cocaine-associated memories. An alternative explanation may be that additional mechanisms are recruited following repeated cocaine treatment that may be resistant to NMDA antagonism. The observed findings further implicate and expand the role of NMDA receptors in mediating the consolidation and reconsolidation of cocaine-associated memories across development. The effectiveness of acute and repeated MK-801 on suppressing and blocking cocaine preferences, despite re-exposure to the drug itself provides further support for the potential therapeutic value for NMDA receptor antagonists in drug relapse and prevention.

Disclosures: **A.R. Uruena:** None. **C.L. Kirstein:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.14/A58

Topic: A.10. Adolescent Development

Support: NSERC's Alexander Graham Bell Masters Award

Title: The effects of chronic unpredictable adolescent stress on fosb expression and anxiety-related behaviors during adulthood

Authors: *J. GARDNER GREGORY[1,2], M. WILKIN[3], E. DUMONT[2], J. MENARD[3]
[1]Queens Univ., Montreal, QC, Canada; [2]Ctr. for Neurosci., Queens Univ., Kingston, ON, Canada; [3]Psychology, Queens university, Kingston, ON, Canada

Abstract: It has been observed in rats that chronic unpredictable stress (CUS) during adolescence can cause a decrease in anxiety-like behaviours and an increase in risk-taking behaviours during adulthood. Currently it is unknown what neurological changes occur to rats exposed to CUS during adolescence that would cause these behavioural changes during adulthood. Therefore the aim of the study is to examine the changes in immediate early gene expression in adulthood after a test of anxiety between rats who underwent CUS during adolescents compared to those that did not in order to identify regions of interest. 18 rats were placed in the CUS group and were exposed randomly from post-natal day 35 to 46, to three different stressors twice that included the elevated platform, water immersion and forced swim. 14 control rats were handled in place of the stressors. At post-natal day 84, 12 CUS rats and 8 control rats were tested on the elevated plus maze (EPM) and perfused 2 hours after the test. The remaining 12 animals, 6 CUS and 6 control were perfused with no manipulation to act as a control for the effects of the EPM. Rats that were exposed to CUS during adolescence displayed a greater percent of open arm time and a lesser amount of closed arm time than controls tested on the EPM indicating a potential decrease in anxiety-like behaviours. This behavioural evidence suggests that CUS during adolescence may cause alterations to anxiety circuitry of the brain. Immunohistochemistry was used to examine FOSB immunoreactive (IR) cell count in anxiety related regions of the brain that include the lateral septum (LS) and the oval and anterior medial bed nucleus of the stria terminalis (BNST). We have observed a greater amount of FOSB-IR cell count in the LS of control rats that were tested on the EPM compared to CUS rats tested on the EPM. Moreover, no differences were observed in the LS between CUS rats that were tested on the EPM and CUS rats that were never exposed to the EPM, while controls exposed to the EPM displayed a statistically significant greater amount of FOSB-IR cell count than control rats never exposed to the EPM. CUS during adolescence appears to blunt neuronal activation of the LS when the rat is exposed to the EPM. CUS did not affect FOSB-IR cell expression in the oval and anterior medial BNST. However, exposure to the EPM increased FOSB-IR cell expression in the anterior medial BNST. Differences found in the LS but not in the BNST, may indicate that CUS during adolescence may selectively alter neuronal activation in specific regions in the anxiety circuitry.

Disclosures: J. Gardner Gregory: None. **M. Wilkin:** None. **E. Dumont:** None. **J. Menard:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.15/A59

Topic: A.10. Adolescent Development

Support: Canadian Institutes of Health Research

Natural Science and Engineering Research Council of Canada

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Fulbright Canada

Concordia University Research Chairs program

Concordia University Office of Research

Title: Altered sensitivity to cocaine enhancement of reward seeking in DCC haploinsufficient mice

Authors: *L. M. REYNOLDS[1,3], A. J. GIFUNI[3], P. SHIZGAL[4], C. FLORES[2,3]
[1]Integrated Program in Neurosci., [2]Psychiatry, McGill Univ., Montreal, QC, Canada; [3]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [4]CSBN, Concordia Univ., Montreal, QC, Canada

Abstract: The receptor for the guidance cue netrin-1, DCC, is involved in the development and adult function of the mesocorticolimbic dopamine system. In adulthood, *dcc* haploinsufficient mice exhibit reduced behavioral response to stimulant drugs of abuse, including blunted response to their locomotor activating effects, reduced conditioned place preference, and lack of sensitization upon repeated drug exposure. To further investigate alterations in sensitivity to rewards in *dcc* haploinsufficient mice, we used the curve-shift method to measure cocaine-induced changes in intracranial self-stimulation (ICSS). We assessed (1) whether *dcc* haploinsufficient mice would acquire ICSS behavior at the same stimulation parameters as wild-type controls; and (2) whether cocaine would modulate performance for brain stimulation reward (BSR) similarly in *dcc* haploinsufficient and wild-type mice. To this end, mice were trained to self-administer electrical stimulation of the lateral hypothalamus, thus activating circuitry important for reward and motivated behavior. The vigor of responding was measured as a

function of pulse frequency, yielding curves analogous to pharmacological dose-response functions. These rate-frequency curves were then used to quantify the effect of cocaine on ICSS. The well-established facilitation of ICSS by stimulant drugs of abuse, such as cocaine, is expressed in a leftward curve shift that reflects a reduction in the strength of stimulation required to sustain performance at a given level. Here, we report that *dcc* haploinsufficient mice acquire ICSS behavior at comparable stimulation parameters to wild-type controls, suggesting that the underlying circuitry is functionally similar between the genotypes. However, due to the small sample size and variability in electrode placement, we cannot completely rule out differences in ICSS sensitivity at baseline. When challenged with 10 mg/kg of cocaine, *dcc* haploinsufficient mice exhibited smaller leftward shifts in rate-frequency curves than wild-type controls. However, a 20 mg/kg dose of cocaine produced leftward curve shifts of similar magnitudes in the two genotypes. The ICSS paradigm is often considered a primary screen for abuse liability. It is thus of interest that our results are consistent with decreased sensitivity to the rewarding effects of cocaine and/or decreased proclivity to invest effort in the pursuit of reward.

Disclosures: L.M. Reynolds: None. **A.J. Gifuni:** None. **P. Shizgal:** None. **C. Flores:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.16/A60

Topic: A.10. Adolescent Development

Support: Research was supported by BP-ENDURE R25-NS080686 (HK) and PSC-CUNY 44 Grants (VL).

Title: Adolescent bisphenol-A exposure leads to enduring decreases in dendritic spine density

Authors: *R. E. BOWMAN[1], V. LUINE[2], H. KHANDAKER[2], J. VILLAFANE[1], M. FRANKFURT[3]
[1]Sacred Heart Univ., Fairfield, CT; [2]Hunter Col., New York, NY; [3]Hofstra North Shore-LIJ Sch. of Med., Hempstead, NY

Abstract: Bisphenol-A (BPA), one of the most common environmental endocrine disruptors, is known to modulate estrogenic, androgenic, and anti-androgenic effects throughout the lifespan. The effects of BPA exposure during early organizational periods of development have been well documented. In order to better understand the mechanisms responsible for BPA's effects on the

adolescent brain, male and female rats were exposed to BPA during adolescence (postnatal days [PND] 42-49) and spine density in the medial prefrontal cortex (mPFC) and CA1 region of the hippocampus (CA1) assessed immediately on day 49 (7 weeks of age), and later in adulthood, at 11 weeks of age. In addition, because perinatal exposure to low-dose BPA alters corticosterone levels under both basal and stress conditions in adolescence, we examined whether adolescent BPA exposure altered serum corticosterone levels in response to a restraint stress challenge at 7 and 11 weeks of age, immediately prior to sacrifice. Stress dependent corticosterone responses were not altered by adolescent BPA exposure at either 7 or 11 weeks of age; however, BPA caused a significant decrease in spine density on apical and basal dendrites of pyramidal cells in both the mPFC and CA1. There was also a sex difference in spine density: females had greater spine density than males on basal dendrites of the mPFC and CA1. This sex difference was further augmented by both age and treatment, with overall results indicating that BPA-dependent decreases in spine density were more pronounced in males than females. These results are the first demonstrating that BPA, at levels below the current U.S.E.P.A. recommended safe daily limit, given during adolescence leads to enduring effects on neural morphology in adulthood. Given that humans are routinely exposed to low levels of BPA through a variety of sources, the decreased spine density reported in both male and female rats after BPA exposure warrants further investigation.

Disclosures: R.E. Bowman: None. **V. Luine:** None. **H. Khandaker:** None. **J. Villafane:** None. **M. Frankfurt:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.17/A61

Topic: A.10. Adolescent Development

Support: Brooklyn Anesthesia Research

Title: Toward a mechanism underlying the effects of neonatal sevoflurane on neuropsychiatric-like behavioral changes

Authors: D. LIN[1], J. LIU[2], J. E. COTTRELL[2], *I. S. KASS[3]
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[3]Anesthesiol. and Physiol. and Pharmacol., SUNY Downstate, BROOKLYN, NY

Abstract: Converging evidence from human and animal studies show that neonatal exposure to volatile anesthetics increases the risk of learning and memory deficits during adolescence. Perturbation of neurons during a critical developmental time window is associated with numerous neuropsychological disorders in addition to learning and memory deficits. Whether exposure to anesthetics during early brain development impose similar risks and the mechanisms underlying the deleterious effects of volatile anesthetics on the developing brain remain unclear. Previous studies from our lab have shown that the signaling pathway kinase mTOR is involved in sevoflurane (sevo) mediated downstream effects. We hypothesized that exposure to volatile anesthetics such as sevo during the early postnatal period would result in changes in mTOR-related downstream signaling pathway, resulting in behavioral changes. Male C57BL6 mice were exposed to 2% sevo for 2 hours on postnatal day 7(P7). Starting at peri-adolescence P27 and continued until adult (2-3 months), untreated and sevo treated mice underwent a battery of behavioral tests. Hippocampal tissue samples were taken after the completion of behavior tests and examined for changes in gene expression using western blot hybridization. The hippocampus-dependent spatial learning and memory task, Active Place Avoidance, showed that sevo treated mice had significantly more entrances into the shock zone. Social-interaction tests showed that sevo treated mice had significantly decreased interest toward novel social targets compared to the non-sevo treated mice. We confirmed that changes in these behaviors were not due to impairment of olfaction. Additional behavioral tests showed no difference in repetitive behaviors. We then examined changes in the expression of two genes, phosphorylated-mTOR and PKMzeta, a gene that is critically associated with learning and memory and a downstream effector gene of mTOR. Although adult brains showed no change in the expression of these two genes, we are still in the process of analyzing postnatal brains immediately after treatment with sevo. Our lab has confirmed that early life anesthetic exposure impairs cognitive function during peri-adolescent age. We initiated a battery of neuropsychiatric behavioral experiments and demonstrated changes in social interaction. These behaviors have been shown to be impaired in mouse models of neuropsychological disorders, such as Autism Spectrum Disorder. In order to understand the mechanisms underlying the observed changes in behavior, we are investigating changes in genes and microRNA expression from postnatal brains immediate after sevo exposure.

Disclosures: **D. Lin:** None. **I.S. Kass:** None. **J.E. Cottrell:** None. **J. Liu:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.18/A62

Topic: A.10. Adolescent Development

Support: Mercer University School of Medcine

MEDCEN Foundation Community Health and Education Grant

Title: Developmental regulation of mouse midbrain dopamine neuron excitability by small-conductance calcium-activated potassium (SK) channels

Authors: C. FAIRCLOTH, C. BAYER, *A. PLACZEK
Mercer Univ. Sch. of Med., Macon, GA

Abstract: In both human and animal studies, adolescence has been shown to be a period of relative vulnerability to drug addiction. There is accumulating evidence for critical periods of altered excitability and synaptic plasticity during the development of reward-related brain systems. The release of dopamine (DA) from ventral tegmental area (VTA) neurons is an important component of behavioral reinforcement and drug addiction. This is particularly true in the shell of the nucleus accumbens (NAc), where addictive substances have been shown to increase DA release. Action potential firing is probabilistically coupled to neurotransmitter release, and both *in vivo* and *ex vivo* studies have shown an important relationship between action potential firing patterns in VTA DA neurons and the release of DA in the NAc. We have previously reported age-dependent variations in the action potential firing patterns of VTA DA neurons in C57BL/6J mice. In patch clamp studies using brain slices, the VTA DA neurons of adolescent (35-42 day-old) mice fire more action potentials than those of adult mice when depolarized with direct current injection. In contrast, adult (90-120 day-old) C57BL/6J mice fire fewer action potentials with sustained depolarization. The latter firing pattern has been associated with a tendency toward phasic activity, a firing mode that has been linked to both natural reward and the response to addictive drugs. While there are multiple factors that can potentially affect action potential waveforms and firing patterns in VTA DA neurons, previous studies have implicated small-conductance calcium-activated potassium (SK) channels as important regulators of both sustained activity and the shift from tonic to phasic firing. We therefore explored the relative contribution of SK channel-mediated conductances on the intrinsic excitability of VTA DA neurons during both adolescence and adulthood in C57BL/6J mice. Adolescent mice displayed a significant increase in both the apamin-sensitive component of the action potential afterhyperpolarization (AHP), and apamin-sensitive tail-current amplitude. This increased apamin-sensitive current was shown to be a key contributor to sustained action potential firing during adolescence, because bath application of 20 nM apamin resulted in reduced action potential firing, shifting the adolescent firing toward a more adult-like pattern. These findings suggest that adolescent action potential firing patterns lead to increased tonic-like activity and thus reduced dopamine release during excitation. This may therefore increase the probability of binge-like consumption of addictive substances during adolescence.

Disclosures: **C. Faircloth:** None. **A. Placzek:** None. **C. Bayer:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.19/A63

Topic: A.10. Adolescent Development

Title: The effects of social isolation in adolescent rats on cognitive function and prefrontal cortex circuitry in adulthood

Authors: ***D. LINDER**, I. GAISLER-SALOMON
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Abstract: The "two-hit" hypothesis of schizophrenia (SZ) states that genetic and/or early environmental factors lead to developmental disruption of the CNS, increasing vulnerability to future environmental stressors. Animal studies have shown that exposing adolescent rats to social isolation leads to the emergence of behavioral and neurochemical changes in adulthood that mimic pathophysiological features of SZ. Currently, few studies have addressed the contributions of individual differences in social and cognitive functions to this effect, although deficits in cognitive and social function are good predictors of patient outcome and exist in milder form in high-risk populations. Here, we screened rats for social preference in pre-adolescence (P25) and subjected them to two weeks of social isolation or social rearing during mid-adolescence. In adulthood, rats that exhibit high social preference in pre-adolescence exhibit deficits in reversal learning, regardless of housing conditions. However, as the cognitive demand increased, i.e. in an extra-dimensional set-shifting task, this effect was moderated by isolation stress, so that rats high in social preference reared in isolation performed set-shifting faster than isolated rats with low social preference. These results suggest that cognitive functions are influenced by individual differences in social preference as well as social stress in adolescence, but in a task-dependent manner. Guided by the hypothesis that predispositional and environmental factors converge to affect the balance between excitatory (E) and inhibitory (I) transmission in the prefrontal cortex (PFC), we studied the effect of early social factors combined with social or isolation rearing on the E/I balance in the PFC. This study could contribute to our understanding of the relative importance of predispositional and environmental influences on SZ risk and resilience.

Disclosures: **D. Linder:** None. **I. Gaisler-Salomon:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.20/A64

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 5P20RR016463

8P20GM103423

Title: Antischizophrenic properties of dietary choline supplementation during adolescence: Comparing outcomes in the DISC1 knockout rat and chronic MK-801 models

Authors: A. A. BATALLAN, N. K. ZIV, W. YU, J. R. MITCHELL, A. S. MOORE, C. EVANGELISTA, *M. J. GLENN
Psychology, Colby Col., WATERVILLE, ME

Abstract: Schizophrenia is a debilitating psychological disorder characterized by a constellation of positive, negative, and cognitive symptoms. While a great deal is known about the underlying neural abnormalities that contribute to these symptoms, the etiological bases for the disorder remains elusive. It is clear, however, that development of the disorder rests significantly on genetic and experiential factors. Reproducing these factors, or the neurochemical abnormalities, in non-human animal models is a powerful research tool with the potential to greatly enhance our understanding of the disorder and enable the investigation of variables that may attenuate or prevent symptoms. Mounting evidence from our lab and others compelling points to the antischizophrenic properties of the essential nutrient choline. In the present work, we continued our investigation into the ways in which choline may be antischizophrenic and did so in two models: with a genetic DISC1 knockout or chronic MK-801 treatment. DISC1, or disrupted in schizophrenia1, is a significant risk gene that codes for proteins that contribute in various ways to neuron migration, function, and plasticity. DISC1-knockout rats lack both copies of the gene and we report here robust positive and negative symptoms in them. Interestingly, choline supplementation in adolescence resulted in mixed effects on adult symptoms: it modestly, though significantly, attenuated the negative symptom of anxiety, failed to combat the positive symptom of hyperactivity, and resulted in worse memory function than seen in standard-fed DISC1 rats. In the pharmacological model, rats were given daily injections of the NMDA antagonist, MK-801 (1 mg/kg) for 14 days. This significantly impaired pre-pulse inhibition (PPI), a hallmark symptom in schizophrenia, and adolescent choline supplementation completely prevented that

effect. Taken together, these results add to the growing literature that dietary choline may exert protective effects on neural systems that are dysfunctional in schizophrenia, yet there may be some specificity to the protection. Adding to this work, we also report that choline supplementation significantly enhanced cognitive flexibility in an attention set-shifting task, an analog of the Wisconsin card-sorting test that reveals the characteristic hypofrontality in humans with schizophrenia. This finding aligns well with our work in the rat schizophrenia models and future directions aim to investigate PPI and cognitive flexibility in DISC1 knockout rats to further characterize their phenotype and to systematically investigate the interaction of the genotype and choline availability.

Disclosures: **A.A. Batallan:** None. **N.K. Ziv:** None. **W. Yu:** None. **J.R. Mitchell:** None. **A.S. Moore:** None. **M.J. Glenn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Sage Labs. **C. Evangelista:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.21/A65

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: R01MH100228

Title: Adolescent cannabinoid treatment and persistent frontostriatal endocannabinoid signaling gene changes in mice and humans

Authors: *S. MUKHERJEE, K. GLEASON, A. SHUKLA, S. BIRNBAUM, S. GHOSE
Dept. of Psychiatry, UT Southwestern Med. Ctr., DALLAS, TX

Abstract: Cannabis is the most commonly abused drug in the United States. 2.1 million Americans used cannabis for the first time in 2007; of them 62.2% were less than 18 years old. This is of particular health concern given the association between adolescent cannabis use and adult onset of psychosis. Schizophrenia did not develop days or weeks after cannabis use, but years later, suggesting that cannabis use during a critical period of brain maturation may lead to long-term effects. We conducted a series of experiments to examine the long-term consequences of adolescent cannabinoid exposure on the endocannabinoid system. Mice were administered with a cannabinoid receptor 1 (CB1) agonist, (WIN 55,212-2) or vehicle for 10 days by I.P. injection at different developmental time points (5, and 9 weeks). At 16-18 weeks of age,

behavioral tests were carried out followed by molecular studies 2 week after the last behavioral test. Mice administered WIN 55,212-2 (WIN) at 5 weeks of age display significant deficits in PPI and fear conditioning learning and memory paradigm. These behavioral deficits were not observed in mice treated with the CB1 agonist at later developmental time points. Group I metabotropic glutamate receptor (mGluR) activation drives endocannabinoid synthesis and release and we previously reported changes in CB1 and mGluR5 expression patterns in the hippocampus. Here, we extend those studies, examining endocannabinoid genes in the frontal cortex and striatum in mice and a human post mortem tissue cohort of control and schizophrenia cases divided into individuals with and without a significant adolescent cannabis use history. Mice treated with WIN 55,212-2 at 5 weeks of age show a distinct profile in CB1 and mGluR5 the frontal cortex and striatum. There is a significant upregulation of CB1 in both the frontal cortex and striatum while mGluR5 protein levels demonstrated a bidirectional expression pattern with significantly increases in the frontal cortex and strong trend to reduction in the striatum. Our pilot human studies (n=5 per group) show a similar profile with higher levels of CB1 and mGLuR5 (effect size >0.6) in the prefrontal cortex (BA9) of schizophrenia cases with adolescent cannabis use history. These data suggest that adolescent cannabinoid administration leads to regionally specific persistent changes in the endocannabinoid pathway. These data may be relevant to understanding the long term sequelae of significant adolescent cannabis use and may be of particular importance in understanding mechanisms by which adolescent cannabinoid exposure leads to molecular changes predisposing to schizophrenia.

Disclosures: **S. Mukherjee:** None. **K. Gleason:** None. **A. Shukla:** None. **S. Birnbaum:** None. **S. Ghose:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.22/A66

Topic: A.10. Adolescent Development

Support: NIH Grant MH099625

Title: The timing of neuroanatomical changes across adolescence in the male and female rat medial prefrontal cortex

Authors: *J. WILLING[1], T. KIM[2], J. M. BRODSKY[3], L. R. CORTES[3], J. JURASKA[1]
[1]Psychology, Univ. of Illinois, Champaign, IL; [2]Psychology, [3]Neurosci. Program, Univ. of Illinois at Urbana Champaign, Champaign, IL

Abstract: Adolescence is a critical period for brain maturation, characterized by the reorganization of many interacting neural networks. The medial prefrontal cortex (mPFC), a region highly involved in executive function, is particularly known to undergo synaptic pruning during this time. Disruptions of normal mPFC development have been associated with a variety of clinical disorders including schizohprenia, ADHD and depression. We have previously shown that rats lose neurons in the mPFC between adolescence and adulthood, with more prominent losses being observed in females. Additionally, previous data from our lab show that an increase in ovarian hormones at puberty play a significant role in neuronal loss in females, as OVX prevented this cellular loss. In the present study, we track neuronal and glial changes in the male and female mPFC at multiple time points from preadolescence to adulthood (P25, P35, P45, P60 and P90). Our preliminary analysis confirmed a greater neuronal loss in the female mPFC between adolescence and adulthood, and that the timing of neuronal loss in females coincides with the onset of puberty (between P35 and P45). The timing of loss was not as obviously discernible in males in this preliminary sample. In addition to adding animals to the analysis of cell number in the mPFC, we will also characterize levels of tyrosine hydroxylase immunoreactivity, representing dopaminergic axons innervating the region, at the time points mentioned previously. Elucidation of the precise timing of cellular pruning of the mPFC may have clinical implications with respect to mental illnesses characterized by mPFC dysfunction and the observed sex differences in prevalence of these disorders.

Disclosures: J. Willing: None. **T. Kim:** None. **J.M. Brodsky:** None. **L.R. Cortes:** None. **J. Juraska:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.23/A67

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Sleep deprivation at juvenile age causes alterations in cortical GAD67 levels in juvenile mice and induces prepulse inhibition deficit in adult female mice

Authors: *C. MONPAYS, A. TREMEY, J. DESLAURIERS, W. RACINE, K. ASLI, P. SARRET, S. GRIGNON
Physiol. and Biophysics, Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: Age at onset plays a significant role in the pathophysiology, clinical presentation and prognosis of bipolar affective disorder (BAD). In the present study, we assessed whether the sleep deprivation (SD) model of mania, which has been well-validated in adult rodents, could be successfully implemented in juvenile animals. We previously demonstrated that SD at juvenile age induced a PPI deficit in both males and females, compared to non-sleep-deprived animals. However, when SD was performed in adulthood (post natal day (PN) 70), we did not observe differences as compared to control. The fact that PPI deficits are only observed when SD is induced at juvenile age, but not during adulthood, supports the hypothetical interaction between age at onset and apparition of pathophysiological mechanisms. To verify this hypothesis, we compared a third group, submitted to juvenile SD and to behavioral tests at adult age, and we evaluated the involvement of relevant neurochemical parameters in the juvenile model. To induce SD, C57BL/6 mice were placed on a small 3-cm-diameter platform. When mice fell asleep, muscle tone relaxation made them fall into the water and provoked awakening. In the control condition, mice were placed on a larger 10-cm-diameter platform, compatible with uninterrupted sleep. Sleep deprivation was induced for 72 hours, from postnatal (PN) days 33 to 35. On PN70, sensorimotor gating deficits, mouse exploratory behaviors and social interaction were determined by investigating the prepulse inhibition of the acoustic startle response (PPI), open-field task and resident-intruder test, respectively. We also evaluated, by immunoblotting and qPCR, the protein and mRNA levels of dopamine D2 receptor and GAD67 in the prefrontal cortex (PFC). SD induced a PPI deficit in juvenile mice, which persisted in adult females (PN70) (-17.48%; $p<0.01$ compared to control group) but not in adult males. Preliminary results in juvenile mice showed a decrease, at trend levels, in dopamine D2 receptor protein levels in the PFC of female mice. Moreover, we observed an increase, at trend levels, in cortical GAD67 protein and mRNA expression in both sexes. We found here that juvenile SD induces a PPI deficit that is persistent only in adult females, in line with some epidemiological features of the condition. Interestingly, we also observed a trend for increased GAD67 protein and mRNA levels in the PFC, in contrast with the schizophrenia model developed in our laboratory (Deslauriers et al, 2013). This work may help to explore the pathophysiological differences between early stage schizophrenia and BAD.

Disclosures: C. Monpays: None. **A. Tremey:** None. **J. Deslauriers:** None. **W. Racine:** None. **K. Asli:** None. **P. Sarret:** None. **S. Grignon:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.24/A68

Topic: E.03. Behavioral Neuroendocrinology

Support: ARRS grant P4-0053

NIH grant MH61376

Title: Altered female sexual receptive behavior after adolescent social isolation in mice

Authors: *G. MAJDIC[1], J. KERCMAR[2], S. A. TOBET[3]
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Abstract: Exposure to stress during puberty can cause long lasting consequences in brain development and consequently changes in behavior in adult rodents. During this time the brain remodels and reorganizes as a function of sex steroid hormone actions. Stress hormones also could influence this development. Social isolation present a stress for social animals like mice, but little is known about effects of such stress during adolescence on reproductive behaviors. The present study examined the sexual behavior of ovariectomized, estradiol and progesterone primed female mice that were individually housed from 25 days of age until testing at approximately 95 days, or individually housed from day 25 until day 60 (during puberty), followed by housing in social groups. Mice in isolated groups were compared to females that were housed in social groups throughout the experiment. Receptive sexual behaviors of females and behaviors of stimulus males were recorded. Females housed in social groups displayed greater levels of receptive behaviors in comparison to both socially isolated groups. Namely, social females had higher lordosis quotients and more often displayed better lordosis postures in comparison to isolated females. No differences between female groups were observed in stimulus male behavior suggesting that female 'attractiveness' was not affected by their social isolation. Females housed in social groups had less area containing immunoreactive estrogen receptor α in the anteroventral periventricular nucleus (AVPV) and the ventromedial nucleus of the hypothalamus (VMH) than both socially isolated groups. These results suggest that isolation during adolescence affects female sexual behavior and re-socialization for one month in adulthood is insufficient to rescue lordosis behavior from the effects of social isolation during the pubertal period.

Disclosures: G. Majdic: None. **J. Kercmar:** None. **S.A. Tobet:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.25/B1

Topic: A.10. Adolescent Development

Title: Developmental fluoxetine affects hippocampal plasticity in juvenile male and female offspring

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Abstract: The estimated prevalence of depression during pregnancy is 20%. Treatment of depression in this critical period raises the question about safeness of antidepressant therapy. The Most common treatment for maternal depression is selective serotonin reuptake inhibitor (SSRI) medications, e.g. Prozac (fluoxetine), which are prescribed to 5-10% of pregnant mothers. SSRIs cross the placental and blood-brain-barrier and are transferred, via breast milk, to the infant. This increased level of serotonin (5-HT) due to prenatal flouxetine treatment may interfere with functional maturation of the brain and can differentially affect neuronal plasticity of both, the mothers and their offspring. Previous work we have done shows that developmental exposure to fluoxetine affects hippocampal neurogenesis in both adolescent and adult offspring. Therefore the aim of this study was to evaluate the effect of flouxetine administration during sensitive functional brain development on hippocampal neurogenesis of the rat dams and their juvenile male and female offspring (P21). Pregnant Sprague-Dawley rats were subject to stress during gestation and were treated with fluoxetine (10 mg/kg/day) prior to parturition and throughout lactation. Animals from the following four groups were used were used: 1. Control+Vehicle (CV), 2. Control+Fluoxetine (CF), 3. Prenatal Stress+Vehicle (PSV), 4. Prenatal Stress+Fluoxetine (PSF). Preliminary results show that developmental fluoxetine exposure, but not maternal prenatal stress, significantly decreases the number of new immature neurons in the dorsal hippocampus of juvenile male and female offspring ($p=.024$). Furthermore, there were no signitifcant effects of prenatal stress or developmental fluoxetine exposure on the number of tryptophan-hydroxylase (TPH) positive cells in the dorsal raphe nucleus in juvenile offspring. Future work will assess the relationship between circulating levels of fluoxetine and norfluoxetine and measures of neural plasticity, as well as any effects on hippocampal plasticity in the dam. Understanding the impact of developmental exposure to SSRI medications will improve our understanding of the benefits and risks of this treatment for maternal mood disorders.

Disclosures: **E. Császárová:** None. **J. Pawluski:** None. **L. Knaepen:** None. **M. Fillet:** None. **V. Houbart:** None. **H. Steinbusch:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.26/B2

Topic: A.10. Adolescent Development

Title: Developmental SSRI exposure decreases serotonin and 5-HIAA levels in the PFC, but not the hippocampus, of prenatally stressed juvenile offspring

Authors: *M. GEMMEL[1], I. RAYEN[2], E. VAN DONKELAAR[2], H. W. STEINBUSCH[2], N. KOKRAS[3], C. DALLA[3], J. L. PAWLUSKI[1,2,4]
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Abstract: Selective serotonin reuptake inhibitor (SSRI) medications are the most frequently used antidepressant treatment for maternal mood disorders during pregnancy and in the postpartum period. The ability of these antidepressant medications to cross the placental barrier suggests possible impact on fetal development. Likewise, serotonin has been shown to play a significant role in stress regulation and fetal development. Thus, investigation of SSRIs and their effects on the serotonergic system is crucial in understanding key aspects of development. The aim of this study was to determine the effect of the SSRI medication, fluoxetine, on both maternal and offspring serotonergic systems. Sprague-Dawley rat dams were subjected to stress during gestation and were treated with fluoxetine (5mg/kg/day) or vehicle via osmotic minipumps. Animals from the following four groups were used: 1.Control+Vehicle (CV), 2.Control+Fluoxetine (CF), 3.Prenatal Stress+Vehicle (PSV), 4.Prenatal Stress+Fluoxetine (PSF). At weaning, brains of male and female offspring and the dam were collected. Half the brain was used to assess serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), levels in the hippocampus and prefrontal cortex. Preliminary results show that at weaning, developmental fluoxetine exposure markedly decreases 5-HT ($p=.06$) and 5-HIAA levels ($p=.02$) in the prefrontal cortex of prenatally stressed offspring ($p=.02$), with no significant differences between groups in 5-HT or 5-HIAA levels in the hippocampus. In dams, the ratio of 5-HIAA/5-HT, an indication of serotonin (5-HT) metabolism, was significantly lower in the

prefrontal cortex of dams administered fluoxetine (p=.036). Furthermore, in the hippocampus there was a significant interaction effect of stress x fluoxetine (p=.006) with PSV dams having significantly higher 5-HT metabolism compared to CV and PSF dams. Further work will investigate the effect of developmental fluoxetine exposure on neuronal and synaptic plasticity in the hippocampus and prefrontal cortex. Understanding the impact of developmental exposure to SSRI medications on the serotoninergic system and neurodevelopmental processes will further enhance our understanding of the benefits and risks of these medications.

Disclosures: **M. Gemmel:** None. **I. Rayen:** None. **E. Van Donkelaar:** None. **H.W. Steinbusch:** None. **N. Kokras:** None. **C. Dalla:** None. **J.L. Pawluski:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

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Program#/Poster#: 210.27/B3

Topic: A.10. Adolescent Development

Support: NIH Grant MH076060

NIH Grant MH080272

Title: Gene expression throughout human postnatal development in single cell populations in the prefrontal cortex

Authors: *E. KOHLBRENNER, *. WOO
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Abstract: Schizophrenia (SZ) is a neurodevelopmental disorder that has been associated with pre- and peri-natal insults such as maternal illness and poor nutrition during fetal development, as well as the season of birth and certain childhood infections. It is likely that hereditary factors also play a role. Together these genetic and environmental insults may explain, at least in part, cognitive deficits throughout childhood in things like attention, memory, and impulse control. For most individuals, however, the onset of the overt symptoms and deficits of schizophrenia do not occur until late adolescence or early adulthood, suggesting that additional events occurring during the peri-adolescent period contribute to the onset of illness. The prefrontal cortex (PFC), an area that has consistently shown severe disturbances in SZ, undergoes a very protracted course of maturation; it does not reaching full competence until the late teens- similar to the age

of onset of the overt symptoms and deficits of schizophrenia. Parvalbumin (PV) inhibitory neurons are found throughout the cerebral cortex and are known to coordinate the critical periods of development, as well as regulate maturation plasticity of the brain, placing them at the forefront of faulty plasticity research that includes neurodevelopmental disorders such as schizophrenia and autism. Malfunction of PV cells during adolescence can dismantle the synchronization of pyramidal neuronal circuits that is critical for the functional maturation of PFC connectional architecture, compounding on previous environmental insults and contributing to the onset of disease.We profiled gene expression (Affymetrix microarray)changes in laser-captured PV-immunolabeled neurons in layer 3 of Brodmann's area 9 of the PFC during normal human peri-adolescent development in order to understand genes and molecular pathways that underlie the maturation of these neurons. We examined a cohort of 18 normally developing individuals, 1-24 years old. Three groups were formed for comparison: 6 Pre-pubescent, 6 pubescent, and 6 post-pubescent. So far in pyramidal neurons, TGFβ signaling, EGFR signaling, folic acid network, cytoplasmic ribosomal proteins, mRNA processing, ubiquitin-mediated proteolysis and SNARE-mediated vesicular transport are among the pathways that are most significantly differentially regulated during peri-adolescent development. Interestingly, TGFβ signaling also appears to be dysregulated in layer 3 pyramidal neurons from the PFC in SZ. Comparison of these findings with gene expression changes in schizophrenia will shed light onto the developmental pathophysiology of schizophrenia onset.

Disclosures: E. Kohlbrenner: None. ***. Woo:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.28/B4

Topic: A.10. Adolescent Development

Support: R01MH094358

Title: Chromatin kinetics in the visual system

Authors: *K. A. CHASE[1], C. HASTY[1], B. M. FEINER[1], N. H. WRAY[2], H. GIN[1], E. HU[1], R. P. SHARMA[1]
[1]Psychiatry, [2]Univ. of Illinois At Chicago, Chicago, IL

Abstract: Background: Epigenetic regulation of gene expression is an important mechanism by which the brain coordinates its transcriptome, with modifications of histone proteins encoding the use or disuse of neuronal projections (Sharma, 2005; 2011; 2012). The best demarcated brain system, from input to encoding, is the visual system, making it ideal to investigate the kinetics of chromatin remodeling. In this system, light deprivation increases H3K27 methylation and decreases H3K9 acetylation binding to the trophic factor BDNF, decreasing gene transcription (Karpova, 2010). Pharmacologically induced increases in acetylated histone modifications can reverse the effects of monocular light deprivation (Silingardi, 2011). It is our hypothesis that chronic misuse or disuse will result in an accumulation of experience-dependent chromatin remodeling, allowing for kinetics of these changes in neuronal systems to be modelled. Methods: C57BL/6 strain male mice were weaned at PND 21 into either ocular deprivation (24h dark) or normal light conditions. Food and water was available ad libitum. Occipital cortices were removed upon euthanasia and snap frozen in 2-methylbutane. mRNA levels were measured through Real-Time RT PCR, and DNA-protein interactions through Chromatin Immunoprecipitation. Results: There were ocular deprivation-dependent changes of selected genes relating to relevant plasticity factors, known chromatin modifying enzymes and transcription factors. Coordinate changes in mRNA expression were associated with significant differences in promoter-specific H3K9me2 binding. Discussion: Through using the visual system as a model, termination of sensory input results in an accumulation of a transcriptionally restrictive landscape, allowing for a characterization of the kinetics of histone modification formation. This experience-dependent chromatin manipulation has important implications for both understanding and identifying potential treatment targets of neurocognitive disorders, stroke, rehabilitation for limb injury, loss and movement disorders.

Disclosures: **K.A. Chase:** None. **C. Hasty:** None. **B.M. Feiner:** None. **N.H. Wray:** None. **H. Gin:** None. **E. Hu:** None. **R.P. Sharma:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.29/B5

Topic: A.10. Adolescent Development

Support: NIMH grant 63104

NIGMS grant 96873

Title: Ontogeny of circadian rhythms in the suprachiasmatic nucleus

Authors: A. T. C. SUN[1], C. L. SIMMS[1], *E. D. HERZOG[2]
[1]Biol., Washington Univ. in St. Louis, St. Louis, MO; [2]Dept. of Biol., Washington Univ. In St. Louis, ST LOUIS, MO

Abstract: The mammalian suprachiasmatic nucleus (SCN), situated in the hypothalamus, is directly above the optic chiasm and a master circadian pacemaker. The SCN produces endogenous rhythms in gene expression to regulate daily rhythms in physiology and behavior. Although the function of the SCN is well studied in adult mammals, the ontogeny of SCN function is less well understood. We hypothesized, based on 2-deoxyglucose data, that SCN neurons initiate intrinsic circadian rhythmicity *in utero* and synchronize to each other when the neuropeptide, vasoactive intestinal polypeptide (VIP) is expressed. We monitored PER2::Luciferase, a bioluminescent reporter for PER2 levels, in fetal SCN explants. We found that SCN explants exhibited rhythms in PER2 expression as early as embryonic day 14.5 (E14.5) and the amplitude of the rhythms increased in later ages. However, PER2 expression was arrhythmic in E13.5 explants. From embryonic day 15.5 to postnatal day 3, most slices were circadian, and, from P5, all slices were circadian. Single cell imaging of fetal SCN explants revealed rhythmic and synchronized cells by E17.5. Consistent with these results, we found that VIP expression was present by E17.5 with more cells expressing VIP in later fetal stages. Our findings support the conclusion that the embryonic SCN is capable of producing daily rhythms in gene expression starting at E14.5 and, by E17.5, VIP expression supports synchrony among SCN cells.

Disclosures: A.T.C. Sun: None. **E.D. Herzog:** None. **C.L. Simms:** None.

Poster

210. Adolescent Vulnerability: Animal Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 210.30/B6

Topic: A.10. Adolescent Development

Title: The effect of metformin on the developing central nervous system (cns), using *Drosophila* melanogaster

Authors: *N. DEWITTE[1], T. WILLIAMS[2], N. LEELANI[1]
[2]Biology/Neuroscience, [1]Trinity Univ., San Antonio, TX

Abstract: Type 2 Diabetes affects more than 26 million Americans, and the number of those diagnosed continues to rise. The Center for Disease Control estimates that 1 in 3 U.S. adults could have diabetes by 2050. A drug commonly prescribed to treat those with Type 2 Diabetes is Metformin. It is used in combination with exercise and diet in order to help control blood sugar levels in patients. While Metformin has shown significant success with treating Type 2 Diabetes, little is known about its long term effects on juveniles, especially as it relates to the developing brain. Therefore, the purpose of our research is to determine the effects of Metformin on the developing central nervous system (CNS), using *Drosophila melanogaster* as an animal model. Our results show flies exposed to high concentrations of metformin (39%), when compared to control (100%) or low-dose metformin (100%) exposed flies, leads to reduced viability in which flies do not develop beyond the third instar larval stage. Furthermore, we have shown the mRNA expression level of Neuroligin 4 is reduced by 50% for flies fed a high-dose of metformin as compared to controls or flies maintained on a low-dose metformin diet. Therefore, we predict daily exposure to metformin during early development can lead to altered CNS development.

Disclosures: **N. Dewitte:** None. **T. Williams:** None. **N. Leelani:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.01/B7

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NINDS/NIGMS 60655

Title: Serotonin suppresses and enhances temporoammonic path-CA1 responses *in vivo*

Authors: *J. GONZALEZ[1], B. E. DERRICK[1,2]
[1]Biol., U Texas San Antonio, San Antonio, TX; [2]The Neurosciences Res. Inst., San Antonio, TX

Abstract: Temporoammonic path (TAP) afferents arising from layer III of the entorhinal cortex convey cortical input directly to area CA1 of the hippocampus, and are thought to contribute to a variety of memory functions in the hippocampus, such as long-term memory and match-mismatch functions. Previous investigations reveal conflicting findings of serotonergic effects on TAP-CA1 synaptic responses, including suppression or enhancement of TAP-CA1 responses *in vitro* (Otmakhova & Lisman 2000; Otmakhova et al. 2005; Cai et al. 2013). Here we examine the neuromodulatory impact of serotonergic re-uptake inhibitors and releasers on medial TAP-CA1

responses *in vivo*. In urethane anesthetized animals, a consistent and reversible reduction in medial TAP-CA1 responses was observed following an acute, systemic injection of fluoxetine (10 mg/kg), a selective serotonin re-uptake inhibitor. Medial TAP-CA1 responses were maximally depressed at 1 hr and approached baseline levels 2 hr after fluoxetine injection. This effect appears to be limited to TAP-CA1 synapses as simultaneous recordings of commissural CA3-CA1 (cCA3-CA1) responses showed no change in amplitude following fluoxetine administration. As vehicle-treated animals did not exhibit decreased amplitudes of medial TAP-CA1 responses, these data suggest serotonin selectively suppresses medial TAP-CA1, not cCA3-CA1, neurotransmission following fluoxetine delivery *in vivo*. By contrast, intraperitoneal injection of d-fenfluramine (10 mg/kg), a selective serotonin releaser and re-uptake inhibitor, in anesthetized animals induced variable observations of either a robust increase in the amplitude of medial TAP-CA1 responses lasting more than 2 hr or a reversible reduction in medial TAP-CA1 responses. Although fluoxetine and d-fenfluramine both augment extracellular serotonin concentration, our data demonstrate serotonergic activity can suppress or enhance TAP-CA1 responses *in vivo*. As d-fenfluramine induces a more robust increase of synaptic serotonin levels when compared to fluoxetine, our data suggest variations in synaptic serotonin concentration may regulate bidirectional changes in TAP-CA1 responses via actions on distinct serotonin receptors.

Disclosures: B.E. Derrick: None. **J. Gonzalez:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.02/B8

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Natural Sciences and Engineering Research Council of Canada

Title: Nitrosylation of vesicular neurotransmitter transporters

Authors: *Y. WANG[1], H. TAN[1], Z. ZHOU[1], Y. SUN[1], S. ZHU[1], X.-M. LI[2], J.-F. WANG[1]
[1]Neurosci. Res. Program, Univ. of Manitoba, Winnipeg, MB, Canada; [2]Dept. of Psychiatry, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Brain is the main site for oxygen consumption (80%), making the brain cells vulnerable to production of reactive oxygen/nitrogen species (ROS/RNS). Vesicular transporters

such as vesicular monoamine transporter 2 (VMAT2) vesicular glutamate transporter 1 (VGluT1) and vesicular glutamate transporter 2 (VGluT2) are key proteins for packaging and uploading neurotransmitters into vesicles. These transporters have high density of cysteine residues that are susceptible to ROS/RNS attack. Therefore we determined whether nitric oxide radical donor S-nitrosoglutathione (GSNO) induces nitrosylation of VMAT2, VGluT1 and VGluT2 and whether GSNO regulates vesicular uptake activity of glutamate in mouse brain. Cysteine nitrosylation of the transporters was measured by biotin-switch method followed by immunoblotting analysis, and tritium labeled glutamate was used to measure the uptake of glutamate by vesicles. We found that GSNO at 80 uM GSNO treatment significantly increased nitrosylation of VMAT2, VGluT1 and VGluT2 and inhibited the uptake of glutamate by vesicles. Our finding suggested that nitrosylation of vesicular transporters may play a role in the regulation of monoamine and glutamate neurotransmission.

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Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.03/B9

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Research Council for Health Sciences, Hungarian Ministry of Health, Grant No ETT-031/2012

Title: Stimulation of glutamate receptors on on- and off-cone bipolar cells leads to increased release of [3h]glycine in rat retina

Authors: *L. G. HARSING, Jr.[1], G. SZENASI[2], A. HANUSKA[1], A. SZABO[1], M. ALBERT[3]
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Abstract: Glutamate release establishes neuronal communication in the synapses of cone photoreceptors and bipolar cells as well as between bipolar and ganglion cells in the retina. This communication exhibits characteristic segregation of glutamate receptors. In the outer plexiform layer of the retina, AMPA ionotropic and mGluR6 metabotropic glutamate receptors are expressed in Off and On bipolar cells, respectively. Moreover, GABAergic and glycinergic

amacrine cells possess AMPA receptors, whereas Off- and On-type ganglion cells contain N-methyl-D-aspartate (NMDA) receptors in the inner plexiform layer. In this study, we have investigated the effects of various glutamate receptor agonists on [3H]glycine release from amacrine cells using posterior eyecup preparations lined by the retina. This preparation was made from rat eye, loaded with [3H]glycine and its release was determined by low-volume superfusion technique combined with scintillation spectrometry. The group III mGluR agonist L-(+)-2-amino-4-phosphonobutyric acid (L-AP4, APB), which blocks neurotransmission between cones and On bipolar cells, suppresses light-evoked activity in the On parallel pathway. Addition of L-AP4 in a concentration of 0.1 mM increased electrical stimulation (20 V, 10 Hz, 2 msec for 3 min)-induced [3H]glycine release. The AMPA glutamate receptor agonist (RS)-α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA), which stimulates neurotransmission between cones and Off bipolar cells, increases dark-evoked activity in the Off parallel pathway. AMPA added in concentrations of 0.01 to 1 mM in combination with the positive allosteric modulator cyclothiazide (0.03 mM) also increased [3H]glycine release from isolated retina preparation. The AMPA receptor antagonist GYKI-53405 (0.05 mM) decreased electrically evoked [3H]glycine release and reversed the AMPA-stimulated [3H]glycine release. In the retinal circuitry, GABAergic amacrine cells negatively influence On pathway and glycinergic amacrine cells exert similar inhibitory effect on Off pathway. The two parallel pathways may communicate via amacrine-amacrine cell interactions. In the dark, when On pathway is inactivated, less GABA and more glycine may be released from the amacrine cells, whereas the activated Off pathway may directly enhance glycine release. An opposite shift in GABA and glycine release from retinal amacrine cells may occur in the light. This dual regulation of [3H]glycine release from amacrine cells may have a role in center-surround properties of the inner retinal neurons.

Disclosures: **L.G. Harsing:** None. **G. Szenasi:** None. **A. Hanuska:** None. **A. Szabo:** None. **M. Albert:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.04/B10

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Spontaneous depolarization waves in medetomidine sedated Sprague-Dawley rats detected by fMRI

Authors: *A. SHATILLO[1], J. HUTTUNEN[1], A. AIRAKSINEN[1], J.-P. NISKANEN[2], O. GROHN[1]
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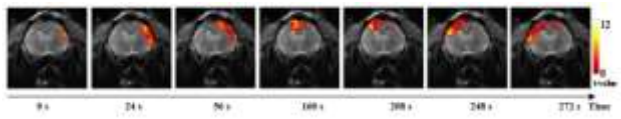
Abstract: Medetomidine is a highly specific a2-adrenoreceptor agonist that has recently been increasingly used as an anesthetic in experimental fMRI in rodents. Spreading depolarization (SD), a wave of neuronal depolarization moving across the cortex with a speed of 2-6 mm/min, is known to take place in number of neurological diseases. Here we report our findings of spontaneous SDs in medetomidine sedated Sprague-Dawley rats during prolonged (1h) BOLD fMRI imaging. Adult male Sprague-Dawley rats (n=9, 455 ± 65 g) were tracheotomized and cannulated prior to imaging for artificial ventilation, physiological monitoring and drugs administration. Bolus injection of medetomidine was given i.v. (Domitor, 0.05 mg/kg) followed by a continuous subcutaneous infusion (0.1 mg/kg/h) 5 min later. The fMRI data were acquired using 9.4T MRI scanner with a single-shot spin-echo echo planar imaging (SE-EPI) sequence (TR 4 s, TE 40 ms, 15x1.5 mm slices, in-plane resolution 0.4 mm) for 1 h 7 min (1000 images). We recorded total of 17 (2.1 ± 1.4 per animal, mean ± std) spontaneously occurring SDs. Site of the onset and propagation patterns were heterogeneous, varying even within one animal between repeated SDs. Most of the waves originated unilaterally from inferior cortical areas in medial and posterior parts of rat brain and subcortically. Mean duration of activation in a ROI was 193.3 ± 58.8 sec propagating in cortical regions with speed of 2.93 ± 0.6 mm/min. Observed features (spreading pattern and involvement of subcortical regions) were not typical for commonly reported spreading depression. There are reports of increased acoustic hyperexcitability in medetomidine sedated animals which can cause a SD upon strong auditory stimulation. Therefore acoustic stimulation during EPI fMRI imaging can be a triggering factor for spreading depression. Spontaneous depolarizations are clearly a potential confounding factor for fMRI studies in rats and origin of them should be studied in more detail. **Figure 1.** BOLD activation maps of one spreading depolarization in one representative case.



Disclosures: **A. Shatillo:** None. **J. Huttunen:** None. **A. Airaksinen:** None. **O. Grohn:** None. **J. Niskanen:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.05/B11

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant 1F31DA037652-01

Title: Fast scan cyclic voltammetric recording of dopamine release in the mouse ventral pallidum

Authors: *K. STOUT[1], G. W. MILLER[2], T. S. GUILLOT, III[2]
[1]Envrn. Hlth., [2]Emory Univ., Atlanta, GA

Abstract: The ventral pallidum (VP) is a region of emerging importance in addiction research that is thought to mediate the translation of limbic motivation into motor output. The VP receives dopaminergic inputs from the ventral tegmental area, as well as GABAergic inputs from the nucleus accumbens. In turn, the VP projects to the pedunculopontine nucleus of the brainstem. Disruption of this pathway via VP lesioning ablates the increased locomotor response to stimulant administration in rodents. Further, rodents self-administer both drugs of abuse and direct electrical stimulation in the VP. Dopamine release in the VP likely plays an important role in these behaviors, though it has not been characterized in the literature. Using fast scan cyclic voltammetry (FSCV), we measured dopamine release in the VP of mouse brain slices. Optimal stimulation parameters (60 pulse, 60 hz, 700 μA, 4 ms, monophasic +) yielded 1.67 μM dopamine release, on average. Electrode placement in the VP was confirmed structurally and pharmacologically. D2 receptor antagonism with eticlopride resulted in a 50.7% increase in stimulated dopamine release, likely due to inhibition of presynaptic autoreceptors. Additionally, dopamine transporter inhibition with nomifensine increased stimulated release by 52.1%, which is substantially lower than nomifensine-induced release potentiation in the dorsal and ventral striatum. This effect is due to low expression of the dopamine transport within the VP. Finally, as dopamine and norepinephrine produce similar voltammagrams, we applied A2A receptor antagonist, idozoxan, which enhances norepinephrine signal in regions rich in noradrenergic innervation. The drug did not significantly increase stimulated release, further suggesting dopamine as the primary electroactive neurotransmitter in the VP. Following FSCV experiments, the slices were lesioned and electrode placement further confirmed using the hydrogel lipid-clearing method, CLARITY. Dopamine transporter immunofluorescence in clarified slices confirmed that the lesioned recording site does not co-localize with the dopamine transporter, verifying that we were not recording in striatal regions. As drugs of abuse affect VP signaling, modulation of VP dopamine signaling by methamphetamine was also investigated. Measurement of dopamine release in this region will provide the basis for further investigation of proteins that may contribute to dopamine neurotransmission, such as the synaptic vesicle glycoprotein 2C, which is highly enriched in the VP. Funded by 1F31DA037652-01.

Disclosures: K. Stout: None. **G.W. Miller:** None. **T.S. Guillot:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.06/B12

Topic: B.03. G-Protein Linked Receptors

Support: DGAPA-PAPIIT Grant IN215813

Title: Neurotransmitter specificity in G-protein signaling cascades revealed by slowed activation of CaV2.2 channels

Authors: *D. E. GARCIA-DIAZ, L. DE LA CRUZ, I. ARENAS
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Abstract: Neurotransmitters and hormones such as noradrenaline, vasoactive intestinal polypeptide, somatostatin and gonadotropine releasing hormone have diverse modulating effects. One common means by which they affect cell function is via voltage-dependent inhibition of CaV2.2 channel. Neurotransmitter-induced calcium channel regulation is often by G-protein coupled receptors (GPCR) activation. However it is unknown which molecules in the GPCR signaling pathway determine a neurotransmitter's specificity in voltage-dependent regulation. As Gβ subunits directly mediate voltage-dependent inhibition of CaV2.2 channels, one possibility is that Gβ subunits mediate this specificity. Several neurotransmitters and hormones acting through GPCR elicit a voltage-dependent regulation of CaV2.2 channels, having profound effects on cell function and the organism. It has been hypothesized that protein-protein interactions define specificity in signal transduction. Yet it is unknown however how the molecular interactions in an intracellular signaling cascade determine the specificity of the voltage-dependent regulation induced by a specific neurotransmitter. Probably, specific effector regions on the Gβ subunits of the G-proteins may account to the voltage-dependent regulation. Therefore, the purpose of this study examines whether a neurotransmitter's specificity can be revealed by simple ion-current kinetic analysis. By using biochemical reagents and patch-clamp methods we analyzed kinetics of barium current activation in Gβ subunit-overexpressed superior cervical ganglion neurons. Therefore, we compared kinetic slowing and willing-reluctant population changes induced by agonist application in barium currents under prepulse facilitation. We found evidence that CaV2.2 channels are specifically modulated by every Gβ subunit isoform. Furthermore, changes in the speed of activation of the current and in the channel population interchange induced by neurotransmitters are mimicked by overexpression of specific Gβ isoforms. Also, that Gβ1-Gβ4

slows CaV2.2 activation kinetics while Gβ5 does not. These results contribute to understand the mechanism by which Gβ subunits specifically mediate neurotransmitter-induced CaV2.2 channel inhibition. In addition, they advance our understanding on the mechanisms by which signals conveying from a variety of membrane receptors are able to display precise homeostatic responses.

Disclosures: D.E. Garcia-Diaz: None. **I. Arenas:** None. **L. de la Cruz:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.07/B13

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Systemic 5-HT7 receptor activation by LP-211 increases LTM and cAMP production in the prefrontal cortex and attenuates scopolamine-induced impairments in rats

Authors: *A. MENESES
Cinvestav - IPN, Mexico, Mexico

Abstract: RATIONALE: The serotonin (5-hydroxytryptamine; 5-HT) 5-HT7 receptor is localized in brain areas mediating memory; however, the role of this receptor on memory remains little explored. OBJECTIVE: First, demonstrating the associative nature of P/I task, rats were exposed (3 sessions) to: CS-US (Pavlovian autoshaping), truly random control, free operant, presentations only of US only or CS and were compared to rats trained-tested 4 sessions to the P/I procedure. Systemic administration of 5-HT7 receptor agonist LP-211 on short- (STM; 1.5 h) and long-term memory (LTM; 24 and 48 h) or memory impairment (by scopolamine) as well as the production of cAMP were determined in other animals. METHODS: Autoshaping and its behavioral controls were studied. In other animals, an autoshaping training session and immediately afterwards, rats were given (IP) vehicle or LP-211 (0.1-10 mg/kg) and/or scopolamine (0.2 mg/kg) and were tested for STM and LTM; their brains were extracted for the cAMP ELISA immunoassay. RESULTS: P/I group produced the higher %CR. LP-211 did not affect STM; nonetheless, at 0.5 and 1.0 mg/kg it improved LTM. The 5-HT7 receptor antagonist SB-269970 (SB; 10.0 mg/kg) alone had not effect; nevertheless, the LP-211 (1.0 mg/kg) LTM facilitation was reversed by SB. The scopolamine (0.2 mg/kg) induced-decrement in CR was accompanied by significant increased cAMP production. Scopolamine-induced amnesia and increments in cAMP, were significantly but not completely reversed by LP-211.

CONCLUSIONS: autoshaping task is a reliable associative memory task and LP-211 is a useful tool in studying the effect of 5-HT7 receptor.

Disclosures: A. Meneses: None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.08/B14

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Characterization of the expression of the synaptic vesicle glycoprotein 2C (SV2C) in the basal ganglia

Authors: M. OZAWA, A. R. DUNN, K. A. STOUT, T. S. GUILLOT, M. WANG, Y. LI, *G. W. MILLER
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Abstract: Vesicular packaging of dopamine has two important roles in the neuron: 1) compartmentalization of dopamine to facilitate rapid neurotransmission, and 2) sequestration of dopamine and other toxicants from the cytosol. Our lab and others have shown that impairment of vesicular function in dopamine neurons is detrimental to cell health, leading to increased cytosolic dopamine and subsequent cell degeneration, as well as increased sensitivity to dopaminergic toxicants. We have also recently shown that enhanced dopamine vesicle function_that is, increased vesicular capacity for dopamine by the overexpression of the vesicular monoamine transporter 2 (VMAT2)_is protective. Identifying additional modulators of dopamine vesicle function is of utmost importance, as they may represent novel pharmaceutical targets for the treatment of a wide range of dopamine-related neurological disorders such as Parkinson's disease (PD), addiction, and depression. The synaptic vesicle glycoprotein 2C (SV2C) is a vesicular protein preferentially expressed in dopaminergic brain regions, such as the basal ganglia and mesolimbic reward pathway. SV2C genotype modulates not only the protective effect of smoking against PD, but also response to some atypical antipsychotics, which suggests an important role of SV2C in dopaminergic neurons. Furthermore, SV2C's close family member SV2A is the specific target for the antiepileptic compound levetiracetam, suggesting that SV2C may be pharmaceutically relevant. A molecular function of SV2C has not yet been identified, and a more thorough characterization of SV2C's expression patterns in dopaminergic and other neurotransmitter systems is crucial to enhancing our understanding of

the protein's role in vesicular function and potential importance as a pharmacological target. Here, we present immunohistochemical data detailing SV2C's expression in mouse and human basal ganglia. We characterize a novel SV2C antibody designed by our lab. SV2C colocalizes with a majority of dopaminergic cells of the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA), as well as some GABAergic areas including the fibers of the substantia nigra reticulata (SNr). Further, we performed coimmunoprecipitation experiments to characterize the association between SV2C and other vesicular proteins. Finally, we describe a novel line of SV2C-knockout mouse. The results from our experiments indicate that SV2C may play an important role in vesicular function in the basal ganglia and may represent a novel pharmaceutical target.

Disclosures: **M. Ozawa:** None. **A.R. Dunn:** None. **K.A. Stout:** None. **T.S. Guillot:** None. **M. Wang:** None. **Y. Li:** None. **G.W. Miller:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.09/B15

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Sara Page Mayo Endowment for Pediatric Pain Research

Title: Two major network domains within the dorsal raphe nucleus

Authors: *K. G. COMMONS
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Abstract: Serotonin neurons in the dorsal raphe nucleus (DR) are clustered into heterogeneous groups that give rise to topographically organized forebrain projections. However, a compelling definition of the key subgroups of serotonin neurons within these areas has remained elusive. In order to be functionally distinct, neurons must participate in distinct networks. Therefore we analyzed subregions of the DR and the median raphe (MR) by their afferent input. Clustering methods and principal component analysis were applied to anterograde tract-tracing experiments in mouse available on the Allen Brain Atlas. The results revealed a major break in the networks of the DR such that the caudal third of the DR was more similar in afferent innervation to the MR than it was to the rostral two thirds of the DR. The rostral part of the DR is associated with networks controlling motor and motivated behavior, while the caudal DR is closely aligned with

regions that regulate hippocampal theta rhythm. Thus a major source of heterogeneity within the DR is inclusion of the caudal component, which may be more accurately viewed as a dorsal extension of the MR.

Disclosures: K.G. Commons: None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.10/B16

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant 5T32ES012870-10

Title: Effects of genetic deletion of the synaptic vesicle glycoprotein 2C (SV2C)

Authors: *A. DUNN[1], K. A. STOUT[1], M. OZAWA[1], M. WANG[1], Y. LI[2], T. S. GUILLOT, III[1], G. W. MILLER[1,2]
[1]Envrn. Hlth., [2]Ctr. for Neurodegenerative Dis., Emory Univ., Atlanta, GA

Abstract: Vesicular storage of dopamine plays two important roles in neurons: to prepare the synapse for rapid neurotransmission, and to sequester dopamine and other toxicants from the cytosol. It is well-established that impaired vesicular function in dopamine neurons, such as that resulting from underexpression of vesicular monoamine transporter 2 (VMAT2), increases susceptibility to oxidative stress and toxicity. Our lab also recently demonstrated that enhanced vesicular function as the result of increased VMAT2 expression is neuroprotective. Identifying additional modulators of dopamine vesicle function is important, as they may represent novel pharmacological targets for the treatment of dopamine-related disorders such as Parkinson's disease (PD). The synaptic vesicle glycoprotein 2C (SV2C) is a vesicular protein enriched in dopaminergic areas, such as the basal ganglia. SV2C genotype modulates the protective effect of smoking against PD. SV2C's close family member SV2A is the specific target for the antiepileptic levetiracetam, suggesting that SV2C is pharmaceutically relevant. SV2C's function is unknown, although there is significant evidence that it plays a role in vesicular function. To characterize the role of SV2C in dopamine vesicles, we created SV2C-knockout mice (SV2C-KO) using the EUCOMM Knockout First construct. Here, we characterize the "SV2C-knockout first" (SV2C-KOF) line, which has disrupted transcription and expression of SV2C. Our results support previous findings that SV2C-KOF mice develop normally and have no gross behavioral

differences from wild type animals. Immunohistochemistry shows that markers of nigrostriatal dopamine pathways (tyrosine hydroxylase, dopamine transporter, and VMAT2) remain unchanged in the SV2C-KOF. SV2C-KO also does not result in upregulation of its family members, SV2A or 2B. However, preliminary data suggest that SV2C-KO alters vesicular dopamine handling. We observed a slight decrease (~20%; p=0.06) in vesicular capacity for dopamine, as well as altered dopamine release as measured by fast-scan cyclic voltammetry. Using the EUCOMM construct, we have developed a line of mice with a floxed SV2C gene; with this, we are currently generating conditional SV2C-KO animals with a CaMKII-driven cre-recombinase. Future experiments will more thoroughly explore the impact of this SV2C-KO on vesicular dopamine dynamics, as well as behavioral effects and susceptibility to dopaminergic toxicants. Given our preliminary results, we hypothesize that manipulation of SV2C modulates dopamine vesicle function. Supported by: 5T32ES012870-10

Disclosures: A. Dunn: None. **K.A. Stout:** None. **M. Ozawa:** None. **T.S. Guillot:** None. **M. Wang:** None. **Y. Li:** None. **G.W. Miller:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

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Program#/Poster#: 211.11/B17

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant MH045817

Title: Simultaneous assessment of cell type-specific activity through Ca2+ imaging in brain slices

Authors: *N. V. POVYSHEVA, J. W. JOHNSON
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Abstract: To assess excitation/inhibition balance in cortical brain slices, it is important to measure activity of excitatory and inhibitory neurons simultaneously in the same experimental conditions. Here we describe an approach that combines imaging of neurons bulk-loaded with cell-permeable Ca^{2+}-sensitive dye with imaging of specific types of inhibitory neurons, parvalbumin (PV)-positive and somatostatin (SST)-positive interneurons labeled with the green fluorescent protein (GFP). Experiments were performed on prefrontal cortical (PFC) slices from 3-7 month old transgenic mice that express GFP in PV-positive interneurons (CB6/Tg(Gad1-

EGFP)G42jh/J mice) and from the mice that express GFP in SST-positive interneurons (FVBTg(GadGFP) 45704Swn/J). Neurons in PFC slices were loaded with the cell-permeable Ca^{2+}-sensitive dye Fura-2 AM as previously described (MacLean & Yuste, 2009). Imaging was performed on a Leica TSC SP-5 multiphoton microscope. Fura-2 was visualized by laser excitation at a wavelength of 815 nm. GFP-labeled PV-positive or SST-positive interneurons were identified using an excitation wavelength of 900 nm. Pyramidal cells were identified by their triangular cell bodies and the initial regions of their apical dendrites. To increase activity of neurons in PFC slices, cells were depolarized by elevating the extracellular concentration of K^+ to 10 mM. The resulting increase in both synaptic excitation and action potential generation resulted in neuronal Ca^{2+} influx through Ca^{2+}-permeable ligand-gated channels (e.g., those associated with NMDA and Ca^{2+}-permeable AMPA receptors) and voltage-gated Ca^{2+} channels. We assessed neuronal activity by measuring the decrease in Fura-2 fluorescence intensity relative to resting fluorescence induced by Ca^{2+} influx. High K^+ produced a substantial decrease in fluorescence, indicating Ca^{2+} influx in pyramidal cells ($-\Delta F/F=31\pm8\%$, $n=10$, $p<0.001$) and in PV-positive interneurons ($21.6\pm10\%$, $n=11$, $p<0.001$) as well as in a SST-positive interneuron (20%). When the extracellular concentration of K^+ was returned to the control concentration (3.5 mM) we consistently observed that Fura-2 AM fluorescence intensity returned to near control values.

Disclosures: **N.V. Povysheva:** None. **J.W. Johnson:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.12/B18

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: RFBR 14-04-31707

RFBR 14-04-00227

Title: Homocysteine-induced integral currents, Ca2+ responses and changes in mitochondrial membrane potential in cortical and trigeminal ganglion neurons

Authors: P. A. ABUSHIK[1], D. A. SIBAROV[1], *S. M. ANTONOV[1], R. GINIATULLIN[2]
[1]Sechenov Inst. of Evolutionary Physiol. and Biochem., Saint-Petersburg, Russian Federation;

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Abstract: Homocysteine (HCY), a sulfur-containing aminoacid, exhibits neurotoxic effects and is involved in the pathogenesis of several major neurodegenerative disorders. In contrast to well studied excitoxicity of glutamate (Glu), the mechanism of HCY neurotoxicity is not clearly understood. To study the neurotoxic action of HCY within the nociceptive system, we analyzed membrane currents, Ca2+ responses and changes in mitochondrial membrane potential induced by this aminoacid in cultured rat cortical and trigeminal ganglion (TG) neurons. We found that in large portion of cortical and trigeminal neurons HCY induced inward currents that could be completely blocked by the selective antagonist of NMDA receptors - AP5 and partially inhibited by MTEP, a selective blocker of mGluR5 receptors. In contrast to cortical neurons, in the minority TG neurons HCY induced currents could be completely blocked only by MTEP but not by AP5 co-application suggesting the predominant expression of mGluR5 receptors in this neurons. In addition, the fraction of TG neurons responding to HCY was relatively small. Comparison of Ca2+ responses to HCY, Glu or NMDA demonstrated that in all cortical neurons and the majority of TG neurons HCY induced short oscillatory type Ca2+ responses. In contrast, NMDA or Glu induced sustained increase of intracellular Ca2+. Interestingly, part of TG neurons had Ca2+ responses with the single peak to HCY and did not respond to Glu or NMDA. Such heterogeneity of Ca2+ responses to HCY might be determined by a distinct pattern of expression of NMDA and mGluR5 receptors in the plasma membrane of TG and cortical neurons. Analysis of changes in mitochondrial membrane potential demonstrated that in contrast to NMDA or Glu, HCY did not induced drop of mitochondrial membrane potential during first minutes of action. Our data suggest that in cortical and TG neurons, HCY induces the neurotoxic action through the activation of NMDA and mGluR5 receptors without significant changes in the mitochondrial membrane potential.

Disclosures: P.A. Abushik: None. **S.M. Antonov:** None. **D.A. Sibarov:** None. **R. Giniatullin:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.13/B19

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR

Title: Axonal segregation and role of the vesicular glutamate transporter type 3 in neuronal survival of serotonin neurons

Authors: *A. N. VOISIN[1], N. GIGUÈRE[1], G. M. FORTIN[1], S. EL MESTIKAWY[2], L.-É. TRUDEAU[1]
[1]Departments of Pharmacol. and Neurosciences, GRSNC, Fac. of Med., Univ. De Montréal, MONTREAL, QC, Canada; [2]Douglas Mental Hlth. Univ. Institute, Univ. McGill, Montréal, QC, Canada

Abstract: A subset of serotonin (5-HT) neurons, as well as of dopamine (DA) neurons, has been shown to release glutamate as a cotransmitter due to specific expression of the vesicular glutamate transporter type 3 (VGLUT3) or type 2 (VGLUT2), respectively. It is now well-established that VGLUT3 enhances 5-HT vesicular loading in a common pool of vesicles through functional synergy with the vesicular monoamine transporter (VMAT2).A recent study also revealed a functional role of glutamate release by mesencephalic DA neurons in promoting their growth and survival. However, whether glutamate co-release play a similar developmental role in Raphe 5-HT neurons and whether glutamate is released from distinct releases sites from 5-HT neurons has not been examined. Using postnatal mouse Raphe cultures, in which 5-HT neurons develop highly arborized axonal processes, we first tested the hypothesis of partial axon terminal segregation. Using immunostaining and confocal microscopy, we found that in contrast with 5-HT, the 5-HT reuptake transporter (SERT) was present in a subset of axon terminals. Furthermore, these 5-HT axonal branches displayed postnatal upregulation. Whereas 25 % of terminals were SERT-positive at day 1 *in vitro*, 48% expressed SERT at day 7. Interestingly, only a subset of SERT- and 5-HT-positive axonal varicosities expressed VGLUT3, with SERT and VGLUT3 being mostly segregated in different axonal domains. Finally, using a VGLUT3 knockout mouse, we found that VGLUT3 deletion did not impair the axonal and dendritic growth of cultured 5-HT neurons but reduced their survival by approximately 15%. In conclusion, our results demonstrate that Raphe 5-HT neurons express SERT and VGLUT3 mainly in segregated axon terminals and that VGLUT3 regulates the vulnerability of these neurons.

Disclosures: A.N. Voisin: None. **N. Giguère:** None. **G.M. Fortin:** None. **L. Trudeau:** None. **S. El Mestikawy:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH grant NS 052818

Title: miRNAs 29b and 181a down-regulate expression of the norepinephrine transporter in PC12 cells

Authors: M. DENG, *G. A. ORDWAY, M.-Y. ZHU
Biomed. Sci., ETSU Quillen Col. of Med., JOHNSON CITY, TN

Abstract: miRNAs 29b and 181a down-regulate expression of the norepinephrine transporter in PC12 cells. M.X. Deng, G. A. Ordway and M.-Y. Zhu. Dept. of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA MicroRNAs are short non-coding RNAs that provide global regulation of gene expression at the post-transcriptional level. Such regulation has been found to play a role in stress-induced epigenetic responses in the brain. The noradrenaline transporter (NET) is a noradrenergic marker and regulates neurotransmitter signaling by rapidly clearing released norepinephrine from synapses. Our previous studies demonstrated that rat NET mRNA and protein levels are regulated by chronic stress and by administration of corticosterone. Whether miRNAs are intermediaries in the regulation of NET expression remains to be elucidated. The present study was undertaken to determine possible regulatory effects of miRNAs on NET expression in PC12 cells, a cell model for noradrenergic neurons. Using computational target prediction, we identified several miRNAs potentially related to regulation of NET expression. Mimics of these miRNAs were transfected into PC12 cells. NET protein expression was assayed by Western blotting 48 hours after transfection. miR29b- and miR181a-transfected cells showed significantly reduced NET protein levels. To identify the exact target loci, the 3'-UTR of NET mRNA was amplified by PCR from PC12 genomic DNA and cloned downstream of the red firefly gene of the pmirGlo vector. The NET 3'-UTR-bearing pmirGlo and miR29b or miR181a were co-transfected into PC12 cells and luciferase signals were measured 48 hours after transfection. Consistent with Western blots, co-transfection of these miRNAs with rat NET3'-UTR-containing plasmids resulted in reduced levels of luciferase activity in PC12 cells. We conclude that miR29b and miR181a can function as negative regulators of NET translation *in vitro*. Further studies to determine whether these miRNAs contribute to the regulation of NET expression induced by antidepressants are under way. (Supported by NIH grant NS052818)

Disclosures: M. Deng: None. **G.A. Ordway:** None. **M. Zhu:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: DA024760 (to SAP)

DA015462-supplement (to MGB, SAP, Mark Greenwald)

Wayne State University Office of Vice President for Research and Department of Psychiatry and Behavioral Neurosciences

Wayne State University of Vice President for Research and Department of Psychiatry and Behavioral Neurosciences, M.D./ PhD Program

Title: Changes in brain norepinephrine and serotonin levels in an animal model of posttraumatic stress disorder

Authors: *D. E. POP[1], R. J. KOHLER[2], M. J. LISIESKI[2], M. BAUER[2], A. L. EAGLE[2], S. A. PERRINE[2], D. E. POP[2]
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Abstract: Posttraumatic Stress Disorder (PTSD) is a debilitating condition that is characterized by re-experiencing the traumatic event, avoidant behavior, hyper-arousal, and negative cognition and mood. Although extensive pre-clinically and clinically research has been done exploring PTSD, its neurobiology remains to be fully understood. The aim of this study was to examine the effects of single prolonged stress (SPS), an animal model of PTSD, on monoamine levels in the brain. Male Sprague-Dawley rats were exposed to SPS treatment, consisting of a consecutive series of stressors (2 h restraint, 20 min group forced swim, and ether exposure until unconsciousness) followed by a 7 d incubation period. Both SPS and control rats were decapitated after the incubation period and their brains dissected for analysis of monoamines (norepinephrine, NE; serotonin, 5-HT) using high pressure liquid chromatography (HPLC). Regions of interest involved in PTSD were examined: dorsal hippocampus (dHC), intermediate-ventral hippocampus (i-vHC), medial prefrontal cortex (mPFC), amygdala, and ventral tegmental area (VTA). Results showed significant increases in 5-HT within the dHC and i-vHC and NE within the i-vHC. A significant decrease in NE was seen within the VTA. There were no SPS-induced differences in the mPFC or amygdala. These data indicate that SPS alters NE and 5-HT levels in key brain regions involved in regulating emotional states in response to environmental stimuli.

Disclosures: **D.E. Pop:** None. **R.J. Kohler:** None. **M.J. Lisieski:** None. **M. Bauer:** None. **A.L. Eagle:** None. **S.A. Perrine:** None. **D.E. Pop:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.16/B22

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH/NIMH F31 MH099807-02

NIH/NIMH 1R01MH099114-01

Title: Kainate receptors mobilize endocannabinoids in striatal spiny projection neurons

Authors: *J. MARSHALL[1], J. XU[2], A. CONTRACTOR[2]
[1]Physiol., Northwestern Univ. - Dept. Of Physiol., Chicago, IL; [2]Dept. of Physiol., Northwestern Univ., Chicago, IL

Abstract: The striatum is the main input structure of the basal ganglia, integrating information from the thalamus and cortex to control planning and modulation of movement. The principal neurons of the striatum, spiny projection neurons (SPNs), express high levels of kainate receptors, members of the ionotropic glutamate receptor family, yet their functional roles within SPNs have not been fully characterized. Kainate receptors play diverse modulatory roles at synapses throughout the brain in regions where they are expressed. In addition to contributing to excitatory post-synaptic currents (EPSCs) they have been shown to presynaptically regulate neurotransmitter release and in some cases are metabotropically coupled to intracellular signaling pathways—activating downstream signaling independent of ionotropic currents. Here we show that kainate receptors are present at postsynaptic sites in SPNs where they are activated by endogenously released glutamate. We also show that activating kainate receptors with a low concentration of agonist, that produces little or no inward current, reduces glutamate release at excitatory synapses onto SNPs. In a subset of cells, this effect is blocked with a cannabinoid type 1 (CB1R) antagonist, indicating that activating kainate receptors leads to endocannabinoid (eCB) mobilization. Cannabinoid signaling itself has a well-established role in the striatum playing a crucial role in different forms of striatal plasticity. Furthermore, this effect of low-agonist activation of kainate receptors is sensitive to a number of downstream pharmacological manipulations that suggest kainate receptors mobilize eCBs through a metabotropic function—

signaling that is independent of ion flux through the channel pore. These experiments suggest a novel and significant role for kainate receptors in tuning striatal synapses and regulating striatal activity.

Disclosures: J. Marshall: None. **J. Xu:** None. **A. Contractor:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: FAPESP

CNPq

PRODOC

AFIP

Title: Relationship between Rearing and Na,K-ATPase

Authors: *R. ALVES[1,2], A. VASCONCELOS[1], C. SCAVONE[1], M. A. C. VENDITTI[3]
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Abstract: The Na^+/K^+-ATPase is of paramount importance for the proper functioning of the organism. The enzyme is involved in several aspects of brain function, such as the repolarization of the neuronal membranes and neurotransmitters uptake/release. Therefore, individual differences in the brain Na^+/K^+-ATPase activity may result in differences in the functioning of the brain, which, in consequence, could lead to behavioral divergences. We have showed a higher hippocampal activity of Na^+/K^+-ATPase in the subgroup of rats of higher rearings. The correlation between ouabain sensitivity and tissue specific expression of various isoforms of the Na^+/K^+-ATPase is impressive. The aim of this work was to verify if subgroups of rats selected according to the number of rearings differ in the isoforms of the Na^+/K^+-ATPase. Naive adult outbred male Wistar rats were used to select subgroups of rats showing low (LR) and high (HR) rearings number in the open field test. Twenty days after the open field session, the rats were sacrificed and the hippocampus was dissected. On the day of the assays, the pellets were

resuspended and the determination of the binding of the [^{3}H]Ouabain was assayed. The results obtained indicated statistically significant difference between LR and HR subgroups in the 1200 nM [^{3}H]Ouabain. This result suggests the involvement of α2 and α3 isoforms in the interindividual differences in rearing behavior. Our data suggest that the difference in the hippocampal Na^+/K^+-ATPase activity is innate and is involved in the expression of the rearing behavior.

Disclosures: R. Alves: None. **A. Vasconcelos:** None. **C. Scavone:** None. **M.A.C. Venditti:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

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Program#/Poster#: 211.18/B24

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIMH K08

NIMH R01

NIDA P50

NARSAD

Title: Glutathione: An antioxidant reservoir of synaptic glutamate

Authors: *T. W. SEDLAK[1], M. KOGA[1], A. KAMIYA[1], S. SNYDER[2], A. SAWA[1]
[2]Neurosci., [1]Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Glutamate is the most abundant excitatory neurotransmitter, utilized at 80-90% of cortical synapses and with 7-10 millimolar brain concentrations. It governs physiologic and pathologic processes ranging from learning and memory to stroke. The tripeptide, glutathione, is one third glutamate and present at 1-3 millimolar intracellular concentrations in brain, participating in antioxidant defense and drug detoxification. Multiple lines of evidence find abberant glutathione levels in neuropsychiatric disorders, including Alzheimer's Disease and Schizophrenia. Because of the substantial amounts of brain glutathione and its rapid turnover, we hypothesized that glutathione is a relevant reservoir of glutamate, and that this has not received systematic interrogation because the glutathione pathway was worked out over a decade prior to

the general acceptance that glutamate was a neurotransmitter. We find that drugs that inhibit liberation of glutamate from the glutathione cycle, acivicin and 2I4C, lead to decreases in cytosolic glutamate, decreased miniature excitatory post synaptic potential (mEPSC) frequency, and diminished depolarization-associated calcium fluxes in cortical neuron cultures. In contrast, pharmacologically decreasing the biosynthesis of glutathione with buthionine sulfoximine leads to increases in cytosolic glutamate, increased mEPSC frequency, and increased depolarization-associated calcium release. The glutathione pathway may complement the glutamate-glutamine shuttle for glutamate neurotransmitter, as restricting delivery of glutamine with MeAIB led to mEPSC decreases that could be rescued by increasing the liberation of glutamate from the glutathione cycle. We suggest that sulforaphane and pyroglutamate, which act on elements of the glutathione cycle, may have applications for human use to modulate oxidative stress and glutamatergic function.

Disclosures: **T.W. Sedlak:** None. **M. Koga:** None. **A. Kamiya:** None. **S. Snyder:** None. **A. Sawa:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH NIDA DA10044

Title: Striatin proteins within STRIPAK complex regulate the dephosphorylation of DARPP-32 as B subunits of PP2A

Authors: ***D. LI**, V. MUSANTE, A. C. NAIRN
Yale Univ., New Haven, CT

Abstract: Dopaminergic signaling is primarily mediated through phosphorylation and dephosphorylation events in downstream signaling targets. While previous studies have focused on the activity of protein kinases, emerging research has begun to investigate the increasing significance of protein phosphatases. In particular, the protein phosphatase PP2A is of interest due to its multiple substrates within dopaminergic pathways. One of these, DARPP-32, is a known mediator of psychostimulant action through regulation of multiple phosphorylation sites. Recent studies have identified a family of proteins, the striatins, as novel regulatory subunits of

PP2A; of particular interest is the fact that striatin is also known to assemble large signaling complexes termed STRIPAK, of which PP2A is a member. However, nothing is known of striatin or STRIPAK function within the striatum, despite known enrichment of these proteins within this brain region. Through the use of immunoprecipitation and affinity purification to interrogate the PP2A-striatin interactome, as well as through *in vitro* assays measuring the ability of striatin and STRIPAK proteins to dephosphorylate 32P-DARPP-32, we have accumulated evidence identifying DARPP-32 as a substrate of PP2A-striatin. More physiological studies within cell culture and primary cultures also indicate that this dephosphorylation activity functions in intact cells. Due to the role of DARPP-32 as a mediator of the action of drugs of abuse, this data implicates striatin and the STRIPAK complex as potential targets of these drugs. Future studies will expand into behavioral paradigms to examine the interactions of the psychostimulant, cocaine, with these proteins of interest.

Disclosures: D. Li: None. **V. Musante:** None. **A.C. Nairn:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.20/B26

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Resveratrol regulates binding activity of specific protein 1 (SP1) and nuclear respiratory factor 1 (NRF1) in an *in vivo* model of cerebral ischemia

Authors: *I. M. ALQUISIRAS BURGOS[1], A. MILLÁN VEGA[4], A. ORTIZ PLATA[2], M. I. SALAZAR[6], M. ESPINOZA ROJO[5], P. AGUILERA[3]
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Abstract: Cerebral ischemia results from reduction of cerebral blood flow in the brain due to a blockage or thrombosis of a major cerebral artery. This event leads to an immediate activation of death or cell survival pathways, depending on the extent of injury after occlusion. Moderate levels of reactive oxygen species (ROS) of mitochondrial origin trigger survival pathways. On the other hand, antioxidants [e.g. resveratrol (RSV)] might support neuronal survival through

ROS level regulation during cerebral ischemia. Importantly, ROS regulates specific protein 1 (SP1) and respiratory factor nuclear 1 (NRF1) which are all together responsible for transcription of genes involved in neuronal function as the N-methyl-D-aspartate (NMDA) receptor subunits and the cytochrome c oxidase (COX) subunits. In this study, we investigated the possible effect of RSV on binding activity of SP1 and NRF1. Methods. Male Wistar rats (250 to 350 g) were subjected to middle cerebral artery occlusion (MCAO) for 2 h and different times of reperfusion (0, 15, 30, 60, and 120 min). RSV was administered (1 mg/kg; i.v; diluted in 50% ethanol) before reperfusion. Nuclear protein was extracted from parietal cerebral cortex and binding activity was analyzed by chemiluminescent electrophoretic mobility shift assay (EMSA). Results. We found that maximal binding activity for Sp1 factor was observed after 15 min of reperfusion (173.01%). RSV returns Sp1 binding activity to basal level (109.95%). Maximal NRF1 binding activity was observed 15 min after reperfusion (240.05%); it was kept elevated until 30 min (234.07%) and returned to basal level after 60 min (116.59%). RSV decreased NRF1 binding activity to 160.46% after 15 min of reperfusion. Our findings indicate that resveratrol decreased Sp1 and NRF1 binding activity. Therefore, it is possible that a RSV modulates mRNA transcription of genes involved in neuronal survival after injury.

Disclosures: I.M. Alquisiras Burgos: None. **A. Millán Vega:** None. **A. Ortiz plata:** None. **M.I. Salazar:** None. **M. Espinoza Rojo:** None. **P. Aguilera:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.21/B27

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Academy of Finland

Title: The role of presenilin1 in neurogenesis in zebrafish, Danio rerio

Authors: *J. M. SUNDVIK, P. PANULA
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Abstract: Abstract Histaminergic neurons play a major role in an array of different behaviors executed by the vertebrate brain. So far, the sole regulator of the development of the histaminergic neurons was recently shown to be the Alzheimer's disease associated *presenilin1* (*psen1*) gene. There are two presenilin genes, presenilin1 and presenilin 2, which both function

as the catalytic subunit of γ-secretase. The γ-secretase has tens of different substrates of which Notch and amyloid precursor protein are best known. We have previously shown that the number of histamine neurons in *psen1-/-* fish is lower than in wild-type control animals during development and that the number of histamine neurons in the old *psen1-/- fish* is increased compared with control animals. We hypothesized that this could be due to either neurotransmsitter respecification or neurogenesis. To answer this, we have done immunohistochemical analysis of markers associated with neurogenesis. We studied sox2, pax6a and NeuroD expression in *psen1-/-* and control zebrafish larvae at three days post fertilization which did not reveal any difference in expression. Neither did we find a difference in neurogenesis of seven days post fertilization old zebrafish larvae as incorporation of 5-bromo-2'-deoxyuridine was not altered between the two different genotypes. Histaminergic neurons are located in the caudal hypothalamus, surrounding the posterior recess of the diencephalic ventricle. Immunohistochemical studies of sox2, pax6a, NeuroD, PCNA and ki67 in the old *psen1-/-* animals revealed an increase in the number of sox2 and pax6a immunoreactive neurons in the inferior lobe of the posterior hypothalamus when compared with control animals. No difference in numbers of neuronal precursor cells was observed in the histaminergic caudal hypothalamus. These findings implicate that the increase in histaminergic neuron number observed in adult *psen1-/-* is likely due to neurotransmitter respecification rather than neurogenesis within the histaminergic caudal hypothalamus.

Disclosures: J.M. Sundvik: None. **P. Panula:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

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Program#/Poster#: 211.22/B28

Topic: B.03. G-Protein Linked Receptors

Support: Grant-in-Aid for Exploratory Research(24659548)

Title: Nuclear factor-Y and specificity protein factors act together to regulate the human beta1-adrenergic receptor gene transcription in human neuroblastoma SH-SY5Y cells

Authors: Y. NAWA[1], R. KUWABARA[1], T. HIROI[1], R. TAKAHASHI[2], *H. MATSUI[3]
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Abstract: The beta1-adrenergic receptor (beta1-AR) mediates a variety of neural functions, including synaptic plasticity and memory formation. Regulation of the human beta1-AR gene is poorly understood. Here, we describe a detailed characterization of the human beta1-AR gene promoter in human neuroblastoma SH-SY5Y cells, which endogenously express the human beta1-AR. Site-directed mutagenesis analyses identified two cis-acting elements involved in the regulation of the gene: an inverted CCAAT box (ICB) (-369/ -365 relative to the ATG start codon) and a proximal non-canonical GC-box (-339/-330), which bind NF-Y and Sp factors (Sp1, Sp3 and Sp4), respectively. Electrophoretic mobility shift assays and chromatin immunoprecipitation assays indicated specific binding of NF-Y and Sp factors to the human beta1-AR promoter. Co-immunoprecipitation experiments revealed physical associations between NF-Y and Sp1, Sp3 or Sp4 in the intact cell. Treatment with a siRNA targeting NF-YA, a transactivation subunit of NF-Y, or mithramycin A, an inhibitor of Sp factors reduced the promoter activity. Whereas NF-Y was absolutely required for expression of human beta1-AR gene, overexpression of Sp1, Sp3 or Sp4 further enhanced NF-Y induced activation of the promoter. Taken together, these data demonstrate that ICB-bound NF-Y plays a pivotal role in regulating expression of the human beta1-AR gene via functional and possible physical interactions with Sp factors.

Disclosures: **Y. Nawa:** None. **H. Matsui:** None. **R. Kuwabara:** None. **T. Hiroi:** None. **R. Takahashi:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.23/B29

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Science Foundation Ireland ((SFI/12/RC/2273, 02/CE/B124 and 07/CE/B1368)

Health Research Board HRA_PHS/2011/32

Brain and Behavior Research Foundation 20771

Title: Rapid antidepressant response to ketamine in treatment-resistant depression is not dependent on normalising kynurenine pathway metabolism

Authors: *G. CLARKE[1], M. NAUGHTON[1], R. O'SHEA[2], J. DOWLING[3], A. WALSH[3], F. ISMAIL[1], G. SHORTEN[3], L. V. SCOTT[1], J. F. CRYAN[4], T. G. DINAN[5]
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Abstract: **Background:** Recently, the NMDA receptor antagonist ketamine has emerged as a fast acting antidepressant with therapeutic potential for treatment-resistant depression (TRD) cohorts but its clinical use is hampered by its psychotomimetic properties. Biological markers of the rapid antidepressant response associated with ketamine are urgently required to understand its mechanism of action. Increased peripheral kynurenine production from tryptophan has been considered as a potential biological marker of major depression and the kynurenine pathway has been suggested as a putative target for ketamine based on preclinical studies. However, the relationship between TRD, kynurenine concentrations and the response to ketamine treatment has not yet been evaluated in clinical populations. **Aim:** We hypothesised that ketamine treatment rapidly reverses abnormal kynurenine pathway metabolism and that this effect mediates the clinical improvement in TRD. **Methods:** 17 patients with treatment-resistant major depression were compared at baseline to 20 healthy control subjects. Subsequently, the TRD cohort received 3 ketamine infusions (0.5mg/Kg) spaced one week apart. Depressive symptoms were evaluated using the 17-item Hamilton Depression Rating Scale (HDRS). Tryptophan and kynurenine were measured in plasma using high-performance liquid chromatography (HPLC). **Results:** Ketamine significantly reduced HDRS scores with response rates ranging from 81% at 24h post infusion 1 to 67% 1 week post infusion 3. Kynurenine concentrations were significantly elevated in the TRD cohort pre-ketamine compared to HC (494 ± 26 vs 639 ± 61 ng/ml, $p < 0.05$). Ketamine treatment did not reduce the elevated kynurenine concentrations in the TRD cohort 2 hours, 24 hours or 1 week following the first ketamine infusion ($p > 0.05$). Moreover, there was no effect of repeated ketamine infusion on kynurenine concentrations at any time point evaluated. There was also no effect of ketamine infusion on plasma tryptophan concentrations ($p > 0.05$). **Conclusions:** To our knowledge, this is the first study to characterise the temporal relationship between the improvement in symptoms following ketamine treatment and tryptophan metabolism along the kynurenine pathway. Our results confirm the rapid efficacy of ketamine in TRD and suggest that this positive treatment outcome is not dependent on normalising kynurenine pathway abnormalities. Further studies are warranted to determine if these findings generalise to the other modalities used to treat TRD such as electroconvulsive therapy.

Disclosures: **G. Clarke:** Other; Science Foundation Ireland, Health Research Board, Brain and Behaviour Research Foundation. **M. Naughton:** Other; Science Foundation Ireland. **R. O'Shea:** None. **J. Dowling:** None. **A. Walsh:** None. **F. Ismail:** None. **G. Shorten:** None. **L.V. Scott:** None. **J.F. Cryan:** Other; Science Foundation Ireland, Health Reseach Board. **T.G. Dinan:** Other; Science Foundation Ireland, Health Research Board.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.24/B30

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH R01MH085666 to WLG

R37MH073853 to MGC

Title: Conditional knockout of gsk-3β in d1 versus d2 receptor-expressing neurons exhibits differential effects on pfc function

Authors: *Y. LI[1], B. XING[1], N. URS[2], M. CARON[2], W.-J. GAO[1]
[1]Drexel Univ. Col. of Med., PHILADELPHIA, PA; [2]Departments of Cell biology, Neurobiology, and Medicine, Duke Univ. Med. Ctr., Durham, NC

Abstract: Glycogen synthase kinase-3β (GSK-3β) is a key regulator of many cellular signaling pathways. In the dopamine (DA) system, GSK-3β is particularly involved in D2R-mediated signaling. Increasing evidence indicates that D1R and D2R are respectively restricted to a subpopulation of neurons in the striatum, hippocampus and PFC. However, how GSK-3β affects synaptic function in D1R- and D2R-expressing neurons in the PFC is unclear. A previous study reported that conditional knockout of GSK-3β in D1R- orD2R-expressing neurons (D1RGSK-3β -/- and D2RGSK-3β-/-) induced a differential antipsychotic response. Here we study whether genetic ablation of GSK-3β in D1R- or D2R-expressing neurons affects PFC cortical neuronal and synaptic activities. Using patch-clamp recording of layer V pyramidal neurons in the PFC, we found that neuronal excitability was increased in both D1RGSK-3β -/- and D2RGSK-3β-/- mice, with spike number increasing even more significantly in D1RGSK-3β -/- vs. D2RGSK-3β-/- mice. Furthermore, the amplitude but not frequency of both sEPSCs and mEPSCs was significantly increased in D1RGSK-3β -/- mice, suggesting a potential increased postsynaptic AMPAR function. Consistently, paired-pulse ratio (PPR) in AMPAR-EPSCs was unaffected; but the PPR in NMDAR-EPSCs appeared to be increased in D1RGSK-3β -/- mice. Interestingly, the frequency of sEPSC was increased whereas the amplitude of neither sEPSCs nor mEPSCs was changed in D2RGSK-3β -/- mice, indicating an increased presynaptic glutamate release. This change of sEPSCs was undetected in A2a (postsynaptic D2R) GSK-3β -/- mice. In addition, there were no changes in short-term plasticity mediated by either AMPAR-EPSCs or NMDAR-

EPSCs in D2RGSK-3β -/- mice. Together, these data suggest GSK-3β ablation exhibits a cell-type-specific action on neuronal and synaptic function in PFC.

Disclosures: **Y. Li:** None. **B. Xing:** None. **M. Caron:** None. **W. Gao:** None. **N. Urs:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.25/B31

Topic: C.05. Aging

Support: American Federation for Aging Research

NIH Grant AG029592

Title: Prefrontal cholinergic overload and attentional capacities in aging

Authors: ***B. YEGLA**, A. KELBAUGH, A. MOOKHTIAR, V. PARIKH
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Abstract: The cognitive reserve hypothesis of aging posits that brain activity attempts to cope with functional age-related changes. Individuals with lower cognitive reserve are considered more susceptible to cognitive decline and age-related pathologies. However, what neuronal mechanisms underlie cognitive reserve, and how these mechanisms provide compensation for age-related decline in attentional capacities remains unknown. The basal forebrain cortical cholinergic input system is a critical component of the brain's attentional system. Healthy older adults show attentional load-dependent posterior-anterior shift in aging (PASA) characterized by higher activation of the prefrontal regions. However, it is not known whether prefrontal cortex (PFC)-driven cholinergic mechanisms compensate for age-related decline in attentional capacities. Here, we investigated the impact of partial cholinergic deafferentation of the PFC on attentional capacities in young and aged rats. The impact of cholinergic depletion on neuronal activation in the PFC and posterior parietal cortex (PPC) was also investigated using a semi-quantitiative c-fos immunohistochemistry procedure. Young and aged rats were trained in an operant sustained attention task (SAT) that required the animals to distinguish between signal and non-signal events to attain a reward. After attaining criterion (70% correct responses on both trial types), animals either received bilateral infusions of 192-IgG saporin or sterile saline into the PFC and the performance was monitored for 4 weeks. Aged rats required more training

sessions to acquire criterion than young rats. However, post-criterion performance prior to lesion surgeries remained similar between the two age groups. Saline-infused aged rats show a greater number of c-fos expressing cells in the PFC but not PPC as compared to the young animals. Restricted loss of prefrontal cholinergic inputs produced attentional impairments in aged rats (SAT scores: 0.43±0.08 vs. 0.63±0.05 in young lesioned rats). Moreover, lesioned aged rats show reduced c-fos positive counts in the PFC as compared to aged intact rats. Collectively, these data suggest that PASA shifts and prefrontal overload foster top-down processes to maintain attentional capacities in aging. Moreover, these compensatory processes are triggered by prefrontal cholinergic inputs.

Disclosures: B. Yegla: None. **A. Kelbaugh:** None. **A. Mookhtiar:** None. **V. Parikh:** None.

Poster

211. Neurotransmitter Signaling in Health and Disease

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 211.26/B32

Topic: C.05. Aging

Support: NIH -1 F31 NS081930-01A

NIH - RO1 GM62328

Title: pH sensitive soluble adenylyl cyclase (sAC) regulates lysosomal pH

Authors: *N. RAHMAN[1], F. R. MAXFIELD[2], T. A. MILNER[3], J. BUCK[4], L. R. LEVIN[4]
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Abstract: In age-related neurodegeneration, damaged proteins and organelles accumulate within neurons. Normally, these proteins and organelles would be degraded in the lysosomes by cathepsins, which are optimally active at acidic pH, but with aging, age-related diseases and lysosomal storage disorders, elevation of lysosomal pH hinders normal degradation and impairs autophagy leading to neuronal dysfunction. The molecular processes and signaling pathways that regulate lysosomal pH are not understood. While it has been shown that addition of exogenous cAMP can lower lysosomal pH, the relationship between cAMP and pH is unclear. Lysosomal pH is dependent upon the V-ATPase, and bicarbonate-regulated, soluble adenylyl cyclase (sAC) is essential for pH-dependent V-ATPase mobilization in both epididymis and kidney. We now

show sAC to be essential for lysosomal acidification. sAC null fibroblasts and primary neurons have several lysosomal alteration, including impaired lysosomal acidification, diminished cathepsin activity, reduced degradation of substrate and reduced lysosome-mediated autophagosome clearance during autophagy. The lysosomal acidification defect in sAC null cells is rescued by exogenous cAMP. Thus, sAC appears to be an essential source of cAMP which regulates lysosomal pH. These results suggest sAC serves as a previously unappreciated sensor of lysosomal pH, and define it as a possible target for designing novel treatment strategies for neurodegenerative disorders.

Disclosures: **N. Rahman:** None. **F.R. Maxfield:** None. **T.A. Milner:** None. **J. Buck:** None. **L.R. Levin:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.01/B33

Topic: B.04. Ion Channels

Support: Ataxia UK grant

Wellcome Trust Senior Investigator award 098360/Z/12/Z

Title: Development of an assay for inhibitors of the deleterious interaction between wild type and Episodic ataxia-2 mutant CaV2.1 channels

Authors: *S. DAHIMENE, K. M. PAGE, J. S. CASSIDY, A. C. DOLPHIN
NPP, UCL, London, United Kingdom

Abstract: Episodic ataxia-2 is an autosomal dominant disorder caused by mutations of the *CACNA1A* gene that encodes for the pore forming calcium channel subunit $Ca_V2.1$. This channel is preferentially expressed in the presynaptic terminals where it plays a central role in neurotransmitter release. The majority of episodic ataxia-2 mutations reported so far are nonsense or deletion/insertion mutations predicted to form truncated proteins. Heterologous expression of wild-type $Ca_V2.1$ channel together with the truncated constructs that mimic episodic ataxia-2 mutants significantly suppressed the wild-type channel function, indicating that the truncated protein produces a dominant- negative effect (Jouvenceau et al.,2001; Page et al.,2004). A similar finding has been shown for $Ca_V2.2$ (Raghib et al.,2001). In addition, the

suppression effect required interaction between the full-length and the mutant construct. We aimed to develop a cellular assay in order to disrupt the destructive interaction between wild-type and episodic ataxia-2 mutant $Ca_V2.1$ channels and therefore restore partially or totally the trafficking and function of the wild-type $Ca_V2.1$. Using a cell imaging assay, we have confirmed that the truncated $Ca_V2.2$ protein (Dom I-II) prevents full-length $Ca_V2.2$ from reaching the plasma membrane. We have also confirmed that the N-terminus residues for both Cav2.1 and Cav2.2 channels which are highly conserved among Ca_V2 family are involved in this process. Additionally, it was possible to prevent the suppressive effect of the truncated protein by coexpressing constructs that mimic the key N-terminal residues. By doing so we were able to restore partially the function of the full-length channel. We postulate that the N-terminus is essential for the truncated proteins to interact with the full-length channel and as a result prevent the correct folding of the wild-type channel. Therefore, coexpressing the key N-terminal residues, would prevent the deleterious interaction. Page KM, Heblich F, Davies A, Butcher AJ, Leroy J, Bertaso F, Pratt WS, Dolphin AC (2004) Dominant-negative calcium channel suppression by truncated constructs involves a kinase implicated in the unfolded protein response. J Neurosci 24: 5400-5409. Jouvenceau A, Eunson LH, Spauschus A, Ramesh V, Zuberi SM, Kullmann DM, Hanna MG (2001) Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. Lancet 358: 801-807. Raghib A, Bertaso F, Davies A, Page KM, Meir A, Bogdanov Y, Dolphin AC (2001) Dominant-negative synthesis suppression of voltage-gated calcium channel $Ca_v2.2$ induced by truncated constructs. J Neurosci 21: 8495-8504.

Disclosures: **S. Dahimene:** None. **K.M. Page:** None. **J.S. Cassidy:** None. **A.C. Dolphin:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.02/B34

Topic: B.04. Ion Channels

Support: PAPIIT UNAM IN205914 to AS

Conacyt 128707-Q to RF

Title: Regulation of Cav2.2 channel functional expression by the light chain 1 (LC1) of the microtubule associated protein B (MAP1B)

Authors: M. A. GANDINI[1], *A. SANDOVAL[2], R. FELIX[1]
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Abstract: We reported recently a new mechanism by which the neuronal N-type Ca2+ (CaV2.2) channel expression may be regulated by ubiquitination. This regulation is mediated by the interaction between the channel and the light chain (LC1) of the microtubule associated protein B (MAP1B) and involves a binding sequence in the N-terminal of LC1 and binding domains within the C terminus of the pore-forming CaV2.2α1 subunit. We also showed that the LC1-mediated CaV2.2α1 down-regulation could be prevented by inhibiting the ubiquitin-proteasome proteolytic pathway and that LC1 could interact with the ubiquitin-conjugating E2 enzyme Ube2L3, playing a role as an ubiquitin-protein E3 ligase or as an adaptor protein for CaV2.2 channel ubiquitination. Here we report that i) LC1 can interact with the two main variants of the CaV2.2 channels (CaV2.2e37a and CaV2.2e37b), ii) the LC1-mediated regulation most likely involves an internalization of the CaV2.2 channels via a dynamin and clathrin-dependent pathway, and iii) the ubiquitination/degradation mechanism triggered by LC1 might be conserved among N-type and P/Q-type channels. Last, consistent with our previous functional analysis, siRNA-mediated LC1 and Ube2L3 silencing significantly increased the amplitude of whole-cell N-type Ca2+ currents expressed in dorsal root ganglion neurons. Therefore, the work reported here provides additional evidence that LC1 reduces N-type Ca2+ channel surface expression.

Disclosures: M.A. Gandini: None. **R. Felix:** None. **A. Sandoval:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.03/B35

Topic: B.04. Ion Channels

Support: FWF grant # P25085 to V.D.B.

Title: Dynamics of CaV1.2 L-type voltage-gated calcium channels and β adrenergic receptors signaling complexes in cultured hippocampal neurons

Authors: *V. DI BIASE[1], A. STEINBERGER[1], M. HEINE[2], C. RAMPRECHT[1]
[1]Med. Univ. of Graz, Graz, Austria; [2]Leibniz Inst. of Neurobio., Magdeburg, Germany

Abstract: In neurons calcium influx via CaV1.2 L-type voltage gated calcium channels (L-VGCCs) controls excitation-transcription coupling, homeostatic plasticity, learning and memory. Therefore, the modulation of the L-VGCCs activity is instrumental to tuning of neuronal functions. Adrenergic stimulation increases the amplitude of CaV1.2 mediated calcium currents. A plausible underlying mechanism implies the release of the inhibitory interaction between the proximal and the distal CaV1.2 C-terminus induced by the adrenergic dependent phosphorylation of the channel. Here, we analyze the distribution of the signaling complexes including CaV1.2 and β adrenergic receptors (ARs) and the effect of adrenergic stimulation on their dynamics at the membrane of cultured hippocampal neurons. Anti-HA live surface staining in neurons transfected with an extracellularly HA-tagged CaV1.2 channel (CaV1.2-HA) shows the known distribution of CaV1.2s in discrete clusters along the dendrites. Similar experiments on FLAG tagged β1 and β2ARs transfected neurons reveal the grainy/homeogeneus pattern of β1ARs and the clustered localization of β2ARs throughout the membrane. Double anti-HA and -FLAG immunolabeling in neurons overexpressing CaV1.2-HA and FLAG-β1ARs or FLAG-β2ARs shows that CaV1.2 and β1ARs randomly overlap whereas CaV1.2 and β2ARs often specifically co-localize on the spines but not along the shaft. To analyze the lateral diffusion of CaV1.2s and β2ARs at the membrane we performed fluorescence recovery after photobleaching (FRAP) analysis on neurons expressing the fluorescently tagged CaV1.2 and β2ARs - eGFP/SEP-CaV1.2 and SEP-β2ARs, respectively. SEP-β2ARs display a FRAP curve significantly higher and faster than eGFP/SEP-CaV1.2 channels, indicating that the mobility is higher for β2ARs than for CaV1.2s. These results are corroborated by single particle tracking data in which the diffusion coefficients of CaV1.2 and βARs differ of at least one order of magnitude. Immunolabeling analysis on CaV1.2-HA transfected neurons suggest that the adrenergic agonist isoproterenol tends to increase the fluorescence intensity of membrane inserted CaV1.2-HA clusters. Finally, we introduced two point mutations at the CaV1.2-HA C-terminal rendering the channel constitutively unable to establish the C-terminal inhibitory interaction (CaV1.2-HA-RRQQ). The CaV1.2-HA-RRQQ mutant displayed significantly higher membrane expression levels than the control CaV1.2-HA. Our data suggest that the signaling complexes formed by CaV1.2 and βARs are restricted to specific subcellular compartments and that their dynamics at the membrane may be subject to activity.

Disclosures: **V. Di Biase:** None. **C. Ramprecht:** None. **A. Steinberger:** None. **M. Heine:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.04/B36

Topic: B.04. Ion Channels

Support: NIH Grant R01 NS22625

Title: Modulation of CaV2.1 channels by neuronal calcium sensor-1 induces short-term synaptic facilitation

Authors: *J. YAN[1], K. LEAL[1], V. G. MAGUPALLI[2], E. NANOU[1], G. Q. MARTINEZ[1], T. SCHEUER[1], W. A. CATTERALL[1]
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Abstract: Modulation of P/Q-type Ca2+ currents mediated by Ca2+/calmodulin binding to CaV2.1 channels contributes to short-term synaptic plasticity. Calcium sensor proteins displace calmodulin from its binding site and differentially modulate P/Q-type Ca2+ currents, resulting in diverse patterns of facilitation and depression of synaptic transmission. Neuronal calcium sensor-1 (NCS-1, frequenin) has been shown to modulate synaptic facilitation. However the underlying mechanism is not clear. In tsA201 cells transfected with NCS-1 and CaV2.1, NCS-1 reduces Ca2+-dependent inactivation of P/Q-type Ca2+ current through interaction with the IQ-like motif and calmodulin-binding domain in the C-terminal domain of CaV2.1 channels without affecting peak current or activation kinetics. This regulation by NCS-1 requires binding to the IQ-like domain and CBD domain of CaV2.1 channels. We also show that activity-dependent modulation of CaV2.1 current by NCS-1 significantly affects short-term plasticity in synapses between cultured superior cervical ganglion neurons. Co-expression of NCS-1 induces facilitation of synaptic transmission in response to paired pulses and trains of depolarizing stimuli, and this effect is lost in CaV2.1 channels with mutations in the IQ-like motif and calmodulin-binding domain. These results demonstrate that NCS-1 inhibits inactivation of CaV2.1 and enhances facilitation of synaptic transmission through direct interaction with CaV2.1 channels and further support the hypothesis that CaS proteins are crucial in fine-tuning short-term synaptic plasticity. Supported by NIH Research Grant R01 NS22625.

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Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Program#/Poster#: 212.05/B37

Topic: B.04. Ion Channels

Support: MRC grant MR/K021303/1

MRC grant G1002183

Title: Substance P inhibits T-type Ca2+ channels in sensory neurons via redox-mediated mechanism

Authors: D. HUANG[1], J. SCRAGG[2], C. PEERS[2], H. ZHANG[1], *N. GAMPER[3,1]
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Abstract: T-type voltage-gated calcium channels (T-type VGCCs; Cav3.1, Cav3.2, Cav3.3) are distinct from other VGCCs since they activate at voltages below -60 mV, have faster kinetics and smaller single channel conductances. The negative activation voltage of T-type VGCCs allows them to control neuronal firing threshold and pattern. T-type calcium channels are expressed in the somata, axons and nerve endings of peripheral somatosensory neurons (including pain-sensing neurons, the nociceptors), suggesting an important role in nociception. However, the mechanisms of T-type channel regulation are still poorly understood. We investigated the regulation of T-type VGCCs by the inflammatory mediator substance P (SP). Acute application of SP (1 μM) inhibited Cav3.2 currents in HEK293 cells co-transfected with NK1 receptor and native T-type Ca2+ currents in cultured small-diameter dorsal root ganglion (DRG) neurons by 30.2±6.8% (n=11, P<0.05) and by 25.9±5.3% (n=11, P<0.05) respectively. Inhibition was not reversible upon washout but was completely reversed by the application of reducing agent dithiothreitol (DTT; 1 mM) suggesting that the effect of SP on T-type channels involves oxidative process. Pre-application of DTT abolished the SP effect. Application of the mitochondrial inhibitor Antimycin A (1 μM), which was shown to induce superoxide anion release from mitochondria, mimicked SP-mediated inhibition of T-type calcium currents in DRG neurons, an effect that was reversed by DTT. Application of SP after Antimycin A did not produce significant further current inhibition. H2O2 (300 μM) also inhibited Cav3.2 currents in HEK293 cells and native T-type Ca2+ currents in cultured DRG neurons by 9.4%±1.7 (n=5, P<0.05) and by 9.3%±2.9 (n=7, P<0.05) respectively. Redox modulation of Cav3.2 is mediated by alteration of its interaction with inhibitory zinc ions; accordingly, mutation of zinc binding histidine (H191Q) in Cav3.2 significantly attenuated the SP-induced inhibition of this channel. Cav3.1 lacks histidine at a position homologous to H191 in Cav3.2 and was insensitive to SP in the expression system. Introduction of homologous histidine (Q172H) bestowed Cav3.1 with sensitivity to SP. Pretreatment of DRG with pertussis toxin completely abolished the effect of SP suggesting that the action of SP in DRG is mediated via Gi/o. Our results identify novel molecular mechanism of T-type Ca2+ channels inhibition by SP which is likely to contribute to

the autocrine/paracrine, anti-excitatory action of this neuropeptide in peripheral nociceptive fibers.

Disclosures: **D. Huang:** None. **N. Gamper:** None. **H. Zhang:** None. **C. Peers:** None. **J. Scragg:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.06/B38

Topic: B.04. Ion Channels

Support: NSF Grant 1121606

Title: A population density and moment-based approach to modeling domain calcium-mediated inactivation of L-type calcium channels

Authors: ***X. WANG**, K. HARDCASTLE, S. H. WEINBERG, G. D. SMITH
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Abstract: We present a population density and moment-based description of stochastic domain calcium-mediated inactivation of L-type calcium channels. Our approach accounts for the effect of heterogeneity of local calcium signals on whole cell calcium currents; however, in contrast with prior work by Sherman et al. [Biophys J. 58(4):985, 1990], we do not assume that calcium domain formation and collapse are fast compared to channel gating. We demonstrate the population density and moment-based modeling approach using a12-state Markov chain model of an L-type calcium channel [Greenstein and Winslow, Biophys J. 83(6):2918, 2002]. Simulated whole cell voltage clamp responses yield an inactivation function for the whole cell calcium current that agrees or disagrees with the classic result of Sherman et al. when domains dynamics are fast or slow, respectively. We analyze the voltage-dependence of calcium inactivation that occurs via slow heterogeneous domains and find that when channel permeability is held constant, calcium inactivation increases as the domain time constant increases. However, when this parameter study is repeated for fixed maximum domain calcium concentration, inactivation decreases as the domain time constant increases. Comparison of simulation results using population densities and moment equations confirms the computational efficiency of the moment-based approach, and enables the validation of several distinct methods of truncating and closing the open system of moment equations. In general, a slow domain time constant requires

higher order moment truncation for agreement between moment-based and population density simulations.

Disclosures: **X. Wang:** None. **K. Hardcastle:** None. **S.H. Weinberg:** None. **G.D. Smith:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.07/B39

Topic: B.04. Ion Channels

Support: CIHR

Title: T-type calcium channels form a calcium-dependent complex with calmodulin

Authors: *H. ASMARA, N. C. HEATH, B. SIMMS, T. BARTOLETTI, R. REHAK, I. MICU, F. X. ZHANG, P. STYS, G. W. ZAMPONI, R. W. TURNER
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Abstract: Calcium entry in neurons can activate a cascade of calcium-dependent second messenger systems that involve specific calcium sensors. We know that calmodulin (CaM) forms an association with high voltage-activated calcium channels that is modulated by calcium entry. CaM binds to L-type (Cav1.2) channels at multiple sites including pre-IQ and IQ motifs in the distal C-terminus, and this interaction is important for calcium-dependent facilitation (CDF) or inactivation (CDI) of Cav1.2 current. Although CaM interactions are thought to occur only with HVA channels, we noticed that the Cav3.1 channel isoform also contains a sequence in the C-terminal region that is homologous to the IQ motif of Cav1.2 channels. Indeed, Cav3.1 and Cav3.3 (but not Cav3.2) coimmunoprecipitate (co-IP) with CaM from lysates of either rat brain or tsA-201 cells expressing hCav3.1 and hCaM cDNA. Moreover, the co-IP from tsA-201 cell lysate was calcium-dependent in that the association was present in 0 calcium medium or in 100 nM resting [Ca] for both CaM or the calcium insensitive CaM1234 mutant, but exhibited a calcium-dependent loss of co-IP in 50 μM or greater [Ca]. Protein pull-down assays between CaM-beads revealed an association with Cav3.1 protein from lysates of tsA-201 cells that was eliminated upon deletion of the C-terminus of the channel. Imaging tsA-201 cells coexpressing cDNA for CaM-mKate and Cav3.1-GFP revealed FRET between the acceptor and donor pair upon excitation at 457 nm under resting conditions. FRET was reduced upon perfusing 0.05 mM

ionomycin to raise intracellular calcium, but not in 0.1 mM BAPTA-AM. FRET was strongly reduced upon depolarization mediated activation of Cav3.1 channels. The depolarization-induced loss of FRET was blocked in the presence of 1 μM mibefradil and 300 μM Ni2+, indicating that calcium influx via Cav3 calcium channels is sufficient to block the Cav3-CaM interaction. These data are important in revealing a constitutive association between the Cav3.1 channel isoform and CaM that is lost upon raising internal calcium, thus providing for an activity-dependent release of CaM from the channel complex.

Disclosures: **H. Asmara:** None. **N.C. Heath:** None. **B. Simms:** None. **T. Bartoletti:** None. **R. Rehak:** None. **I. Micu:** None. **F.X. Zhang:** None. **P. Stys:** None. **G.W. Zamponi:** None. **R.W. Turner:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Program#/Poster#: 212.08/B40

Topic: B.04. Ion Channels

Support: Telethon Italy Grant GGP06234

MIUR PRIN07,10

UNIPD Strategic Project08,INVS

Title: Differential effect of familial hemiplegic migraine mutations on excitatory and inhibitory synaptic transmission between cortical pyramidal cells and somatostatin-expressing interneurons

Authors: *N. PILATI[1], A. FORLI[1], M. SESSOLO[1], D. PIETROBON[1,2]
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Abstract: Familial hemiplegic migraine type 1 (FHM1) is a subtype of migraine with aura caused by gain-of-function mutations in CaV2.1 (P/Q-type) Ca channels. Knockin (KI) mice carrying FHM1 mutations show increased neuronal P/Q-type Ca current and facilitation of induction and propagation of cortical spreading depression (CSD), the phenomenon that underlies migraine aura and may activate the migraine headache mechanisms. We previously reported gain-of-function of excitatory synaptic transmission at cortical pyramidal cell (Pyr) synapses but unaltered inhibitory synaptic transmission at cortical fast-spiking interneuron synapses in FHM1 R192Q KI mice, suggesting that the balance between excitation (E) and

inhibition (I) could be altered in FHM1 (Tottene et al, 2009, Neuron 61:762). Another polysynaptic inhibitory subcircuit important for dynamic regulation of the cortical E-I balance involves somatostatin (SOM+) interneurons. To study the effect of the FHM1 mutation on synaptic transmission and short-term plasticity at the excitatory and reciprocal inhibitory synapses between layer 2/3 Pyrs and SOM+ interneurons we created a new transgenic mouse line by crossbreeding R192Q KI with GIN mice expressing GFP in specific SOM+ interneurons. Dual patch-clamp recordings in slices of somatosensory cortex revealed that i) P/Q-type Ca channels have a dominant role in controlling neurotransmitter release at both the Pyr-SOM+ interneuron excitatory synapse and the SOM+ interneuron-Pyr inhibitory synapse; ii) the FHM1 mutation enhances the strength of excitatory synaptic transmission and the probability of glutamate release at Pyr->SOM+ synapses, without affecting short-term facilitation during brief action potential trains at 25 Hz; iii) by contrast, the FHM1 mutation does not affect inhibitory synaptic transmission at SOM+ ->Pyr synapses. These findings further support the hypothesis that the dynamic regulation of the cortical E/I balance may be altered in FHM1.

Disclosures: **N. Pilati:** None. **A. Forli:** None. **M. Sessolo:** None. **D. Pietrobon:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Program#/Poster#: 212.09/B41

Topic: B.04. Ion Channels

Support: R01NS084190

R01DC009433

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Carver Foundation Research Program of Excellence

Title: A non-canonical role for Cav1.4 Ca2+ channels in photoreceptor synaptogenesis

Authors: ***V. KEROV**[1], J. LAIRD[1], B. WILLIAMS[1], F. HAESELEER[2], S. BAKER[1], A. LEE[1]
[1]Univ. of Iowa, Iowa City, IA; [2]Univ. of Washington, Seattle, WA

Abstract: Voltage-gated $Ca_v1.4$ Ca^{2+} channels are localized in the synaptic terminals of retinal photoreceptors (PRs) and mediate Ca^{2+} influx that triggers synaptic vesicle release. Mutations in

the genes encoding $Ca_v1.4$ or the $Ca_v1.4$-regulatory protein, CaBP4, lead to visual disorders including congenital stationary night blindness type 2 (CSNB2), which has largely been attributed to loss- or gain- of function in $Ca_v1.4$ Ca^{2+} signaling. However, PR synapses do not form in mice lacking $Ca_v1.4$ ($Ca_v1.4$ KO), indicating an essential developmental role for $Ca_v1.4$ that may be affected in CSNB2. Here, we probed the requirement for $Ca_v1.4$ in PR synaptogenesis by examining the consequences of heterologous expression of recombinant $Ca_v1.4$ channels in $Ca_v1.4$ KO mouse retina. Remarkably, expression of either a wild-type or non-conducting mutant channel rescued mature PR synapses in $Ca_v1.4$ KO retina. This effect of Cav1.4 depended on its interaction with CaBP4: expression of a $Ca_v1.4$ mutant incapable of binding CaBP4 did not result in synapse rescue, whereas expression of a $Ca_v1.4$ channel tethered to CaBP4 restored PR terminals in $Ca_v1.4$ KO retina. Our results support a novel, non-conducting role for $Ca_v1.4$ channels in organizing PR synapse assembly that relies on association with CaBP4, and suggest that abnormal PR synapse development may contribute to vision impairment in some cases of CSNB2.

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Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: NIH Grant AR059397

Title: The Ca2+ channel:mu opioid receptor signaling pathway in rat sensory neurons is altered following chronic femoral artery occlusion

Authors: M. FARRAG[1], J. KIM[2], B. HASSAN[1], M. P. KAUFMAN[2], K. SEDEEK[1], *V. RUIZ-VELASCO[1]
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Abstract: The exercise pressor reflex (EPR), a crucial component of the cardiovascular response under physiological and pathophysiological states, is activated via metabolic and mechanical mediators that originate from contracting muscles and stimulate group III and IV afferents. We previously reported that stimulation of mu opioid receptors (MOR), expressed in both afferents,

led to an attenuation of the pressor reflex in rats whose femoral arteries had been occluded for 72 hr. The present study examined the effect of arterial occlusion on the signaling components involved in the opioid-mediated modulation of Ca2+ channel currents in rat dorsal root ganglion (DRG) neurons (L4-L6) innervating the triceps surae muscle. Moreover, we focused on neurons that were transiently transfected with cDNA coding for EGFP whose expression is driven by the putative NaV 1.8 promoter region, a channel primarily expressed in nociceptive neurons. Employing the small interference RNA (siRNA) approach, our results show that the pertussis toxin (PTX)-sensitive Galphai3 subunit couples MOR and Ca2+ channels. On the other hand, this pathway does not employ Galphai2 and GalphaO while Galphai1 subunits do not appear to be expressed. We also observed a significant ($P<0.05$) leftward shift of the MOR agonist, DAMGO, concentration-response relationship in neurons isolated from rats with occluded arteries compared to those that were freely perfused. The mean IC50 (nM) values for the freely perfused and ligated groups were 283 and 146, respectively. Western blotting analysis indicated that the leftward shift resulted from a moderate increase in MOR expression but not in Galphai3 levels. Additionally, the Ca2+ channel density in DRG neurons was not altered as a result of arterial occlusion. We found that all neurons from both groups exhibited an inward current following exposure to the TRPV1 agonist, capsaicin. Our findings suggest that sensory neurons mediating the EPR express NaV 1.8 and TRPV1 channels and exhibit MOR upregulation following a 72 hr occlusion of the femoral artery.

Disclosures: **M. Farrag:** None. **J. Kim:** None. **B. Hassan:** None. **M.P. Kaufman:** None. **K. Sedeek:** None. **V. Ruiz-Velasco:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Program#/Poster#: 212.11/B43

Topic: B.04. Ion Channels

Support: NIH Grant AR059397

NIH Grant DA025574

Title: Voltage-dependent inhibition of Ca2+ currents mediated by substance P-stimulated neurokinin-1 receptors in rat celiac-superior mesenteric ganglion neurons

Authors: *S. SUGINO, M. FARRAG, V. RUIZ-VELASCO
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Abstract: The celiac-superior mesenteric ganglia (CSMG) contain postganglionic sympathetic neurons that regulate renal, gastrointestinal, hepatic, and pancreatic function. Previous reports have indicated that CSMG express neurokinin-1 receptors (NK-1R). NK-1R are G protein coupled receptors that are activated by the tachykinin known as substance P and signal via Gαq/11 protein subunits. In sympathetic neurons, such as superior cervical and stellate ganglion neurons, NK-1R activation modulates Ca2+ channel currents in a voltage-independent (VI) manner. However, the modulation by NK-1R of Ca2+ currents and the signal transduction elements are currently unknown. Therefore, the purpose of the present study was to examine coupling mechanisms between NK-1R and Ca2+ channels in acutely isolated male rat CSMG neurons employing the whole-cell variant of the patch-clamp technique. Ca2+ currents were evoked with a double-pulse voltage protocol. Surprisingly, the application of SP (1 μM) led to a voltage-dependent (VD) inhibition of Ca2+ currents as did exposure to NE (10 μM). The VD modulation of Ca2+ currents is generally associated with coupling of either pertussis toxin (PTX)-sensitive Gαi/o or cholera toxin (CTX)-sensitive GαS protein subunits. Thus, in one set of experiments, CSMG neurons were pretreated overnight with either PTX (500 ng/ml) or CTX (100 ng/ml). Under these conditions, exposure of the neurons to SP did not affect the coupling of NK-1R with Ca2+ channels (36±4, n=21 vs. 36±3, n=12), yet the modulation of the currents remained VD. On the other hand, PTX significantly ($P<0.05$) attenuated the NE-induced modulation of Ca2+ currents (56±4, n=14 vs. 5±1, n=13). Furthermore, CTX pretreatment significantly ($P<0.05$) eliminated the VIP-induced modulation of Ca2+ currents (26±4, n=11 vs. .2±1, n=10). In a separate set of experiments, we transfected CSMG neurons with the cDNA coding for phospholipase Cβ1 c-terminal construct. In neurons expressing the construct (n=3), the SP-mediated Ca2+ current inhibition was eliminated, while application of NE resulted in VD inhibition of Ca2+ channels. These preliminary findings suggest that in CSMG neurons, Gαq/11-coupled NK-1R modulate Ca2+ channels in a VD manner, indicative of a novel signaling pathway in sympathetic neurons.

Disclosures: S. Sugino: None. **V. Ruiz-Velasco:** None. **M. Farrag:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: Austrian Science Fund (FWF F4402, FWF W11)

Tyrolean Department of Education

Title: Human Cav1.3 (CACNA1D) calcium channel mutations associated with hyperaldosteronism or autism risk

Authors: *A. PINGGERA[1], A. LIEB[1], S. MONTELEONE[2], M. J. BROWN[3], K. R. LIEDL[2], P. TULUC[1], J. STRIESSNIG[1]
[1]Pharmacol. and Toxicology, [2]General, Inorganic and Theoretical Chemistry, Ctr. for Mol. Biosci., Univ. of Innsbruck, Innsbruck, Austria; [3]Clin. Pharmacol. Unit, Ctr. for Clin. Investigation, Addenbrooke's Hosp., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: The L-type voltage gated calcium channel family comprises four isoforms (Cav1.1-1.4) and its members are crucial for a variety of physiological functions in electrically excitable cells. Cav1.2 and 1.3 are expressed in various tissues, including the brain, heart and endocrine cells. Gain of function mutations in Cav1.2 (CACNA1C) cause Timothy syndrome which includes autism and QT prolongation. Recently we identified four somatic mutations in aldosterone-producing adenomas (APA) which all showed a gain of function phenotype. A mutation in Cav1.3 has also been associated with increased risk of autism. Here we studied the functional consequences of three more APA mutations and a mutation associated with autism risk by expressing them together with β3 and α2δ1 subunits in tsA-201 cells. ICa (15 mM Ca2+) and ON-gating currents (QON) were recorded using the whole cell patch-clamp technique. Moreover we generated a 3D model of Cav1.3 α1 subunit using homology (MOE) and Rosetta ab initio modeling. Then we simulated the model in a membrane environment using Molecular Dynamics simulations to predict the consequence of human mutations on channel structure. APA mutation F747L, located in the activation gate of the channel (IIS6), activated at about 15 mV more negative potentials than the wild type (WT) and inactivated more slowly and less completely thus resulting in a gain of function. APA mutation R990H (IIIS4) showed neither a shift in the voltage dependence of activation nor altered inactivation kinetics. It significantly increased the ratio of peak tail current vs QON 1.6-fold suggesting a gain of function induced by higher open probability. Homology modeling of the voltage sensor revealed that R990H mutation could result in a gating-pore current during the resting state. This possibility is currently investigated. No phenotype was found for APA mutation M1354I. The autism risk mutation caused a strong gain of function phenotype evident from a shift of 10 mV towards more negative potentials and a 2-fold higher ratio of peak tail versus QON. Our data demonstrate that 6 out of 7 APA mutations are associated with a Cav1.3 channel gain of function phenotype. Cav1.3 gain of function may also contribute to autism risk in humans.

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Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Program#/Poster#: 212.13/B45

Topic: B.04. Ion Channels

Title: Dual modulation of R-type CaV2.3 currents by M1 muscarinic receptor

Authors: J.-Y. JEONG, *B.-C. SUH
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Abstract: Many of high voltage-gated Ca2+ channels (VGCCs) are modulated by Gq-coupled M1 muscarinic acetylcholine receptors through diverse signaling pathways. R-type CaV2.3 currents are known to be increased by M1 receptor activation. According to the previous studies, M1 receptor-induced increase in the amplitude of R-type current is mediated by phosphorylation of CaV2.3 channel via the activation of protein kinase C (PKC). Here we report that M1 muscarinic receptors are also able to inhibit CaV2.3 currents when the channels are fully activated by PKC. In the whole-cell configuration of tsA201 cells, phorbol 12-myristate 13-acetate (PMA, 1 μM), a PKC activator potentiated CaV2.3 currents by ~2-fold. We found that after the PMA-induced potentiation of CaV2.3 currents, application of the M1 receptor agonist oxotremorine-M (Oxo-M, 10 μM) decreased the currents by ~66%. We examined if the hydrolysis of membrane phosphoinositides (PIs) are involved in the muscarinic suppression of R-type currents. We used rapamycin-induced translocatable pseudojanin (PJ) system which can directly dephosphorylate 4- and 5-phosphates from membrane PI(4)P as well as PI(4,5)P2. After the addition of rapamycin, R-type currents dramatically and irreversibly decreased to ~34.2% of the initial level. Taken together, our results suggest that R-type CaV2.3 currents are modulated by M1 receptor in a dual mode, such as potentiation via PKC activation and suppression by poly-PI depletion. Activation of M1 receptor can solely decrease R-type currents in the PKC-activated but not in control cells and PJ-induced inhibition of CaV2.3 currents demonstrates that poly-PIs are important in the maintenance of R-type channel activity.

Disclosures: J. Jeong: None. **B. Suh:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning :2012-0009525

Title: Suppression of peripheral sympathetic outflow underlies agmatine-mediated hypotension

Authors: J.-H. JOENG, *S. CHUNG
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Abstract: Agmatine, an imidazoline deriviatives, suppress the vasopressor sympathetic outflow to produce hypotension. This effect has been known to be mediated in part by suppressing sympathetic outflow via acting imidazoline I_2 receptors (IR_2) at postganglionic sympathetic neurons. But, the cellular mechanism of IR_2-induced inhibition of noradrenaline (NA) release is still unknown. We investigated effect of agmatine activation on contraction evoked by electrical field stimulation (EFS) in superior mesenteric arterial. We also directly tested effect of agmatine activation on N-type Ca^{2+} current in isolated neurons of celiac ganglion (CG) which regulate vascular sympathetic tone of mesenteric artery. agmatine suppressed neurogenic contractions evoked by EFS in endothelium-denuded mesenteric arterial strips and did not affect contraction by external application of NA. In addition, ω-conotoxin GVIA (CgTx), a selective N-type Ca^{2+} channel blocker, significantly inhibited EFS-evoked contraction and this blockade nearly completely occluded suppression of EFS-evoked contraction by agmatine. Moreover, agmatine diminished I_{Ca} measured using patch-clamp method in an irreversible manner in rat CG neurons. In addition, blockage of N-type Ca^{2+} channel with w-CgTx nearly completely occluded I_{Ca} inhibition by agmatine. The present findings demonstrated that activation of agmatine suppresses peripheral sympathetic outflow by modulating N-type Ca^{2+} channel activity located in peripheral sympathetic nerve terminal, which appears to involve agmatine-induced hypotension. This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2012-0009525)

Disclosures: J. Joeng: None. **S. Chung:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: NICHD Intramural

Title: Fragile x mental retardation protein regulates cav2.3 mrna

Authors: *E. E. GRAY, Y. LIU, I. TRANG, J. LEE, D. A. HOFFMAN
NICHD, NIH, Bethesda, MD

Abstract: Fragile X syndrome (FXS) is the most common form of inherited intellectual disability in humans, and is further characterized by autistic behavior, childhood seizures, and abnormal dendritic spine formation. FXS arises from the loss of function of the fragile X mental retardation protein (FMRP), an RNA binding protein that can regulate mRNA localization and translation in neurons. Loss of FMRP alters neuronal excitability and dendritic calcium signaling, and this may arise from the ability of FMRP to regulate the mRNA of voltage-gated ion channels. Recently it was shown that FMRP can bind the mRNA of the voltage-gated calcium channel Cav2.3 (Darnell et al, 2011), although the consequences of this interaction have not been investigated. Cav2.3 is highly expressed in the dendrites of hippocampal and cortical neurons, where it is capable of generating large calcium spikes in response to both back-propagating action potentials and synaptic activity. Thus, alterations in Cav2.3 mRNA localization and translation could have a dramatic impact on cellular excitability and calcium signaling. We seek to investigate the possibility that Cav2.3 mRNA can be regulated by FMRP, and to determine potential consequences of this interaction. Toward this goal, we performed real time PCR on mRNA isolated from the hippocampi or cortex of wild-type and FMR1-/- male mice, and examined the mRNA levels of several dendritic proteins. When comparing hippocampi from FMR1-/- and wild-type mice at 3- or 8-weeks of age, we found no significant difference in the mRNA levels of Cav2.3 (95 ± 5.3%; 103 ± 7.6%), Kv4.2 (100 ± 1.6%; 114 ± 11.1%), PSD-95 (96 ± 3.1%; 86.9 ± 5.7%), and HCN-1 (93 ± 2.2%, 3-weeks only) (n=6 and n=4; 3- and 8-weeks old, respectively). However, in cortical tissue isolated from 3-weeks old mice, we found a significant decrease ($p<0.05$, n=4) in the mRNA levels of Cav2.3 (79 ± 3.9%), Kv4.2 (77 ± 2.4%), PSD-95 (53 ± 18.6%), and HCN-1 (77 ± 1.2%) in FMR1-/- compared to wild-type mice. This data suggests that Cav2.3 mRNA is regulated by FMRP in cortex but not in

the hippocampus. We will further characterize this regulation by identifying FMRP binding sites on Cav2.3. Furthermore, we will determine how FMRP might affect Cav2.3 protein expression and ultimately cellular physiology.

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Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Topic: B.04. Ion Channels

Support: NIH grant R01 NS22625

SSMF postdoctoral fellowship

Title: Altered synaptic plasticity and spatial learning in knock-in mice with a mutation that prevents regulation of $Ca_V2.1$ channels by calmodulin and neuronal calcium sensor proteins

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Abstract: A key component of short-term synaptic plasticity is regulation of P/Q-type Ca^{2+} currents through presynaptic $Ca_V2.1$ channels by Ca^{2+} binding to calmodulin (CaM) and CaM-like calcium sensor (CaS) proteins. Ca^{2+}-dependent facilitation and inactivation of the P/Q-type Ca^{2+} current translates into facilitation and rapid depression of synaptic transmission in cultured neurons that transiently express $Ca_V2.1$ channels. To understand the functional role of the regulation of $Ca_V2.1$ channels by CaS proteins *in vivo*, we introduced a mutation (IMAA) into the CaS protein binding site in the C-terminal domain of $Ca_V2.1$ channels. P/Q-type Ca^{2+} currents from hippocampal pyramidal cell bodies of WT and IMAA mice were indistinguishable, and basal neurotransmitter release in hippocampal autaptic synapses and in CA3-CA1 synapses in hippocampal slices was unchanged in amplitude and frequency. However, synaptic facilitation during paired-pulse stimulation of autaptic synapses in hippocampal cultures and CA3-CA1 synapses in hippocampal slices was significantly reduced. The ratio of amplitudes of AMPA- to NMDA-mediated EPSCs was not significantly different between WT and IMAA. Taken together, these results show that the $Ca_V2.1$-IMAA mutation alters short-term synaptic plasticity

in CA3-CA1 synapses without affecting basal excitatory transmission or postsynaptic function. Strikingly, CA3-CA1 synapses in hippocampal slices from IMAA mice show impaired long-term potentiation induced by a 100-Hz tetanus or by theta-burst stimulation. Because hippocampal plasticity is important for spatial learning, we tested the IMAA mice in context-dependent fear conditioning and found that they have impaired learning and memory for the spatial context of a mild foot shock. To assess spatial learning and memory without a component of fear, we utilized the Barnes Maze, in which mice learn to find a dark hole on the edge of a bright circular field. IMAA mice learned the location of the escape hole more slowly than WT, and they spent greater time to find the escape hole. Altogether, our results suggest that regulation of $Ca_V2.1$ channels by CaS proteins is essential for normal synaptic facilitation, long-term potentiation, and spatial learning and memory in mice.

Disclosures: E. Nanou: None. **J.M. Sullivan:** None. **T. Scheuer:** None. **W.A. Catterall:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Program#/Poster#: 212.17/B49

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Title: Densin-180 regulates cell-surface density of voltage gated Cav1.2 Ca2+ channels

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Abstract: $Ca_v1.2$ and $Ca_v1.3$ are the major voltage-gated L-type Ca^{2+} channels in the nervous system, and regulate Ca^{2+} -dependent gene transcription and synaptic plasticity. Polymorphisms in the *CACNA1C* gene encoding $Ca_v1.2$ are risk factors for multiple neuropsychiatric disorders. Thus, factors that regulate $Ca_v1.2$ are expected to play an important role in the neural control of cognitive/affective functions. Densin-180 is a protein enriched at excitatory synapses and functionally interacts with Ca^{2+}/calmodulin-dependent protein kinase II and $Ca_v1.3$ channels. Mice lacking densin-180 (densin KO) exhibit behavioral phenotypes of mental illness. Here, we report that densin-180 interacts with and regulates $Ca_v1.2$ channels, which contributes to their roles in modulating Ca^{2+} signaling in the brain. Densin-180 coimmunoprecipitates with $Ca_v1.2$ channels in both HEK293T cells and in mouse brain. In biochemical and electrophysiological experiments in HEK293T cells, densin-180 enhances the level of $Ca_v1.2$ at the plasma membrane. The density of cell-surface $Ca_v1.2$ channels is increased in dendrites of mouse hippocampal neurons overexpressing densin-180, and decreased in the brain of densin KO mice. Moreover, Ca_v1-dependent phosphorylation of the transcription factor CREB is decreased in neurons from densin KO mice. We conclude that densin-180 is an important determinant of $Ca_v1.2$ Ca^{2+} signaling in neurons and that further studies of densin KO mice may reveal insights into how dysregulation of $Ca_v1.2$ may lead to mental illness.

Disclosures: S. Wang: None. **R. Stanika:** None. **G. Obermair:** None. **M. Kennedy:** None. **R. Colbran:** None. **A. Lee:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: R01AA016852

Tab Williams Family Fund

Title: Enhanced function of native T-type Calcium Channels in DBA2J mice undergoing alcohol withdrawal

Authors: *H. SHAN, M. RIEGLE, M. MASICAMPO, D. MOLINA, D. W. GODWIN
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Abstract: Chronic ethanol exposure can profoundly alter brain rhythms. T-type calcium channels are an important contributor to these rhythms. Our lab has previously shown that one isoform of the channel, CaV3.2, is inhibited by acute ethanol administration (Shan et al, 2013). Upon withdrawal (WD), gene expression and gating properties of Cav3.2 channels in midline thalamus are upregulated in c57Bl/6 mice (Graef et al, 2011). Our lab previously demonstrated that alcohol withdrawal increased spike and wave discharge (SWD) events in DBA/2J mice. The number and duration of events increased with each successive withdrawal period. Ethosuximide, a T-type calcium channel antagonist, significantly reduced the seizure severity in DBA/2J mice undergoing withdrawal. It suggests that enhanced T-type calcium channel function contributes to hyperexcitability observed during withdrawal. Here, we examined T-type channel physiology in the thalamus of DBA/2J mice, a seizure prone strain, during a 4-week schedule of chronic intermittent ethanol exposures in a vapor chamber. Blood alcohol levels were maintained in the chamber at (180mg/dl) and verified through blood sampling. 24 hours after the 4th WD, slices containing midline thalamus were prepared. T-type currents were recorded at 32°C using visualized whole-cell patch clamp recordings. Native currents were isolated by voltage dependency, and sodium currents were blocked by tetrodotoxin. Inactivation currents were recorded by 1s voltage step commands from -135 mV to -60 mV followed by a 500 ms test pulse at -50 mV in a standard inactivation protocol. Currents were analyzed with the Boltzmann function fit to the channel inactivation curve, described by I/I_{max}. Recordings from mice undergoing 4 cycles of intermittent alcohol WD showed a depolarizing shift in voltage dependence of inactivation, measured by a shift in the V_{50} from -105.3+/-2.367 mV to -98.57+/-2.102 mV (n=13 per group; $p< 0.05$, unpaired t test). Current density was not significantly affected. Our study illustrates that hyperexcitability induced by multiple intermittent withdrawals from ethanol may be caused by enhanced channel function due to a depolarizing shift in inactivation kinetics. This shift is qualitatively similar to the observations made previously by Graef et al (Graef et al, 2011), and indicates that T-channel upregulation is a common feature of alcohol WD in seizure prone and seizure resistant animals.

Disclosures: H. Shan: None. **M. Riegle:** None. **M. Masicampo:** None. **D. Molina:** None. **D.W. Godwin:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.19/B51

Topic: B.04. Ion Channels

Support: Revson Foundation

Title: Control and plasticity of the presynaptic action potential waveform at small CNS nerve terminals

Authors: *M. B. HOPPA[1,2], G. GOUZER[2], M. ARMBRUSTER[3], T. A. RYAN[2]
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Abstract: The steep dependence of exocytosis on Ca^{2+} entry at nerve terminals implies that voltage control of both Ca^{2+} channel opening and the driving force for Ca^{2+} entry are powerful levers in sculpting synaptic efficacy. Using fast, calibrated, genetically-encoded optical voltage indicators we show that unlike at cell somas or certain giant nerve terminals, the peak of the presynaptic action potential (AP_{SYN}) at small nerve terminals typically reaches only ~ +7 mV, a value that optimizes the balance of voltage-dependent open-probability versus driving force for Ca^{2+} entry. Furthermore, we find that key AP_{SYN} parameters show adaptive plasticity: manipulations that increase presynaptic Ca^{2+} channel abundance and release probability result in a commensurate lowering of the AP_{SYN} peak and narrowing of the waveform while manipulations that decrease presynaptic Ca^{2+} channel abundance do the opposite. Our studies thus reveal that adaptive plasticity in the AP_{SYN} waveform serves as an important regulator of synaptic function.

Disclosures: M.B. Hoppa: None. **G. Gouzer:** None. **M. Armbruster:** None. **T.A. Ryan:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

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Topic: B.04. Ion Channels

Support: MIUR PRIN07,10

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Title: Role of voltage-gated calcium channels in spontaneous recurrent cortical activity

Authors: C. CAPUANI[1], A. TOTTENE[1], *D. PIETROBON[1,2]
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Abstract: Slow synchronous fluctuations between periods of persistent activity (up-state) and periods of relative quiescence (down-state) are observed *in vivo* in the cortex during slow-wave sleep and anesthesia. The up-states are driven by large bursts of spontaneous excitatory and inhibitory synaptic currents arising from correlated activity of a large population of presynaptic connected neurons, due to recurrent network activity. Given the prominent role of P/Q- and the N-type Ca2+ channels in controlling synaptic transmission in the cerebral cortex, we investigated the contribution of these two types of Ca2+ channels to the spontaneous cortical synaptic activity underlying the up-state-like oscillations that can be recorded in acute brain slices. We performed single and double patch-clamp experiments on slices of mouse somatosensory cortex to study the up-state-like activity in layer 2/3 pyramidal cells before and after application of the specific inhibitors of P/Q- and N-type Ca2+ channels ω-AgatoxinIVA (400 nM) and ω-conotoxinGVIA (1 μM). The block of N-type Ca2+ channels strongly reduced the up-states frequency to 39% of control, without affecting up-states duration. In contrast, inhibition of the P/Q-type Ca2+ channels transformed the up-states into simil-epileptiform events. We show that this transformation is due to a larger reduction of the inhibitory compared to the excitatory synaptic conductances underlying the up-states and to a consequent shift of the cortical excitatory-inhibitory balance towards excitation. This finding suggests that P/Q-type Ca2+ channels play a dominant role in controlling inhibitory synaptic transmission in the cerebral cortex.

Disclosures: C. Capuani: None. **D. Pietrobon:** None. **A. Tottene:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Title: $Ca_V1.3$ C-terminal interaction with PDZ-domain scaffold proteins regulates channel membrane expression and dendritic spine morphology in hippocampal neurons

Authors: *R. I. STANIKA[1], M. CAMPIGLIO[1], A. LEE[2], J. STRIESSNIG[3], B. E. FLUCHER[1], G. J. OBERMAIR[1]
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Abstract: The L-type Ca^{2+} channel $Ca_V1.3$ critically contributes to neuronal excitability and pacemaking. Ca^{2+} entry through $Ca_V1.3$ may lead to the increased susceptibility of substantia nigra dopaminergic neurons in Parkinson's disease (PD). Alternative splicing gives rise to a long ($Ca_V1.3_L$) and two short ($Ca_V1.3_{42A}$, $Ca_V1.3_{43S}$) C-terminal variants, which differ with respect to voltage-dependence of activation and Ca^{2+}-dependent inactivation and may thus differentially contribute to the neuronal loss in PD. Only the long splice variant contains a cytoplasmic PDZ ligand (ITTL) that binds the synaptic scaffolding proteins densin-180, shank, erbin. Here we analyzed the role of the $Ca_V1.3$ C-terminus and its interaction with PDZ-domain scaffold proteins for membrane expression and the stability of dendritic spines in cultured hippocampal neurons. Immunofluorescence staining of HA-tagged full length $Ca_V1.3_L$ revealed a clustered distribution on the soma, dendrites, and spines. Surface expression levels of the short splice variants reached only 50% of $Ca_V1.3_L$, indicative of a role of the C-terminus in membrane expression. Consistent with a role in membrane trafficking or anchoring of the channel, deletion of the C-terminal ITTL sequence ($Ca_V1.3_{\Delta ITTL}$) reduced $Ca_V1.3_L$ membrane expression to the level of the short splice variants (45% of full length) by decreasing individual cluster size and cluster density. Coexpression of $Ca_V1.3_L$ with either densin-180 or shank1b significantly reduced overall surface expression of $Ca_V1.3_L$, whereas coexpression of erbin had no effect. At the same time, any of these proteins strongly affected dendritic spine morphology by increasing spine length and spine volume. Morphologically altered dendritic spines still contained functional synaptic sites as revealed by the correct apposition of pre- (synapsin) and postsynaptic (PSD-95) proteins. Importantly, expression of either the short splice variants or a $Ca_V1.3$ construct with a deleted ITTL sequence ($Ca_V1.3_{\Delta ITTL}$) in $Ca_V1.3$ prevented regulation of membrane expression and spine morphology by densin-180, shank1b, and erbin. Expression of dihydropyridine insensitive (T1033Y mutation) $Ca_V1.3$ channel constructs and pharmacological block of all endogenous Ca^{2+} currents in cultured hippocampal neurons allowed to exclusively isolate $Ca_V1.3$-mediated Ca^{2+} and Ba^{2+} currents. Preliminary data suggest differential effects of densin on $Ca_V1.3_L$ and $Ca_V1.3_{\Delta ITTL}$. Together our results indicate a critical role of C-terminus and particularly the ITTL sequence for $Ca_V1.3$ membrane trafficking and the regulation of dendritic spine morphology.

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Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Topic: B.04. Ion Channels

Support: FNRS (Belgium) grants 9.4560.03 and T.0015.13

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Title: Investigating the role of various types of Ca2+ channels on dopamine neuron firing patterns and their variability

Authors: V. A. J. DE VRIND[1,2], F. PHILIPPART[1], G. DRION[1], *J. J. MOREAU[1], D. ENGEL[1], V. SEUTIN[1]
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Abstract: In rat brain slices, dopaminergic (DA) neurons of the medial substantia nigra pars compacta fire in a very homogenous pattern in control conditions. However, when their SK channels are blocked by a maximally active concentration of apamin (300 nM) (all synaptic inputs being blocked), their firing pattern becomes very heterogeneous, with a broad spectrum of "irregularity" (the coefficient of variation (CV) of the interspike interval (ISI), which is 0.056 $\pm$ 0.007 in control conditions becomes 0.528 $\pm$ 0.073 (upper range of CVs becomes ~1.8). This variability is very robust, since we confirmed it repeatedly throughout the last years (N total = 48). The increase in irregularity is due to a block of the apamin-sensitive medium duration afterhyperpolarization (mAHP). We asked what the role of various Ca^{2+} channel species is in either promoting irregularity (by generating an inward current opposing the SK current) or opposing it (by providing the source of Ca^{2+} for SK channels). We first investigated the mechanism of the irregularity by applying supramaximal (but still specific) concentrations of organic Ca^{2+} channel blockers. As already suggested by others (Shepard and Stump, 1999; Johnson and Wu, 2004), only a L-type Ca^{2+} channel blocker (5 µM nifedipine), but not a N-, T-, P/Q- or R-type blocker, was able to significantly decrease the CV in apamin and bring it back close to control values. We next asked whether any other single Ca^{2+} channel blocker could

induce irregularity, similarly to apamin. No single agent was able to do so, which is consistent with previous intracellular data from our lab, showing that no single Ca^{2+} channel blocker completely blocked the mAHP (Scuvée-Moreau et al., poster at 2005 SFN meeting), contrary to what had been found by others in young mice (Wolfart and Roeper, 2002). In our former experiments, the most active compound, omega-conotoxin (N-channel blocker) reduced the surface of the mAHP by only ~40 % (this is very different from the situation in serotonergic neurons: Alix et al., 2014). We are currently evaluating whether 1) combinations of Ca^{2+} channel blockers could mimick the apamin effect on firing pattern 2) in a computational semi-quantitative model, SK channels indeed need to be blocked almost completely to generate bursting-like irregularity. In conclusion, our data suggest opposite effects of various Ca^{2+} channels on regularity in these neurons, which is probably due to variable spatial relationships between different Ca^{2+} channel species and SK channels.

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Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Topic: B.04. Ion Channels

Support: Wellcome Trust Senior Investigator award (098360/Z/12/Z)

BBSRC Doctoral Training account PhD Studentship

Title: Differential mechanisms in trafficking of N-type voltage-gated calcium ion channel (Cav2.2) alternative splice variants

Authors: *N. MACABUAG, J. S. CASSIDY, W. PRATT, A. C. DOLPHIN
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Abstract: The N-type voltage-gated calcium ion channels (Cav2.2) play an important role in presynaptic neurotransmitter release, but how these channels are targeted to the presynaptic terminals is not well known. In this study, we compare intracellular trafficking of Cav2.2 isoforms, which are produced from alternative splicing of two mutually exclusive exons 37a (e37a) and 37b (e37b). The significance of the Cav2.2 e37a isoform is thought to be that it is

preferentially expressed in nociceptive dorsal root ganglion (DRG) neurons and gives rise to larger N-type calcium currents, with enhanced voltage-independent G-protein coupled receptor (GPCR) mediated inhibition (Raingo et al., 2007). Here we demonstrate that cell surface expression of the Cav2.2 e37a isoform is larger than for the e37b isoform by 35.4% (p=0.005, n=114 and n=104 respectively) without a significant increase in whole-cell protein expression, using exofacially tagged Cav2.2 channels with the auxiliary subunits (α2δ-1 and β1b) in a heterologous expression system (N2a cells). This increased expression at the cell membrane gives rise to a larger whole-cell current for Cav2.2 e37a. We compare the anterograde and retrograde trafficking of these Cav2.2 isoforms to examine the mechanism that accounts for the difference in the surface expression. We also investigate the mechanism of endocytosis of these Cav2.2 isoforms, including channel endocytosis due to GPCR activation, to examine whether there are differences in GPCR-mediated endocytosis of these Cav2.2 isoforms. Reference Raingo, J., Castiglioni, A.J., and Lipscombe, D. (2007). Alternative splicing controls G protein-dependent inhibition of N-type calcium channels in nociceptors. Nat. Neurosci. 10, 285-292.

Disclosures: **N. Macabuag:** None. **J.S. Cassidy:** None. **W. Pratt:** None. **A.C. Dolphin:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Topic: B.04. Ion Channels

Support: funded by the Ministry of Science, ICT and Future Planning(2012-0009525)

Title: Suppression of N-type Ca2+ current by protease-activated receptor 2 activation in rat coeliac ganglia neurons

Authors: ***Y. KIM**, J.-H. JOENG, S. CHUNG
Physiol., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Protease-activated receptor (PAR)-2, highly expressed on endothelial cells and vascular smooth muscle, plays a crucial role in regulating blood pressure via modulating peripheral vascular tone. Although several mechanisms to explain the PAR-2-induced hypotension have been suggested, an exact mechanism for the hypotension has not been elucidated yet. We investigated effect of PAR-2 activation on contraction evoked by electrical field stimulation (EFS) in superior mesenteric arterial. We also directly tested effect of PAR-2

activation on N-type Ca2+ current in isolated neurons of celiac ganglion (CG) which regulate vascular sympathetic tone of mesenteric artery. PAR-2 agonists suppressed neurogenic contractions evoked by EFS in endothelium-denuded mesenteric arterial strips and did not affect contraction by external application of NA. On the contrary, thrombin, a potent PAR-1 agonist, had no effect on EFS-evoked contraction. In addition, ω-conotoxin GVIA (CgTx), a selective N-type Ca2+ channel blocker, significantly inhibited EFS-evoked contraction and this blockade nearly completely occluded suppression of EFS-evoked contraction by PAR-2 agonists. Moreover, PAR-2 agonists diminished ICa measured using patch-clamp method in an irreversible manner in rat CG neurons. Meanwhile, thrombin had little effect on ICa. In addition, blockage of N-type Ca2+ channel with w-CgTx nearly completely occluded ICa inhibition by PAR-2 agonists. The present findings demonstrated that activation of PAR-2 suppresses peripheral sympathetic outflow by modulating N-type Ca2+ channel activity located in peripheral sympathetic nerve terminal, which appears to involve PAR-2-induced hypotension.

Disclosures: Y. Kim: None. **J. Joeng:** None. **S. Chung:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Program#/Poster#: 212.25/B57

Topic: B.04. Ion Channels

Support: NSF Grant 1022075

Title: LVA Calcium channels regulate excitability of cultured hypothalamic POMC+ neurons and indirectly affect sensitivity to leptin

Authors: P. P. PERISSINOTTI[1], Y. HE[3], E. A. ETHINGTON[2], M. D. KOOB[3], *E. S. PIEDRAS-RENTERIA[2]
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Abstract: Low voltage-activated (LVA) T-type calcium channels play critical roles in neuronal excitability. Here we confirm the involvement of T-type channel activation in the excitability and generation of action potentials (APs) in cultured hypothalamic POMC neurons. The specific T-type channel blocker NCC 55-0396 reduced neuronal excitability and prevented post-inhibitory

rebound (PIR) responses. The Kelch-like 1 (KLHL1) KO mouse model displays homeostatic changes in the expression of LVA calcium channels, resulting in a down-regulation of the $Ca_V3.2$ α_{1H} isoform in whole brain and cerebellum, in line with this protein's function as a positive modulator of $Ca_V3.2$. In the hypothalamus, however, α_{1H} signal did not decrease significantly, and biophysical and western blot analysis show that the $Ca_V3.1$ α_{1G} isoform is up-regulated, with a 40 % increase in T-type current density in cultured hypothalamic KLHL1-KO neurons compared to WT. A leftward shift and slight increase in window currents was observed, but not a change in resting membrane potential. We found that the excitability and PIR probability of KLHL1-KO neurons was increased compared to WT, and that the spontaneously firing neurons displayed a decreased mean-intraburst interval. T-type channels are potential therapeutic targets for treatment of obesity, and interestingly, the KLHL1-KO mice were adult-onset overweight. Leptin treatment (100 nM) resulted in membrane depolarization and increased excitability in WT neurons; this effect was prevented by treatment with NCC 55-0396. In contrast, KLHL1-KO neurons exhibited a basal excitability similar to the leptin-treated WT group, and leptin treatment did not elicit further depolarization. Leptin action occurred *via* TRPC1 channels, as pre-treatment with 100 μM 2-APB decreased the number of APs induced by leptin by 20%, whereas its application after leptin treatment produced a 50% reduction. Our data suggest that leptin activation of TRPC1 channels induces neuronal depolarization, which recruits T-type channel activity. Hypothalamic neuronal excitability in KLHL1-KO POMC neurons is increased, rendering these neurons leptin-resistant. This mouse model provides insights into the modulation of the hypothalamic excitability by α_{1G} and the potential impact of α_{1G} in obesity.

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Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

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Title: Single channel and whole cell analysis of an exonic mutation in human CACNA1B linked to Myoclonus Dystonia syndrome

Authors: *A. S. ANDRADE[1], J. GROEN[2], K. RITZ[2], M. HAAGMANS[2], T. BRADLEY[2], H. JALALZADEH[2], P. NUERNBERG[2], F. BAAS[2], D. VERBEEK[3], M. AJ TIJSSEN[3], S. DENOME[1], D. LIPSCOMBE[1]
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Abstract: The CACNA1B gene encodes CaV2.2 voltage-gated calcium channel α1 subunits that underlie N-type calcium currents in neurons. CaV2.2 channels are expressed at central and peripheral presynaptic nerve terminals where they regulate transmitter release. Here we describe the functional consequences of a single point exonic mutation in CACNA1B that is linked to a human disorder. R1389H was identified by exome sequencing and exclusion linkage in a 3-generation pedigree with a unique familial Myoclonus Dystonia syndrome. R1389H is located in the outer region of the CaV2.2 ion pore of domain III. We compared the biophysical properties of macroscopic and unitary currents of human wild-type (WT) and R1389H mutant CaV2.2 channels transiently expressed in the human cell line, tsA201. We used 2 mM calcium (whole cell) or 110 mM barium (single channel) as charge carrier. A comparison of whole cell CaV2.2 currents suggests that R1389H has little or no influence on ion selectivity or the voltage-dependence and kinetics of channel activation and inactivation. However, current densities in cells expressing R1389H were larger on average compared to wild-type (R1389H: 97.9 ± 15.0 pA/pF, n = 21 cells; WT: 55.1 ± 9.1 pA/pF, n = 26 cells; p = 0.014, Student's t-test). This could reflect differences in overall expression levels or in single channel properties between mutant and WT. Single channel current amplitudes of R1389H CaV2.2 were significantly smaller (0.77 ± 0.01 pA, n = 8 patches) compared to WT (1.03 ± 0.02 pA, n = 8 patches; p = 9.1 × 10-10 at +20 mV, 110 mM Ba). Further analysis showed that human CaV2.2 channels open to two different current amplitudes the larger of which, O2 (1.03 pA), dominates and represents about 85% of all openings, compared to O1 (0.82 ± 0.02 pA, 8 patches). We observed direct transitions between O1 and O2 within a single opening, suggesting that CaV2.2 channels can exist in two open states that conduct ions at different rates. By contrast, single R1389H channels open predominantly to a smaller amplitude level that is not significantly different from the O1 amplitude of WT channels (p = 0.35, Student's t- test). Our data suggest that R1389H stabilizes the smaller amplitude open state as compared to WT, however, this does not explain the larger whole cell CaV2.2 currents in cells expressing R1389H. Although more analyses are necessary, our preliminary data suggest that the activation duration of single R1389H mutant channels may be longer on average, at the single channel level, compared to WT channels. The different biophysical properties of R1389H channels compared to WT may be linked to the observed hyperexcitability phenotype of the Myoclonus Dystonia syndrome.

Disclosures: **A.S. Andrade:** None. **J. Groen:** None. **K. Ritz:** None. **M. Haagmans:** None. **T. Bradley:** None. **H. Jalalzadeh:** None. **P. Nuernberg:** None. **F. Baas:** None. **D. Verbeek:** None. **M. AJ Tijssen:** None. **S. Denome:** None. **D. Lipscombe:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.27/B59

Topic: B.04. Ion Channels

Support: NIH Grant R21 MH099448 (JP)

NIH Grant NS055251 (DL)

NIH Grant MH099405 (AA)

Stanley Medical Foundation (AA, JH, JP)

Title: Functional analyses of human CaV3.3 calcium channel variants corresponding to rare disruptive mutations in CACNA1I in schizophrenia

Authors: ***J. Q. PAN**[1], A. ANDRADE[2], A. ALLEN[1], J. HOPE[1], D. LIPSCOMBE[2]
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Abstract: Voltage-gated calcium channels are key regulators of gene expression, neurotransmission, excitability and many other neuronal functions. Large-scale genome wide association studies have implicated common alleles in *CACNA1C* and other genes as contributing to the risk of schizophrenia and other psychiatric disorders (PMID: 23453885). More recently, a collaborative exome sequencing study identified rare disruptive exonic variations in calcium channel genes of schizophrenia cases (PMID: 24463508). Collectively, common alleles and rare disruptive variations in calcium channel genes appear to contribute to psychiatric disease susceptibility. Two *de novo* mutations in *CACNA1I* were reported in schizophrenia proband by exome sequencing of 105 schizophrenia trios (PMID:23911319). *CACNA1I* encodes low threshold $Ca_V3.3$ channels with relatively slow deactivation kinetics that are expressed in brain, and that regulate oscillatory bursting. Here we compare the biophysical features of $Ca_V3.3$ mutants T797M and R1346H to wild-type (WT) channels, transiently expressed in tsA201cells. T797 and R1346 are located in the pore region in domains II and III of

$Ca_V3.3$, respectively. Currents were recorded using 5 mM calcium as the charge carrier. Interestingly, 24hrs after transfection, tsA201 cells expressing $Ca_V3.3$ channels developed slow, spontaneous, repetitive oscillatory spiking as the membrane potential was hyperpolarized, consistent with the pacemaking role of Ca_V3 channels in excitable cells. Calcium channel current densities in cells expressing R1346H were smaller on average (22.5 ± 3.4 pA/pF, n = 13) compared to currents in cells expressing WT or T797M channels (65.0 ± 18.3 pA/pF, n = 12 and 86.5 ± 11.2 pA/pF, n = 17). Kinetics of deactivation and inactivation, and ionic reversal potentials were not different among $Ca_V3.3$ channels. Activation thresholds for WT and T797M channels were similar to each other (-36.9 ± 1.8 mV, n = 12 and -35.9 ± 0.9 mV, n = 16) but slightly more hyperpolarized relative to R1346H (-33.3 ± 0.9 mV, n = 12). The steepness of WT and T797M channel activation curves were also similar (5.3 ± 0.4, n = 12, and 5.0 ± 0.2, n = 16) but both differed from R1346H (6.2 ± 0.4 mV, n = 12). Our analyses suggest that R1346H mutants have biophysical properties different from WT $Ca_V3.3$ channels. Further analyses of T797M and R1346H $Ca_V3.3$ channels in neurons are necessary to assess their influence on neuronal function including oscillatory bursting, and their potential role in schizophrenia.

Disclosures: J.Q. Pan: None. **A. Andrade:** None. **A. Allen:** None. **J. Hope:** None. **D. Lipscombe:** None.

Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.28/B60

Topic: B.04. Ion Channels

Support: NSF Grant 1022075

Title: Modulation of CaV3.2 T-type calcium channels by GM1

Authors: G. M. KEELING[1], S. S. SHANKARAPPA[3], C. WEISSMANN[1], P. P. PERISSINOTTI[1], *L. L. DONCARLOS[2], L. CRIBBS[1], E. B. STUBBS, Jr.[1,4], E. S. PIEDRAS-RENTERÍA[1]
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Abstract: Gangliosides are sialic acid-containing, complex glycosphingolipids naturally enriched in neuronal membranes. They are important in the regulation of signaling protein

function, cell morphology, cell cycle and neurotransmission; and they also function as membrane receptors for compounds such as cholera, tetanus and botulinum toxin. Alterations in ganglioside concentration are associated with gangliosiodoses. The monosialoganglioside G_{M1} is particularly abundant in the plasma membrane of neurons. The segregation of gangliosides within restricted lipid rafts provides the molecular basis for lateral interactions between them and membrane proteins. G_{M1} is a known modulator of L-type calcium channels in neuroblastoma cells (Carlson et al, 1994) and cerebellar granule neurons (Wu et al, 1996); and of $Ca_V2.3$ channels in sperm (Cohen et al, 2014). Here we report the modulation of $Ca_V3.2$ channels by $G_{M1.}$ We analyzed the effect of G_{M1} on $Ca_V3.2$ channels constitutively expressed in HEK293 cells. Treatment with 1 μM G_{M1} decreased current densities by 32%, an effect that could be completely reversed by addition of the B subunit of Cholera toxin (ChTxB), a specific binding ligand for G_{M1}. This effect was specific for $Ca_V3.2$, as G_{M1} had no effect on currents arising from $Ca_V3.1$. Moreover, G_{D1a}, a ganglioside with similar structure to G_{M1} induced no changes in $Ca_V3.2$ current densities. Treatment with G_{M1} also resulted in a hyperpolarizing shift in steady state inactivation, not reverted by ChTxB. Co-localization analysis exhibits a strong association between $Ca_V3.2$ and G_{M1} (Pearson's coefficient 84 ± 0.02 %), yielding a Manders' coefficient of 75 ± 0.03 % of the G_{M1} signal associated with $Ca_V3.2$. Overall, hyperglycemic conditions in culture (25 mM glucose) resulted in a reduction of G_{M1}-induced inhibition due to a decreased ganglioside presence compared to normoglycemic conditions (5 mM). Our studies show that G_{M1} is an endogenous, reversible, negative modulator of T-type $Ca_V3.2$ channels and that conditions leading to changes in G_{M1} levels at the membrane may affect neuronal excitability, and could contribute to the beneficial effects of G_{M1} therapies. Dis-inhibition of $Ca_V3.2$ channels by decreases in G_{M1} could contribute to increased excitability in diabetic neuropathy and other diseases.

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Poster

212. Calcium Channels: Functions and Regulation of Voltage-Gated Calcium Channels

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 212.29/C1

Topic: B.04. Ion Channels

Support: CONACYT México 167790

PAPIIT-DGAPA, UNAM IN207614

Title: Differential contribution of S4 voltage sensors to the low-voltage activation of CaV3.3 channel

Authors: A. L. SANCHEZ-SANDOVAL[1], Z. HERRERA-CARRILLO[1], C. E. DIAZ-VELASQUEZ[1], J. F. HIGUELDO-GARCIA[1], H. M. RIVERA[2], *J. GOMORA[3]
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Abstract: The family of voltage-activated ion channels (VGIC), i.e., Na_V, K_V and Ca_V channels exhibits four highly conserved transmembrane -helix known as S4 segments, which play the role of voltage sensing. In each of these S4 segments or voltage sensors, there is a positive charged residue (lysine or arginine) every third position of the -helix. However the voltage range of activation is quite different among the whole family of VGIC. In the case of high-voltage (HVA) and low-voltage (LVA) activated Ca_v channels, their activation is around 30-40 mV apart, despite they show S4 segments with similar sequences. To investigate the contribution of S4 segments to the gating of $Ca_v3.3$ (LVA) and $Ca_v1.2$ (HVA) channels we have constructed several chimeras by swapping the S4 segments between these channels. Wild-type channels and chimeras were transiently transfected in HEK-293 cells and the whole-cell patch clamp technique was used to characterize the biophysical properties of the constructs. Our results indicate that the substitution of S4 segment of domain II (IIS4) of $Ca_v3.3$ for that of $Ca_v1.2$ (named chimera ICIIS4) induced a 30 mV positive shift in the *I-V* peak with respect to the $Ca_v3.3$ wild type (WT). Also, the IVS4 segment shifted the *I-V* by 15 mV to depolarized potentials, while substitution of segments IS4 and IIIS4 moved the gating of $Ca_v3.3$ to more negative potentials (~ 5-10 mV). There were also significant changes in steady-state inactivation and current kinetics. An unexpected result was a drastic decrease (< 95%) in the current density of the ICIIS4 channel at physiological potentials, although current was very robust at more positive potentials (up to +150 mV). Western blot and immunofluorescence evidences demonstrate that there were no a substantial reduction in the amount of channel protein in HEK-293 cells expressing the chimera ICIIS4, as compared with the $Ca_V3.3$ WT channel. A likely explanation is that the IIS4 segment (the whole or some residues of it) of $Ca_v1.2$ is interacting with the rest of the channel protein in such way that makes more stable the closed state of the channel, requiring stronger depolarizations to get the channels open. The results suggest that IIS4 and IVS4 voltage sensors contributed significantly to the low-voltage activation of $Ca_V3.3$ channels.

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Poster

213. Postsynaptic Structure II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 213.01/C2

Topic: B.07. Synaptic Transmission

Support: NINDS intramural funds

Title: Differential distribution of Shank and GKAP at postsynaptic density

Authors: *J.-H. TAO-CHENG, Y. YANG, T. S. REESE, A. DOSEMECI
NINDS, NIH, Bethesda, MD

Abstract: Shank and GKAP are both scaffold proteins and binding partners at the postsynaptic density (PSD) of glutamatergic synapses. The distribution and dynamics of Shank and GKAP were studied in dissociated hippocampal cultures by pre-embedding immunogold electron microscopy. The PDZ domain of Shank is known to bind to the C-terminal of GKAP. Here, antibodies against epitopes containing their respective binding sites were used with the expectation that if all Shank molecules are bound to GKAP, distribution of label for the two proteins would coincide. However, labels for the mutual binding sites on Shank and GKAP showed significant differences in distribution, with label for GKAP located closer to the postsynaptic membrane, and label for Shank extending deeper into the cytoplasm. Under basal conditions, label for GKAP resided in a narrow band (~20-50 nm) with a median distance of ~30 nm from the postsynaptic membrane. In contrast, label for Shank occupied a wider band (~30-100 nm) with a median distance from the membrane of ~ 55 nm. Approximately 40% of label for Shank was located in the distal area of the PSD complex, 60-120 nm from the postsynaptic membrane, where less than 5% of GKAP resided. These observations indicate the existence of a population of Shank molecules at the PSD complex that are not bound to GKAP. Upon depolarization with high K+ (90 mM, 2 min), the intensity or distribution of label for GKAP did not change, but the intensity of label for Shank at PSD increased to ~150% of controls while the median distance of label increased from 54 to 61 nm. These results indicate a preferential recruitment of Shank to more distal parts of the PSD complex. Conversely, upon incubation in Ca2+-free medium containing EGTA (5 min), labeling intensity of Shank at the PSD decreased to ~75% of controls and the median distance of label from postsynaptic membrane decreased from 53 to 46 nm, indicating a preferential loss of Shank molecules in the distal area of the PSD complex. Additionally, a subpopulation of synapses emerged after EGTA treatment in which label for Shank appeared in a narrow band close to the postsynaptic membrane, a labeling pattern resembling that for GKAP. Thus, in these synapses under low calcium conditions, Shank

molecules are absent in the distal area of the PSD complex but persist in the proximal area near GKAP. Altogether these observations identify two pools of Shank at the PSD complex, one relatively stable and presumably bound to GKAP, and another more dynamic and situated at a more distal location that would preclude binding to GKAP.

Disclosures: J. Tao-Cheng: None. **Y. Yang:** None. **T.S. Reese:** None. **A. Dosemeci:** None.

Poster

213. Postsynaptic Structure II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 213.02/C3

Topic: B.07. Synaptic Transmission

Support: National Natural Science Foundation of China

Title: Palmitoylation-dependent CDKL5-PSD95 interaction regulates synaptic targeting and function of CDKL5

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Abstract: The X-linked gene cyclin-dependent kinase-like 5 (CDKL5) is mutated in severe neurodevelopmental disorders, including some forms of atypical Rett syndrome, but the function and regulation of CDKL5 protein in neurons remain to be elucidated. Here, we show that CDKL5 binds to the scaffolding protein postsynaptic density (PSD)-95, and that this binding promotes the targeting of CDKL5 to excitatory synapses. Interestingly, this binding is not constitutive, but governed by palmitate cycling on PSD-95. Furthermore, pathogenic mutations that truncate the C-terminal tail of CDKL5 diminish its binding to PSD-95 and synaptic accumulation. Importantly, down-regulation of CDKL5 by RNA interference (RNAi) or interference with the CDKL5-PSD-95 interaction inhibits dendritic spine formation and growth. These results demonstrate a critical role of the palmitoylation-dependent CDKL5-PSD-95 interaction in localizing CDKL5 to synapses for normal spine development and suggest that disruption of this interaction by pathogenic mutations may be implicated in the pathogenesis of CDKL5-related disorders.

Disclosures: Z. Xiong: None.

Poster

213. Postsynaptic Structure II

Location: Halls A-C

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Program#/Poster#: 213.03/C4

Topic: B.07. Synaptic Transmission

Support: NIH intramural award to CJM

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Title: Neuronal pentraxins coordinate excitatory synapse maturation of parvalbumin expressing interneurons

Authors: *K. A. PELKEY[1], E. BARKSDALE[2], M. T. CRAIG[2], X. YUAN[2], M. SUKUMARAN[2], G. A. VARGISH[2], P. F. WORLEY[3], C. J. MCBAIN[2]
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Abstract: Afferent driven recruitment of widespread perisomatic inhibition through parvalbumin expressing basket cells (PVBCs) critically dictates the synaptic integration properties of downstream principal cells (PCs). At the network level such feedforward inhibition provides temporal constraints upon PC excitation-spike coupling to coordinate and bind firing rates within PC assemblies. Indeed deficient recruitment of PVBCs disrupts PC synchronization and promotes cognitive deficits associated with disorders like schizophrenia. To reliably coordinate network activity PVBCs exhibit specialized synaptic and membrane properties that promote efficient afferent recruitment such as the expression of high conductance, kinetically fast GluA4-containing AMPARs. Here, we demonstrate that at the neonatal stage interneurons fated to become PVBCs lack GluA4 only upregulating this subunit during the second and third postnatal weeks coincident with the developmental profiles of other PVBC hallmark proteins such as Kv3.1b, synaptotagmin 2 (Syt2), and PV itself. This developmental time course is also shared by the AMPAR interacting proteins neuronal pentraxin 2 (NPTX2/NARP) and neuronal pentraxin receptor (NPTXR/3). While previous work has implicated neuronal pentraxins in the synaptic localization of AMPARs we report a complete loss of PVBC GluA4 in NPTX2-/-/NPTXR-/- double knockout mice indicating a role beyond synaptic clustering. Quantitative PCR analyses revealed comparable levels of GluA4 mRNA between wild type and NPTX2-/-/NPTXR-/- brains suggesting normal GluA4 transcription with disrupted translation and/or protein stabilization. Moreover, development of parvalbumin, Syt2, and perineuronal nets proceeds normally in PVBCs of NPTX2-/-/NPTXR-/- mice indicating a selective deficit in AMPAR maturation during circuit integration. Functionally, adolescent NPTX2-/-/NPTXR-/- mice exhibit profound deficits

in basal PVBC AMPAR function with consequent disruption of hippocampal inhibition/excitation (I/E) balance and rhythmogenesis. In addition neonatal NPTX2-/-/NPTXR-/- display a protracted developmental window for hippocampal circuit maturation as revealed by prolonged expression of giant depolarizing potentials (GDPs) compared to wild type animals. These findings implicate neuronal pentraxins in controlling PVBC synaptic integration within cortical circuits providing a novel potential therapeutic target for disorders associated with altered I/E dynamics.

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Poster

213. Postsynaptic Structure II

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Topic: B.02. Ligand-Gated Ion Channels

Support: The John T. Reid Charitable Trust

HFSP Long-Term Fellowship

NHMRC Australia CJ Martin Fellowship

HHMI

NIH

Title: Activity-dependent ubiquitination of GluA1 and GluA2 regulates AMPA receptor intracellular trafficking and degradation

Authors: *V. ANGGONO[1], J. WIDAGDO[2], Y. CHAI[1], M. RIDDER[2], P. SAH[2], R. L. HUGANIR[3]
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Abstract: AMPA receptors (AMPARs) have recently been shown to undergo posttranslational ubiquitination in mammalian neurons. However, the underlying molecular mechanisms are poorly understood and remain controversial. Here we report that all four AMPAR subunits

(GluA1-4) are rapidly ubiquitinated upon brief applications of AMPA or bicuculline in cultured neurons. This process is Ca2+-dependent and requires the activity of L-type voltage-gated Ca2+-channel and Ca2+/calmodulin-dependent kinase II. The ubiquitination of all subunits occurs exclusively on AMPARs located on the plasma membrane post-endocytosis. We further mapped the sites of ubiquitination on lysine residues in GluA1 and GluA2 carboxy-terminal tails. Mutation of these lysines did not affect basal surface expression or AMPA-induced internalisation of GluA1 and GluA2 subunits. Instead, it reduced the intracellular trafficking of AMPARs to the late endosomes and thus, protein degradation. These data indicate that ubiquitination is important for endosomal sorting and stability of AMPARs, which may be crucial for synaptic plasticity.

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Poster

213. Postsynaptic Structure II

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Topic: B.07. Synaptic Transmission

Support: NSF GRFP

R01 NS060847-01A2

Title: The de-ubiquitinating enzyme, USP8, is regulated distinctly by NMDA receptor signaling

Authors: *M. GOO, S. SCUDDER, A. MOLTENI, G. PATRICK
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Abstract: Ubiquitination has emerged as a key post-translational modification to regulate trafficking and turnover of synaptic proteins. We have previously determined that the ubiquitination of AMPA receptors (AMPAR) by the HECT E3, ubiquitin ligase Nedd4-1 mediates a distinct internalization and endocytic sorting pathway to lysosomes for degradation. Specifically, we found that AMPAR ubiquitination occurs exclusively under AMPAR activation while NMDA receptor (NMDAR) activation, which also promotes AMPAR internalization, does not lead to AMPAR ubiquitination. Additionally, in recent studies we show that application of glutamate and glycine does not induce AMPAR ubiquitination. Since glutamate and glycine not

only activates AMPAR but also NMDAR, we hypothesized that NMDAR signaling negatively regulates AMPAR ubiquitination by activating a de-ubiquitinating enzyme (DUB) leading to the deubiquitation of AMPARs. To further support this hypothesis, we found that glutamate stimulation with subsequent blockage of NMDAR with APV induced robust AMPAR ubiquitination. We then identified that USP8/UBPY, a DUB which functions in the endosomal sorting complex required for transport (ESCRT) pathway, is specifically activated by NMDAR activation but not AMPAR activation. NMDAR activation leads to a rapid dephosphorylation of USP8, which increases its activity in a calcium dependent manner. Functionally, we found that overexpression of USP8 in hippocampal neurons significantly increases synaptic strength while knockdown of USP8 had significant decrease in synaptic strength. Taken together, we provide the first evidence for diametric and activity-dependent control of not only a ligase but a DUB at synapses in the regulation of surface AMPAR levels and synaptic strength.

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Poster

213. Postsynaptic Structure II

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Topic: B.07. Synaptic Transmission

Support: NIH Developmental Biology Training Grant (5T32 HD07491)

Title: The rho-family gtpase cdc-42 mediates trafficking of α7 nachrs

Authors: ***A. J. KALLARACKAL**, M. JENSEN, D. M. MADSEN, A. V. MARICQ
Dept Biol., Univ. of Utah, Salt Lake City, UT

Abstract: Regulated expression of synaptic nicotinic acetylcholine receptors (nAChRs) is vital for the development, maintenance and plasticity of cholinergic neurotransmission. Our lab has previously identified a Wnt-mediated signaling pathway that is responsible for the translocation of ACR-16, the *C. elegans* homolog of the vertebrate α7 nAChR, from subsynaptic stores to the surface of muscle cells. Mutations that disrupt this signaling pathway selectively decrease postsynaptic currents mediated by α7 nAChRs. In contrast, currents mediated by GABA receptors, or by non- α7 nAChRs (UNC-29) are unaffected. To further identify the molecules that participate in the trafficking of α7/ACR-16, we screened for genes that phenocopy acr-16 mutants when knocked down using RNAi. Similar to acr-16 mutants, RNAi against the Rho

GTPase gene cdc-42, produced a significant movement defect when in the unc-29 genetic background, but did not appreciably affect movement in wild type worms. Furthermore, we found that cdc-42 null mutants also produced a severe movement defect, but only in combination with the unc-29 mutation. This so-called synthetic defect suggested that the trafficking or function of α7/ACR-16, but not UNC-29 receptors, is dependent on CDC-42. We measured *in vivo* ACh-gated current in muscle cells and found that cdc-42 mutants had a specific decrease in ACR-16-mediated current, similar to that found in Wnt mutants. Interestingly, we found that cdc-42 mutants exhibited a decrease in ACR-16::GFP in muscle arms, contrary to what we observed in Wnt mutants. This result, in addition to data from informative cdc-42/Wnt double mutants, suggests that CDC-42 functions upstream of Wnt signaling by trafficking ACR-16 to subsynaptic stores, but does not mediate translocation of receptors to the cell surface. Together, these findings aid in understanding the pathway through which α7 nAChRs are targeted to synapses.

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Poster

213. Postsynaptic Structure II

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Program#/Poster#: 213.07/C8

Topic: B.02. Ligand-Gated Ion Channels

Support: DFG Grant EN 948/1-1

Title: CKAMP44 and TARP γ-8 modulate AMPA receptor-mediated currents in dentate gyrus granule cells

Authors: ***E. JACOBI**[1], P. FARROW[1], K. KHODOSEVICH[2], H. MONYER[2], J. V. ENGELHARDT[1]
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Abstract: Auxiliary subunits modulate gating properties, subcellular localization and surface trafficking of AMPA receptors (AMPARs). In dentate gyrus granule cells two prominently expressed AMPAR auxiliary subunits, CKAMP44 and TARP γ-8, show overlapping as well as opposing influences on AMPAR expression and function. TARP γ-8 and CKAMP44 both

increase the rate of deactivation, but have an opposite effect on receptor desensitization and recovery from desensitization. Interestingly, the slow recovery from desensitization affects synaptic short-term plasticity in dentate gyrus granule cells. This stands in contrast to the majority of synapses in the CNS, where changes in release probability of transmitter vesicles shape short-term plasticity. Furthermore, paired pulse ratio of synaptic AMPAR-mediated EPSCs, as a measurement of short-term plasticity, is increased and decreased in *CKAMP44*$^{-/-}$ and *TARP γ-8*$^{-/-}$ mice, respectively, when compared to wildtype mice. Paired pulse ratios did not differ between the genotypes when recordings were performed in the presence of cyclothiazide, a potent inhibitor of AMPAR desensitization. Thus, TARP γ-8 and CKAMP44 modulate short-term plasticity by their influence on the recovery from desensitization of AMPARs. Beside their effects on AMPAR gating, extrasynaptic and synaptic AMPAR-mediated currents are reduced in granule cells of *CKAMP44*$^{-/-}$ and *TARP γ-8*$^{-/-}$ mice, indicating that both auxiliary proteins promote the trafficking of AMPARs to the cell surface and their integration into synapses. Finally, extrasynaptic and synaptic AMPAR-mediated currents were strongly reduced in *TARP γ-8/CKAMP44* double-knockout mice, suggesting that these two co-expressed auxiliary proteins are the main auxiliary proteins, that traffic AMPAR to the cell surface of dentate gyrus granule cells. In conclusion, we demonstrate the importance of TARP γ-8 and CKAMP44 in influencing both AMPA receptor gating and trafficking and thereby fine-tuning the excitatory drive onto dentate gyrus granule cells.

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Poster

213. Postsynaptic Structure II

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 213.08/C9

Topic: B.07. Synaptic Transmission

Support: BBSRC (BB/H018344/1)

BBSRC-FAPESP (BB/J02127X/1)

Title: Arc regulates AMPA receptor-mediated synaptic transmission via direct interaction with the endocytic machinery

Authors: *S. A. CORREA[1], J. MULLER[1], M. J. WALL[1], S. C. WAUTERS[1], Y. JANUARIO[2], L. P. DE ALMEIDA[2], L. L. DASILVA[2]
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Abstract: Activity-regulated cytoskeleton (Arc) is a neuron-specific immediate early gene required for learning and memory. As such, Arc protein expression is critical for group 1 metabotropic glutamate receptor-dependent long-term depression and homeostatic synaptic scaling by promoting endocytosis of AMPA receptors (AMPAR). To map the steps linking Arc expression to endocytosis of AMPAR, we immunoprecipitated endogenous Arc from C57BL/6 mouse hippocampal lysates and identified unknown components of the endocytic machinery as Arc-binding proteins using mass spectrometry. To characterize the Arc/endocytic proteins interaction we used several strategies: **a)** Arc co-IPs with components of the endocytic machinery in hippocampal lysates from adult C57BL/6 mice and **b)** recombinant Arc directly binds to the GST-containing the specific components of the endocytic machinery. To determine the Arc amino-acid (aa) sequence that mediates the Arc-endocytic proteins interaction, we generated Arc mutants with successive 50 aa up to 200 aa deletions from either the Nt or Ct and performed pull-down assays using GST-containing endocytic proteins. Next, we tested whether Arc regulates AMPAR endocytosis via the interaction with newly identified components of the endocytic machinery. To do this, we recorded AMPAR-dependent miniature excitatory postsynaptic currents (mEPSCs) from primary hippocampal cultures (PHCs) at 15-18 days *in vitro*. PHCs co-expressed microRNAs-eGFP-tagged to knockdown the endocytic proteins with either Arc-wild-type (Arc-WT) or Arc-mutants, which do not interact with the endocytic machinery. Scrambled miRNAs were used as a control. The significant decrease in mEPSC amplitude observed in PHC expressing Arc-WT is reduced in cells expressing Arc-mutants, suggesting that the Arc-dependent endocytosis of AMPAR requires interaction with the new identified components of the endocytic machinery. In conformation, the increase in AMPAR-endocytosis promoted by Arc was reduced in cells where the expression of these endocytic proteins was depleted. Together, the findings from this study identified the mechanistic link in which neuronal activity regulates constitutive trafficking to control synaptic strength.

Disclosures: S.A. Correa: None. **J. Muller:** None. **M.J. Wall:** None. **S.C. Wauters:** None. **Y. Januario:** None. **L.P. de Almeida:** None. **L.L. daSilva:** None.

Poster

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CIHR MOP-38090

PMSHE 4245/B/P01/2010/38

Title: Cognitive flexibility and LTD are impaired following β-catenin stabilization *in vivo*

Authors: *F. MILLS[1], T. BARTLETT[2], L. DISSING-OLESSEN[3], M. WISNIEWSKA[4], J. KUZNICKI[4], B. MACVICAR[3], Y. WANG[2], S. BAMJI[1]
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Abstract: The cadherin/β-catenin adhesion complex is a key mediator of the bidirectional changes in synapse strength which are believed to underlie complex learning and memory. In the present study, we demonstrate that stabilization of β-catenin in the hippocampus of adult mice results in significant impairments in cognitive flexibility and spatial reversal learning, including impaired extinction during the reversal phase of the Morris Water maze and deficits in a delayed non-match to place T-maze task. In accordance with this, β-catenin stabilization was found to abolish long-term depression (LTD) by stabilizing cadherin at the synaptic membrane and impairing AMPA receptor endocytosis, while leaving basal synaptic transmission and long-term potentiation (LTP) unaffected. These results demonstrate that the β-catenin/cadherin adhesion complex plays an important role in learning and memory, and that aberrant increases in synaptic adhesion can have deleterious effects on cognitive function.

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Poster

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Support: NIH Grant R01DA013680

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Title: Aggressive experience increases PSD-95 in the nucleus accumbens of female hamsters via the Fragile X Mental Retardation Protein Signaling Pathway

Authors: *L. E. BEEN, K. M. MOORE, B. C. KENNEDY, R. L. MEISEL
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Abstract: Abstract: We recently discovered that aggressive experience increases dendritic spine density on medium spiny neurons in the nucleus accumbens (NAc) of female hamsters, suggesting that, like in males, the NAc may be a key brain region for modulating plasticity associated with aggressive experience in females. How aggressive experience produces changes in dendritic spine formation is, however, unknown. One possible mechanism underlying this synaptic plasticity is the Fragile X Mental Retardation Protein (FMRP) signaling pathway. Indeed, *in vitro* studies have demonstrated that binding at G-protein coupled receptors leads to activation of FMRP and dendritic protein synthesis consistent with spine formation. We therefore hypothesized that aggressive experience activates metabotropic glutamate receptor 5 (mGluR5), resulting in a decreased phosphorylation of FMRP and increased expression of spine scaffolding proteins, such as PSD-95, in the NAc. To test this hypothesis, adult female hamsters were randomly assigned to one of two behavioral conditions: experienced subjects received five consecutive days of aggressive experience, whereas naïve control subjects remained in their home cage. Thirty minutes prior to each aggressive or control experience, females received an i.p. injection of MPEP, an mGluR5 antagonist, or vehicle control. Following the last aggressive experience, subjects were sacrificed and bilateral tissue punches were taken from the NAc. PCR and Western blot analyses revealed significant increases in PSD95 mRNA and protein in the NAc of experienced subjects compared with naïve control subjects. This increase was blocked by systemic administration of MPEP. In addition, Western blot analysis revealed a significant decrease in the phosphorylation of FMRP protein in the NAc of experienced subjects compared with naïve controls. Together, these data suggest that the FMRP pathway is involved in regulating synaptic plasticity in the NAc following aggressive experience in female hamsters.

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Poster

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Topic: B.07. Synaptic Transmission

Support: NINDS F32NS067712

Title: Triad3A regulates synaptic strength by ubiquitination of Arc

Authors: *A. M. MABB[1], H. S. JE[2], M. J. WALL[3], C. G. ROBINSON[4], R. S. LARSEN[5], Y. QIANG[2], S. A. L. CORREA[3], M. D. EHLERS[6]
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Abstract: Activity-dependent gene transcription and protein synthesis underlie many forms of learning-related synaptic plasticity. At excitatory glutamatergic synapses, the immediate early gene product Arc/Arg3.1 couples synaptic activity to postsynaptic endocytosis of AMPA-type glutamate receptors. Although the mechanisms for Arc induction have been described, little is known regarding the molecular machinery that terminates Arc function. Here we demonstrate that the RING domain ubiquitin ligase Triad3A/RNF216 ubiquitinates Arc, resulting in its rapid proteasomal degradation. Triad3A associates with Arc, localizes to clathrin-coated pits, and is associated with endocytic sites in dendrites and spines. In the absence of Triad3A, Arc accumulates, leading to the loss of surface AMPA receptors. Furthermore, loss of Triad3A mimics and occludes Arc-dependent forms of synaptic plasticity. Thus, degradation of Arc by clathrin-localized Triad3A regulates the availability of synaptic AMPA receptors and temporally tunes Arc-mediated plasticity at glutamatergic synapses.

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Poster

213. Postsynaptic Structure II

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Topic: B.07. Synaptic Transmission

Title: Systematic analysis of purification process of postsynaptic densities (PSDs) and postsynaptic membrane rafts (PSRs) by solubilization of synaptic plasma membrane with different types of detergents

Authors: *T. SUZUKI[1], L. ZHAO[1], G. WEIHENG[1], H. SAKAGAMI[2]
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Abstract: Both postsynaptic density (PSD) and postsynaptic membrane rafts (PSR) are isolated separately. However, PSD and PSR are considered to make a complex *in vivo* (Suzuki et al., J. Neurochem. 2011, Liu et al., J. Neurogenetics, 2013). To elucidate the more detailed structural relationship between PSD and PSR, we investigated purification process of PSD and PSR from rat forebrain synaptic plasma membrane (SPM) using three different detergents, TX-100, n-octyl β-D-glucoside (OG) and CHAPSO at varied concentrations, and examined the distribution of subsynaptic structures and PSD proteins (both type I and type II) on sucrose density gradient by SDS-PAGE, electron microscopy and western blotting. This type of systematic examination has not been carried out before. Three types of detergents used showed distinct separation profiles of the synaptic subdomains. After TX-100 treatment, type I PSD was recovered in two fractions: pellet (fraction 12) and insoluble fraction 8, the latter of which contained membrane raft-PSD complex that was partially broken. Conventional PSD prepared after TX-100 treatment was suggested to be a mixture of these two types of type I PSD pools (pellet and fraction 8). It was found that the conventional PSD does not contain inhibitory type II PSDs. Association of type I PSD with PSR was identified in the TX-100-treatment but not in the OG-treatment. Association of type II PSD with membrane rafts was suggested in OG- and CHAPSO-treatments but not in the TX-100-treatment. Treatment with relatively high concentration of detergents, in particular OG, solubilized the type I PSD proteins. CHAPSO-treatment produced various novel fractions containing unique subsynaptic structures. Besides pellet/fraction 12, novel PSD-containing structures were isolated in an insoluble fraction 11. Tow pools of GluA were identified in the synaptic region in the OG- and CHAPSO-treatments; one was associated with type I PSD and the other possibly with membrane rafts. The knowledge obtained in this study is useful in understanding the studies using isolated PSDs and PSRs. Furthermore, novel subsynaptic structures obtained in this study may become useful materials for future analyses to understand the organization of synapses at the molecular level.

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Poster

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Title: Confined diffusion of AMPA receptors in the postsynaptic density

Authors: Y. SONG[1], T. LI[2], T. A. BLANPIED[3], *S. RAGHAVACHARI[4]
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Abstract: Mechanisms that regulate the insertion, movement, positioning, and removal of AMPA-type glutamate receptors within the postsynaptic density (PSD) determine the strength of excitatory neurotransmission at excitatory synapses. Two key processes that control synaptic AMPAR number are receptor diffusion within the synaptic and extrasynaptic space and interactions between receptors and PSD scaffold proteins. Electron microscopy suggests that the PSD is highly crowded, potentially limiting the ability of receptors to diffuse and interact with scaffold proteins. However, the contribution of macromolecular crowding to receptor retention remains to be tested systematically. Here, we combine experimental and computational approaches to test the effect of synaptic steric hindrance on receptor mobility and enrichment. To examine the distinct contributions of crowding and receptor-scaffold binding, we developed a computational model for AMPAR diffusion in the synaptic and extrasynaptic space, which contains immobile obstacles, representing scaffolding, receptor and adhesion molecules in the PSD. The spatial distribution of scaffold proteins was determined directly from photo-activated localization microscopy measurements that mapped molecular positions with a resolution of ~30 nm. The AMPAR/scaffold association and dissociation rates were adjusted by computer simulations to fit single-particle tracking and fluorescence recovery after photobleaching measurements. The model predicts that variation of receptor size has the most influence on the recovery curves while variation of kinetic rates did not significantly alter receptor residence time or mobility. In order to directly address these questions experimentally and to verify model predictions, we used single-molecule tracking and bulk imaging techniques such as fluorescence recovery after photobleaching to quantify the diffusion dynamics of AMPARs and a set of uniquely designed transmembrane (TM) proteins that mimic receptors on living synapses. We find that diffusion of TM proteins is slowed in the synapse even in the absence of binding

interactions, while adding a single synaptic binding motif to a small TM protein also slows its diffusion within the synapse, consistent with modeling results. We also examined the effect of acutely increasing the protein bulk of the TM intracellular domain on its diffusion and exchange within dendritic spines. These results suggest that both protein size and binding play important roles in retaining surface-diffusing TM proteins within the excitatory synapse and shed light on the biophysical mechanisms that lead to high density of AMPARs at synapses.

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Poster

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Title: Tomosyn participates in synaptic plasticity and is subject to activity-dependent proteasomal regulation

Authors: ***J. J. SALDATE**, V. A. CAZARES, A. J. MANLY, E. L. STUENKEL
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Abstract: Transmission of information through neural circuits is dynamically modifiable and dependent upon neuronal activity, a process referred to as synaptic plasticity. Modulation of post-synaptic receptors is an extensively studied mechanism by which synaptic plasticity is implemented. However, presynaptic mechanisms have also been elucidated as integral to the modulation of synaptic strength through the regulation of vesicle release. The exocytosis of synaptic vesicles is largely mediated by the SNARE family proteins, which form trans-complexes between the vesicle and presynaptic plasma membrane. These proteins are essential to regulated molecular mechanisms underlying the efficacy of neurotransmission. Tomosyn is a unique SNARE protein in that it is cytosolic and serves as a negative regulator of presynaptic release. The level and activity-state of tomosyn in nerve terminals affects release probability by negatively regulating the availability of vesicles in the active zone. What remains unknown are the mechanisms and signaling pathways by which tomosyn activity is modulated. Our studies

support that the ubiquitin-proteasome system (UPS) is particularly influential in modulating synaptic strength. Preliminary data presented here, from cultured hippocampal neurons (21+ DIV), indicate that tomosyn proteostasis and activity are subject to regulation by the UPS. Tomosyn protein levels increase upon acute pharmacological proteasome blockade. This regulation may be mediated through a novel interaction with the E3 ubiquitin-ligase HRD1. Immunoprecipitation of tomosyn results in co-precipitation of HRD1, which has the ability to ubiquitinate tomosyn *in vitro*. This interaction is activity-dependent and subject to homeostatic plasticity and increases upon induction of homeostatic plasticity via AMPAR blockade using CNQX. Homeostatic activity subsequently decreases the overall endogenous tomosyn levels in our hippocampal culture system. As a whole, preliminary evidence presented here indicates that the UPS may induce presynaptically-mediated homeostatic plasticity in an activity-dependent fashion through the precise regulation of tomosyn.

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Poster

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Topic: B.07. Synaptic Transmission

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Title: Synaptic protein distribution organized by a PSD nanocolumn

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Abstract: Scaffold proteins are abundant and essential components of the postsynaptic density (PSD). They form the foundation of excitatory synaptic transmission by establishing the architecture of the PSD; deletion or mutations in their human genes cause severe neuropsychiatric disorders including autism, mental retardation, and schizophrenia. Though the

characteristics of individual constituents of the PSD has been extensively studied with genetic, biochemical, and molecular analysis, it is still unclear how these constituents are arranged within individual PSDs. Previously, by measuring the internal structure of single PSDs in live neurons using photoactivated localization microscopy (PALM), we have found that four major PSD scaffold proteins, PSD-95, GKAP, Shank and Homer1, were each organized in distinctive ~80 nm ensembles, and that PSD-95 ensembles were enriched for both GluA2-containing AMPARs and GluN2B-containing NMDARs. We now report that ensembles of PSD-95 undergo NMDAR-triggered reorganization: in particular, activation of NMDARs in an LTD induction protocol prompted the diminishment of PSD-95 nanoclusters. This effect is predicted to accentuate the decrease in synaptic strength during LTD by dispersing receptors within the synapse to regions of less efficacious glutamate exposure. To examine how the nanoclustered PSD-95 distribution may affect other synaptic constituents, we used multiple-color 3D stochastic optical reconstruction microscopy (STORM). We find that nano-ensembles of other PSD scaffold molecules are highly correlated to PSD-95, indicating that multiple proteins distributed along the axial extent of the PSD are co-enriched at specific points across the face of the synapse. This relatively compact, vertically oriented molecular organization within the bounds of the synapse suggests a “PSD nanocolumn” that may be important for establishing and modulating synapse function.

Disclosures: **A. Tang:** None. **H.D. MacGillavry:** None. **T.A. Blanpied:** None.

Poster

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Title: Epigenetic editing of the PSD95 gene promoter impacts neuronal architecture

Authors: *F. J. BUSTOS[1,2,3], L. VARELA-NALLAR[3], R. AGUILAR[3,2], B. HENRIQUEZ[3], F. FALAHI[4], M. G. ROTS[4], M. MONTECINO[2,3], B. VAN ZUNDERT[3]
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Abstract: Epigenetic editing aims for long-term modulation of gene expression through changes in the epigenetic state of genes. To achieve specific and local epigenetic reprogramming, diverse epigenetic effector domains can be fused to zinc finger proteins (ZFPs). Artificial transcription factors (ATFs) containing such ZFPs have recently been successfully used to specifically and efficiently modulate various endogenous cancer genes. We first established a histone modification pattern of the neuronal plasticity gene PSD95, and then we designed ZFPs to induce targeted re-writing of the original histone signature of the PSD95 gene promoter and hence modulate bi-directionally its expression in primary hipocampal neurons. We focused on the PSD95 gene because it is the most abundant scaffolding protein in the post-synaptic density that regulates spine maturation, dendritic stabilization, and LTP induction. A ZFP was generated to target the PSD95 gene promoter (PSD95-ZFP) and directly cloned into lentiviral vectors alone (NoED; no effector domain), or fused to activation (VP64) or repression (SKD, G9a, Suv39H1del76) domains. Primary hippocampal neurons were infected with these lentiviral particles at 7 days *in vitro* (DIV) and processed at 12 DIV. ChiP-seq assays in hippocampal neurons demonstrate that PSD95-ATF-NOED binds specifically and efficiently to the PSD95 gene promoter (chromosome 10q24). Furthermore, we find that PSD95-VP64 increased histone H3 acetylation (H3Ac) at the promoter, concomitant with increased global and synaptic upregulation of PSD95 protein levels. Conversely, PSD95-SKD, -G9a or -Suv leads to decreased H3Ac and enhanced H3K9 methylation at the promoter, which parallels with a repression in the global and synaptic PSD95 protein levels. Additionally, morphological analyses showed that similar as for overexpression of PSD95, PSD95-VP64 decreases dendritic branching, whereas PSD95-SKD, -G9a or -Suv increase dendritic arborization relative to control neurons and those transfected with PSD95-NoED. Our data shows that we were able to generate a locus-specific ZFP that binds to the PSD95 promoter. This ZFP was able to direct epigenetic enzymes to edit histone tail modifications associated to the promoter thereby altering endogenous PSD95 gene expression. Additionally, modulation of PSD95 expression by epigenetic editing impacts dendritic arborization and spine morphology in hippocampal neurons.

Disclosures: F.J. Bustos: None. **L. Varela-Nallar:** None. **R. Aguilar:** None. **B. Henriquez:** None. **F. Falahi:** None. **M.G. Rots:** None. **M. Montecino:** None. **B. van Zundert:** None.

Poster

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DK32948

Title: Developmental regulation of Kalrn promoter usage alters protein localization, lipid binding and intracellular trafficking at the PSD

Authors: *M. B. MILLER, K. S. VISHWANATHA, R. E. MAINS, B. A. EIPPER
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Abstract: Kalirin (Kal) is a dual Rho GDP/GTP exchange factor (GEF), highly expressed in the central nervous system (CNS). Alternative splicing generates 3 major Kalirin isoforms, which are developmentally regulated and serve essential roles in CNS development and function. While Kal9 and Kal12 are crucial for normal neurite outgrowth, Kal7 plays important roles in dendritic spine formation and synaptic plasticity. Full-length Kalirin expression is initiated at one of four alternate promoters, resulting in four unique initial exons (Ex1A, 1B, 1C, 1D). While roles of alternatively spliced Kalirin molecules have been studied extensively, little is known about the use and functional significance of the alternate promoters. We found that Kalrn promoter usage is developmentally regulated and varies across brain regions. Ex1B is used early in development, with Ex1C expression increasing postnatally. Promoters A and D are rarely used in brain, but Ex1D predominates in endocrine tissues. Interestingly, structural modeling and biophysical studies predict that Ex1C encodes an amphipathic helix, potentially influencing the adjacent Sec14 lipid-binding domain. We previously found that KalSec14 generated using Ex1A (aSec14) binds most strongly to PI(3,4,5)P3, with some binding to other phosphoinositides (PIPs), but not to more prevalent membrane lipids. Here, we show that alternative promoter usage confers differential lipid binding properties to KalSec14: bSec14 does not bind strongly to any of the lipids tested and cSec14 binds preferentially to PI(4)P. PI(4)P is enriched in the membranes of the trans-Golgi network (TGN) and when GFP-fusion proteins were expressed in neuroendocrine cells, cSec14, but not a- or b-Sec14, localized to the TGN. Since PIPs mediate localized protein interactions necessary for endocytic pathway functions, like AMPA receptor recycling, we used a transferrin uptake assay to evaluate KalSec14 interaction with endocytic pathway function. cSec14 expression interferes with receptor-mediated endocytosis in non-neuronal cells to a greater extent than bSec14. Lastly, we used an antibody specific to Ex1C to evaluate subcellular localization of Ex1C-containing proteins. In cultured neurons, most of the Ex1C signal was localized to dendritic spines and juxtaposed to Vglut1 puncta. In subcellular fractions of adult

mouse brain, the PSD is enriched in Ex1C-containing Kal7, while most extrasynaptic Kal7 is immune-negative for Ex1C. Thus, developmental regulation of Kalrn promoter usage modulates the lipid binding properties of the Sec14 domain, contributing to protein localization and interactions with membrane trafficking machinery.

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Poster

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Title: Immunoisolation and characterization of the PSD-95 interactome from postmortem human tissue with LC MS/MS

Authors: ***A. FUNK**[1], S. O'DONOVAN[1], J. MEADOR-WOODRUFF[2], R. MCCULLUMSMITH[1]
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Abstract: PSD-95 is the most abundant scaffolding protein in the postsynaptic density (PSD) and plays a major role in trafficking of ionotropic glutamate receptors. Through its PDZ domain, PSD-95 interacts with many synaptic proteins that regulate synaptic function. We developed a modified immunoisolation protocol using PSD-95 as the target to determine the PSD-95 interactome in four human brain regions (DLPFC, ACC, STG, and Hippocampus). We verified the specificity of this immunoisolation with Western blot analysis and mass spectrometry. Western blot analysis indicates a significant enrichment of PSD-95, capturing all available PSD-95 from 1500ug of total brain homogenate. Our PSD-95 immunoisolations were free of non-PSD proteins such as calreticulin, but included the GluA4 AMPAR subunit. Preliminary data from mass spectrometry show co-isolation of key postsynaptic components in order of abundance:

PSD-93, SAP97, SAP102, GluA2, Shank3, SynGAP, GluN1, GluA4, Homer1, GluA1, CaMKII, AKAP1, and Shank1. Previous studies indicate an immunoisolation of PSD-95 to be a de-facto postsynaptic fraction, containing putative PSD proteins. Our technique shows promise as a preparation of PSDs from human postmortem brain which would facilitate assessment of the PSD-95 interactome for postmortem studies of psychiatric, developmental, and neurodegenerative diseases with synaptic pathology.

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Poster

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Title: Activity-mediated synaptic trafficking of the palmitoyl-acyl transferase DHHC5

Authors: *S. BRIGIDI, B. SANTYR, J. SHIMELL, S. X. BAMJI
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Abstract: Neuronal activity can regulate the palmitoylation of synaptic proteins. However, the enzymatic activity of palmitoyl-acyl transferases (PATs) is not believed to be activity-regulated, suggesting that the precise control of palmitoylation may depend on the dynamic subcellular localization of PATs. The neuronal PAT, DHHC5, palmitoylates a number of synaptic proteins, including PSD-95, GRIP1b, and δ-catenin. We demonstrate that chemical LTP (cLTP) mediates the rapid and transient translocation of GFP-DHHC5 out of dendritic spine heads, followed by an increased interaction with δ-catenin and the co-transport of DHHC5/δ-catenin back into synapses. cLTP did not impact the localization of DHHC5 at inhibitory synapses. These results indicate that the activity-regulated dynamic trafficking of neuronal PATs allows for the rapid palmitoylation and delivery of substrate proteins to synaptic compartments.

Disclosures: S. Brigidi: None. **B. Santyr:** None. **J. Shimell:** None. **S.X. Bamji:** None.

Poster

213. Postsynaptic Structure II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 213.20/C21

Topic: B.07. Synaptic Transmission

Support: NIMH grant MH-10028395

NIH grant K23MH079498

Title: Dynamic developmental changes and gender differences in the abundance of postsynaptic NMDA signaling proteins in the mouse frontal cortex

Authors: *D. SINCLAIR[1], M. MCMULLEN[2], J. CESARE[1], G. C. CARLSON[2], C.-G. HAHN[1], K. BORGMANN-WINTER[1,3]
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Abstract: Background: Normal development of glutamatergic neural circuitry and underlying NMDA receptor (NR) signaling are critical for healthy brain function, while abnormal NR signaling has been implicated in neurodevelopmental psychiatric illnesses such as schizophrenia. The postsynaptic microenvironment plays host to, and modulates, critical NR signaling events, but it is not clear how the postsynaptic environment changes across the lifespan and how these changes may contribute to gender- and age-related vulnerabilities to psychiatric illness. We investigated the postsynaptic abundance of NR1/2A/2B (NR subunits), Src (a key modulator of NR activity), Psd95 (a PSD scaffolding protein) and PLCγ (an NR signaling target) in total membranes and the postsynaptic density (PSD) from the frontal cortex of neonatal, juvenile, adolescent and adult mice. Methods: PSD and membrane fractions were isolated biochemically from the frontal cortex of wildtype C57BL/6 and heterozygous DTNBP1 mutant mice aged 7, 14, 28 and 56 days (males; n=6 per age group, females; n=4 per age). Proteins were quantified by Western blotting, normalized to Psd95 (PSD) and actin (membranes) and analyzed by factorial ANOVA. Results: In the PSD, striking gender differences and developmental changes in the ratio of NR2A:NR2B and the abundance of Src in the PSD were observed. The ratio of NR2A:NR2B was greater in males than females ($p<0.0005$) and increased across development in both genders ($p<1x10-5$), consistent with an increasing role for NR2A-containing complexes later in life. In contrast, the abundance of Src in the PSD was lower in males than females

($p<0.0005$), and decreased across development in both genders ($p<0.001$). In membranes generally, there were no gender differences in NR2A:NR2B ratio or Src. PSD expression of NR1 ($p<0.0001$) and NR2B ($p<0.05$) decreased across development, despite increasing in membranes generally ($p<0.05$ and $p<0.00005$ respectively). PLCγ also decreased in the PSD across development ($p<0.01$). No differences between wildtype and DTNBP1 het mice were seen. Conclusions: Postsynaptic NR signaling machinery changes dynamically during development, and striking gender differences in key components of the NR signaling pathway exist throughout. Experiments are underway using SILAM mass spectrometry, immunoprecipitation, electrophysiology and behavioral assays to confirm and extend these findings. Observed gender differences in NR signaling in the PSD may be relevant to schizophrenia, a developmental disorder in which disrupted NR signaling and Src hypofunction have been implicated and sex differences in age-of-onset and symptom severity have been observed.

Disclosures: D. Sinclair: None. **M. McMullen:** None. **J. Cesare:** None. **C. Hahn:** None. **K. Borgmann-Winter:** None. **G.C. Carlson:** None.

Poster

213. Postsynaptic Structure II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 213.21/C22

Topic: B.08. Synaptic Plasticity

Support: R01NS058867

R01MH095948

Title: AC5-PKA signaling pathways are required for a stress-induced AMPA receptor switch in cerebellar stellate cells

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[2]Dept. of Cell Biol. & Mol. Med., New Jersey Med. Sch., Newark, NJ

Abstract: An acute natural stimulus, fox urine, can alter the synaptic AMPA receptor (AMPAR) phenotype from GluA2-lacking to GluA2-containing AMPARs in cerebellar stellate cells. This stress-induced AMPAR switch requires gene transcription and can be blocked by a beta-adrenergic receptor blocker (Liu, et al. 2010). However, the mechanism underlying this

transcription-dependent switch in AMPAR phenotype is not known. We hypothesize that adenylyl cyclase (AC)-PKA-CREB signaling pathways are involved. AC5 appears to be expressed in cerebellar stellate cells (Allen Brain Atlas). We found that the frequency of spontaneous action potentials in stellate cells increased during the application of noradrenaline or the AC activator forskolin (50 μM). This change was blocked by the AC5 antagonist NKY80 (20 μM), suggesting the presence of functional enzyme. Next, we determined the role of AC5 in the stress-induced AMPAR switch. Mice were exposed to fox urine for 5 min, and 3 hrs later we recorded spontaneous EPSCs in stellate cells at different potentials using a pipette solution that contained spermine. We found the I-V relationship of EPSCs changed from inwardly rectifying to linear in wild type mice, indicating that synaptic AMPARs had switched from GluR2-lacking to GluR2-containing receptors. In contrast the I-V relationship did not change in AC5 KO mice. To further investigate the underlying cellular mechanisms, we incubated cerebellar slices with noradrenaline (NA) at 37°C for 3 hrs to mimic the stress-induced AMPAR switch. We found that the NA treatment failed to change the I-V relationship of EPSCs to linear in AC5 KO mice. The presence of NKY80 during the incubation of WT cerebellar slices with NA also abolished the NA-induced switch. Thus activation of AC5 is required for the stress-induced switch in synaptic AMPAR phenotype. Activation of AC5 is known to elevate cAMP levels, thereby enhancing PKA activity. In the presence of a PKA inhibitor, PKI, NA failed to alter the synaptic AMPAR subtype in stellate cells. A second PKA inhibitor, H89, also abolished the NA-dependent switch in synaptic AMPA receptors. We also found that acute stress increased the pCREB level in cerebellar stellate cells from wild type animals. Together these results indicate that noradrenaline acting via adrenergic receptors activates AC5 and elevates cAMP levels, which increases PKA activity, thereby promoting GluA2 transcription.

Disclosures: Q. Yang: None. **S. Liu:** None. **Y. Ishikawa:** None.

Poster

213. Postsynaptic Structure II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 213.22/C23

Topic: B.02. Ligand-Gated Ion Channels

Support: NSF CAREER award: NS#065374

Tulane COR Summer Fellowship

Title: NMDA receptor-mediated signaling regulates NeuroD2 expression in developing cortex to control neuronal excitability and synaptic E/I balance

Authors: *F. CHEN[1], J. T. MORAN[2], K. ATES[2], Y. ZHANG[1], P. DAS[1], F. JONES[1], B. J. HALL[1]
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Abstract: Activity-dependent regulation of gene transcription is critical for proper cortical development by promoting cell proliferation and differentiation as well as mediating regimes of synaptic plasticity involved in sculpting neural circuits. N-methyl-D-aspartate receptors (NMDARs) are critical regulators of gene expression. To determine how regulation of transcription by NMDARs is involved in cortical development, we first carried out genome-wide microarray analysis in cultured mouse cortical neurons treated with the competitive NMDAR antagonist APV. Neurogenic differentiation factor 2 (NeuroD2), a late-phase, calcium-activated, basic helix-loop-helix transcription factor, was identified as negatively regulated by NMDAR-mediated signaling both in this *in vitro* assay, and *in vivo*. Our data show that NeuroD2 mRNA expression is regulated by NMDARs via CaMKII, at the level of transcription but not mRNA stability. To examine its role in cortical development we over-expressed NeuroD2 in cultured cortical neurons. This resulted in an increase in spontaneous action potential firing rates, along with a decrease in mIPSC (mini inhibitory postsynaptic current) amplitudes. Furthermore, knocking-down NeuroD2 using siRNA increased mIPSC amplitudes. Genetic removal of NeuroD2 *in vivo* also resulted in an overall decrease in the excitatory/inhibitory synaptic ratio in cortical layer II/III pyramidal neurons. These results show that NMDAR signaling regulates NeuroD2 expression to control synaptic E/I balance and neuronal excitability.

Disclosures: **F. Chen:** None. **J.T. Moran:** None. **K. Ates:** None. **Y. Zhang:** None. **P. Das:** None. **F. Jones:** None. **B.J. Hall:** None.

Poster

213. Postsynaptic Structure II

Location: Halls A-C

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Program#/Poster#: 213.23/C24

Topic: B.07. Synaptic Transmission

Support: MH067229

DA007254

Title: Role of ER calcium release in the mechanism of mGluR-LTD

Authors: *J. PICK, L. KHATRI, E. ZIFF
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Abstract: Although insertion and removal of AMPARs at the synapse has been extensively studied, little is known about the signals required to initiate AMPAR trafficking from the endoplasmic reticulum (ER) towards the synapse. Recent studies from our lab have demonstrated that release of calcium from ER stores leads to GluA2 trafficking out of the ER and accumulation on the cell surface via a PICK1-CaMKII mechanism. In order to explore the physiological relevance of this mechanism we tested the hypothesis that induction of mGluR-LTD, a process that leads to the release of calcium from ER stores, causes the trafficking of GluA2 containing AMPARs from the ER towards the synapse. We used biochemical and immunoflorescent techniques to examine medium spiny neurons in a cultured neuron system, and we provide evidence that release of calcium from the ER upon DHPG induced activation of group 1 mGluRs leads to increased trafficking of GluA2 out of the ER and accumulation at the cell surface. Furthermore, activation of group 1 mGluRs in certain brain regions including the nucleus accumbens leads to removal of synaptic calcium-permeable AMPARs (CPARs) and replacement with GluA2-containing AMPARs that are not permeable to calcium. Using electrophysiological recordings we show that this calcium-induced GluA2 release is involved in the replacement of CPARs with GluA2-containing AMPARs. This mechanism may underlie recent behavioral findings that inducing mGluR-LTD in the nucleus accumbens can help alleviate incubation of cocaine craving by replacing CPARs with GluA2-containing AMPARs.

Disclosures: J. Pick: None. **L. Khatri:** None. **E. Ziff:** None.

Poster

213. Postsynaptic Structure II

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 213.24/C25

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS057266

Georgia Regents University Startup fund

Title: Subunit-specific regulation of NMDA receptor trafficking by SAP102 splice variants

Authors: *B.-S. CHEN, Z. WEI
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Abstract: Synapse-associated protein 102 (SAP102) is a scaffolding protein abundantly expressed early in development and mediates glutamate receptor trafficking during synaptogenesis. Mutations in human SAP102 have been reported to cause intellectual disability, which is consistent with its important role during early development. SAP102 binds directly to N-methyl-D-aspartate receptors (NMDARs), anchors receptors at synapses and facilitates transduction of NMDAR signals. Proper localization of SAP102 at the postsynaptic density (PSD) is essential to these functions. However, how SAP102 is targeted to synapses is unclear. In the current study, we find that synaptic localization of SAP102 is regulated by alternative splicing. SAP102 splice variant that possesses a C-terminal insert is highly enriched at dendritic spines. Previously, we have shown that SAP102 expression promotes spine lengthening. We now find that the spine lengthening effect is independent of the C-terminal alternative splicing of SAP102. In addition, expression of SAP102 splice variants containing the C-terminal insert is regulated developmentally. Finally, knockdown of endogenous SAP102 splice variants containing the C-terminal insert differentially affect NMDAR surface expression in a subunit specific manner. These data reveal a novel role for SAP102 in the regulation of NMDAR trafficking.

Disclosures: B. Chen: None. **Z. Wei:** None.

Poster

213. Postsynaptic Structure II

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Topic: B.07. Synaptic Transmission

Support: NIH HD056370

NIH HD052731

NIMH T32-MH076690

Title: Postsynaptic mGluR5 promotes evoked glutamatergic synaptic transmission in neocortical Layer 2/3 Pyramidal Neurons

Authors: K. W. LOERWALD, A. B. PATEL, K. M. HUBER, *J. R. GIBSON
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Abstract: The role of metabotropic glutamate receptor 5 (mGluR5) in regulating synaptic function has been demonstrated in various glutamatergic neocortical synaptic pathways. mGluR5's role has primarily been examined in acute paradigms in which either specific patterns of synaptic activity or chemical stimulation of the receptor are used to induce mGluR5-dependent forms of synaptic plasticity, and is generally thought to weaken synaptic transmission. However, the baseline role of mGluR5 in regulating the strength of evoked synaptic transmission in various pathways throughout early postnatal development has not been precisely examined. Previous studies seeking to address this question have employed large-scale deletions of mGluR5 in entire cell populations or throughout the entire brain. This approach is limited by secondary, indirect effects which complicate conclusions made about mGluR5-mediated synaptic regulation at the cell-autonomous level. To circumvent these issues inherent to manipulating an entire network of neurons, here we have deleted mGluR5 in a minority of individual layer 2/3 neocortical pyramidal neurons using *in utero* electroporation and examined the effects on excitatory synaptic transmission. Based on the well characterized role of mGluR5 as a negative regulator of synaptic function in the cortical pathways we examined, we hypothesized that deletion of mGluR5 throughout development would result in an increase in excitatory synaptic function. Electrophysiological measurements were made in acutely prepared slices to obtain an accurate snap shot of the *in vivo* effects due to loss of mGluR5. Loss of postsynaptic mGluR5 results in an increase in the frequency of action potential-independent synaptic events, but paradoxically, results in a decrease in evoked transmission in 2 separate synaptic pathways providing input to these pyramidal neurons. In the local L2/3 pathway, the decrease in evoked transmission appears to be largely due to a decrease in cell-to-cell connectivity and not in the strength of individual cell-to-cell connections. Our data suggest a new role for mGluR5, different from what has been observed in studies examining its acute function, where baseline mGluR5 function promotes synaptic pathway strength in multiple cortical glutamatergic pathways.

Disclosures: K.W. Loerwald: None. **A.B. Patel:** None. **J.R. Gibson:** None. **K.M. Huber:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

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Program#/Poster#: 214.01/C27

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS078645

BYU Mentoring Environment Grants

Title: The putative cannabinoid receptor GPR55: Expression, modulation of hippocampal plasticity and behavior

Authors: *K. M. HURST, C. BADGLEY, S. BELL, B. PRINCE, J. G. EDWARDS
BYU, Provo, UT

Abstract: Learning and memory occur due to adaptive brain changes in response to our environment. These changes are mediated by synaptic plasticity, particularly within the hippocampus. Plasticity can either strengthen or weaken synapses, known as long-term potentiation (LTP) or long-term depression (LTD) respectively. While many forms of plasticity are NMDA-dependent, recently endocannabinoids were identified to mediate several new forms of hippocampal synaptic plasticity. Endocannabinoids bind to receptors such as cannabinoid receptor 1 (CB1) and transient receptor potential vanilloid 1 (TRPV1) in several brain areas including the hippocampus. However, research has demonstrated a non-CB1/TRPV1-dependent endocannabinoid synaptic plasticity in the hippocampus. While the receptor(s) involved is currently unknown, several potential candidate receptors that bind the endocannabinoid anandamide have been identified. These are among the orphan G-protein coupled receptors (GPRs) whose distribution in the brain and/or function is less well known. GPR55 is of particular interest as it activates second messenger systems, including IP3-mediated increases in intracellular calcium. Using quantitative RT-PCR, electrophysiological and memory behavioral tasks we examined hippocampal GPR55 expression and function. GPR55 is expressed in hippocampus of both rats and mice. Cellular expression is currently being examined and appears to be rare in interneurons and more likely expressed by pyramidal cells. Interestingly, application of the GPR55 agonist LPI (2 μM) to wild-type mice demonstrates a significant enhancement of LTP in brain slices. This LPI effect was not noted in GPR55 knock-out (KO) mice, which exhibit significantly ($p < 0.05$) smaller LTP (146%) than wild-type (WT) (181%). We are also examining CA1 LTD and preliminary data illustrates no significant difference to this point between KO and wild-type mice. GPR55 also appears to increase release probability (Sylantyev et al., PNAS, 2013), denoting a presynaptic role. Paired-pulse ratios are now being analyzed between GPR55 KO and WT mice to confirm this finding; however we did not note a change in EPSCs in CA1 in response to 2μM LPI. Behaviorally, KO and WT mice did not differ substantially in the novel object recognition test, but we are investigating spatial navigation to examine differences. These data suggest GPR55 is expressed and physiologically relevant in the hippocampus. Because enhanced LTP is usually associated with better memory performance in rodents, this provides a potential target to enhance the cellular mechanism associated with memory formation.

Disclosures: **K.M. Hurst:** None. **C. Badgley:** None. **S. Bell:** None. **B. Prince:** None. **J.G. Edwards:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 214.02/C28

Topic: B.08. Synaptic Plasticity

Support: Conacyt-Mexico CB166241

Title: Senescence-related impairment in short and long-term potentiation at the Mossy Fiber-CA3 synapse of the hippocampus

Authors: ***A. VILLANUEVA**, E. J. GALVAN
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Abstract: In the hippocampal formation senescence is accompanied by a progression of biochemical modifications that ultimately affects its ability to generate and consolidate long-term potentiation (LTP), the physiological process that underlies learning and memory. However, the aging effects on the CA3 feed-forward inhibitory circuit or the posttetanic potentiation (PTP) and LTP at the mossy fiber to CA3 synapse (MF-CA3) are poorly understood. To explore the age-dependent alterations of this synapse, extracellular recordings were performed in acute hippocampal slices from aged (34 ± 2 months old, Wistar rats) and young rats (1 month old). Paired pulse facilitation (PPF; 60 ms ISI) was dramatically reduced in aged rats (1.1 ± 0.1; n=9 vs. 2.2 ± 0.1 in young rats). To assess for changes in the CA3 feed-forward inhibitory circuit, a paired-pulse inhibition protocol (PPI, 8 ms ISI) was applied. Aged rats showed decreased PPI ratio (0.7 ± 0.3 and 1.1 ± 0.1; for young and aged animals, respectively; n=7). Next, we examined induction of MF-CA3 LTP. HFS (3 trains 100 Hz, at 10 sec interval), produced smaller posttetanic potentiation (PTP) in aged (157.6 ± 33%; n=6) vs. young animals (450.7 ± 57.8%; n=11). Furthermore, MF LTP induction in aged animals was smaller (85.0 ± 17.1) and was not induced in 8 out of 10 slices. In contrast, young animals consistently exhibit robust LTP (229.9 ± 44.2%; n=11). In all cases, DCG-IV suppressed the evoked responses (MF depression: 96.9 ± 10.3%). Because MF-LTP induction requires adenylyl cyclase/PKA activity, in another set of experiments, forskolin in combination with IBMX was bath-applied for 15 min. In aged animals, FSK+IBMX induced a fast but transient enhancement of MF fEPSPs, which gradually returned to baseline values in 5 out of 6 slices (MF fEPSP during FSK+IBMX wash in 248.4 ±

30.5%; at 30 min, 105.08 ± 5.9% and at 90 min, 81.1 ± 8.6). Taken together, these data show that hippocampal senescence is associated with significant weakening of the CA3 feed-forward inhibitory circuit, and biochemical changes that compromise the integrity of the signaling cascades responsible for MF PTP and LTP.

Disclosures: A. Villanueva: None. **E.J. Galvan:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: NIAID IAA number AOD12058-0001-0000

DTRA JSTO Medical S&T grant CBM.THRTOX.01.10.RC.021

Title: Cellular and molecular correlates for neurotrophic and neurotoxic long-term potentiation in networked neurons derived from stem cell populations

Authors: *A. B. BRADFORD[1], P. H. BESKE[1], M. E. LYMAN[1], K. S. HUBBARD[1], I. M. GUT[2], P. M. MCNUTT[1]
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Abstract: Synaptic strengthening through long-term potentiation (LTP) underlies learning and memory. However, changes in synaptic weighting caused by trauma such as brain injury, epileptic seizure or chronic stress can increase susceptibility to activity-related pathophysiologies. An *in vitro* population of networked neurons that exhibits canonical responses to altered synaptic activity will provide a platform for controlled, focused studies of the roles of aberrant signaling mechanisms in neuropathologies. We have developed methods to reproducibly generate and characterize mouse embryonic stem cell-derived neurons (ESNs) for use as such a platform. ESNs develop active glutamatergic and GABAergic synapses and exhibit emergent network behavior with an excitatory:inhibitory balance and synaptically driven action potential propagation within 21 days after differentiation. Increasing excitatory synaptic signaling by addition of presynaptic (3,4-DAP) or post-synaptic (bicuculline) modulators rapidly activates the MAPK/ERK pathway, causing increased CREB phosphorylation within 1 min and upregulated transcription of a wide array of immediate early genes (IEG) within 30 min,

including *c-Fos, JunB, Homer-1a, Arc* and *Egr1-4*. Within 4 h, expression of brain-derived neurotrophic factor (BDNF) and phosphorylation of its canonical receptor TrkB is detected, suggestive of late-phase LTP. These data suggest that 3,4-DAP and bicuculline are acting as neurotrophic modulators in networked cultures of ESNs. In contrast, treatment with as little as 1 uM glutamate evokes excitogenic processes that lead to neurotoxicity within 6 h, mediated by extrasynaptic NMDAR Ca^{2+} uptake. These glutamate treatments evoke extrasynaptic signaling pathways that interrupt MAPK/ERK signaling, elicit acute energetic failure and prevent BDNF expression. These data confirm that ESNs exhibit canonical functional and molecular correlates of early and late phases of LTP as well as excitogenic responses to pathophysiologic conditions. Further temporal characterizations of LTP in ESNs include elucidation of electrophysiological markers, AMPA receptor trafficking and phosphorylation, involvement of the mTOR pathway and changes in synapse architecture. Determining the scope and types of treatments that distinguish between neuroprotection and excitotoxicity in a defined population of networked neurons will provide a reproducible, highly synchronized platform for more complex studies, such as characterizing susceptibilities to excitotoxicity based on development stressors, alteration of signaling pathways and other gene-environmental interactions.

Disclosures: A.B. Bradford: None. **P.H. Beske:** None. **M.E. Lyman:** None. **K.S. Hubbard:** None. **I.M. Gut:** None. **P.M. McNutt:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant 5R01MH092926-04

Title: Electric fields boost long term potentiation *in vitro*

Authors: *G. KRONBERG[1], M. BRIDI[2], T. ABEL[2], L. PARRA[1]
[1]Biomed. Engin., The City Col. of New York, New York, NY; [2]Biol., Univ. of Pennsylvania, Phildelphia, PA

Abstract: Transcranial direct current stimulation (tDCS) is a promising neuromodulatory technique that has been shown to induce lasting changes in cognitive function. Generally, a weak direct current (<2mA) is applied via electrodes on the scalp for an extended period of time

(usually 20 minutes). tDCS has already been shown to elicit a number of desirable cognitive effects that are relevant to the treatment of many diseases, ranging from motor deficits to depression. Depending on the duration, intensity, location of scalp electrodes, and the cognitive or training task being performed, the effects of tDCS can outlast the duration of the stimulation for hours or even days. tDCS induces electric fields in the brain and the observed long-term effects suggest that electric fields influence training-induced plasticity. However, the exact mechanisms of action on plastic changes are not well understood. There are a number of human, animal and in-vitro studies showing lasting physiological effects that point to altered synaptic plasticity as the substrate for long term effects. However, there is not direct evidence that endogenous plastic mechanisms, such as Hebbian long term potentiation (LTP) are altered by electric fields. Here we studied frequency dependent LTP in the Schaffer collateral pathway of hippocampal slices in rat and mice. Tetanus consisted of 900 pulses at 20 Hz and occurred after 20 minutes. During the tetanus a DC electric field was applied across the hippocampal slice. We found a significant increase in the amount of LTP at 60 minutes after tetanus for anodal stimulation (in rat: 123.08 +/-14.4% control vs 151.86 +/-17.48% anodal, $p = 0.042$, N=5,3; in mouse: 127% +/- 12.4% control vs 167.9 +/- 6.6% anodal, $p = 0.037$ N=8,7). Thus, the electric fields induced during tDCS may lead to training-specific long-term changes in cognitive function by boosting Hebbian synaptic plasticity.

Disclosures: **G. Kronberg:** None. **L. Parra:** None. **T. Abel:** None. **M. Bridi:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

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Program#/Poster#: 214.05/C31

Topic: B.08. Synaptic Plasticity

Title: Hippocampal overexpression of tau affects synaptic transmission, plasticity, and network function

Authors: *F. JOW, M. LI, S. BROWNE, T. BUSSIERE, M. WITTMANN
Biogen Idec, Cambridge, MA

Abstract: The microtubule associated protein tau is implicated in neurodegenerative diseases, such as Alzheimer's Disease (AD). During disease progression, hyperphosphorylation of tau leads to somatodendritic mislocalization, intracellular accumulation of fibrillar soluble tau species and eventually the formation of neurofibrillary tangles. Importantly, tau pathology

correlates well with the progression of cognitive decline in AD. It has been previously shown that in transgenic mice overexpressing mutant human tau, hyperphosphorylated soluble tau proteins are mislocalized to intact dendritic spines where they impact synaptic function and plasticity and thus might impair cognitive processes. In addition, tau has been implicated in seizure susceptibility and a recent publication demonstrated that mice overexpressing human mutant tau develop spontaneous seizures and have a higher sensitivity to seizures induced by the GABAA antagonist pentylenetetrazol (PTZ). Since it is not clear whether developmental processes are involved in synaptic and network disturbances in tau-overexpressing transgenic mice, we used an AAV vector expression system to increase tau expression in the hippocampus of adult wild-type mice. C57BL/6 mice were injected into the hippocampus with AAV expressing a Dual-2N4R-P301L/K18DK280 construct or empty vector and sacrificed 3 months and 6 months later. Acute hippocampal slices were prepared and extracellular field recordings conducted to measure synaptic function, long term potentiation, and seizure susceptibility. We found that long term potentiation was increased at 3 months and 6 months after injection. While synaptic transmission was comparable between groups at 3 months after injection, at 6 months it was significantly decreased in mice expressing mutant tau. In addition, overexpression of tau increased seizure susceptibility in a bicuculline-induced seizure-like activity assay. These data suggest that overexpression of tau is responsible for synaptic and network dysfunction and that some changes occur in the absence of loss of synapses. The decrease in synaptic transmission at 6 months suggests that overexpression and/or aggregation of tau protein over time could trigger a potential loss of functional synapses. Immunohistochemical analysis is ongoing to determine whether these changes correlate with synaptic loss or other synaptic changes.

Disclosures: F. Jow: None. **M. Li:** None. **S. Browne:** None. **T. Bussiere:** None. **M. Wittmann:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

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Program#/Poster#: 214.06/C32

Topic: B.08. Synaptic Plasticity

Title: Evaluation of presynaptic actions using two different fluorescent probes for neurotransmitter release

Authors: *T. HIKIMA, G. ARBUTHNOT
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Abstract: Neurotransmitter release provides a powerful influence in neuronal network activity. It is a major resource and a source of reserve capacity for neuronal network processing. Typically an increase in synaptic efficacy is accompanied by an increase in presynaptic neurotransmitter release and imaging methods are an effective tool to investigate the kinetics for neurotransmission in cortical presynaptic terminals. We used two fluorescent probes for neurotransmitter release, synaptophysin-pHluorinx2 (SypHx2) and intensity-based glutamate-sensing fluorescent reporter (iGluSnFR) to optically visualize neurotransmitter release. SypHx2 is the fusion of synaptic vesicle protein synaptophysin with two molecules of the super ecliptic pHluorin, pH-sensitive green fluorescent protein (GFP), iGluSnFR is a sensor for synaptically released glutamate constructed using a bacterial periplasmic binding protein and circularly permulated GFP. We could detect the fluorescent change of SypHx2 (ΔSypHx2) with 5 pulses electrical stimulation and estimate the size of the readily releasable pool (RRP) with high stimulation rates. On the other hand, the fluorescent change of iGluSnFR (ΔiGluSnFR) was much higher. ΔiGluSnFR signals to single pulse field stimulation were reliably detected and reached a plateau after 5 pulses. Both signals were dependent on external calcium concentration. We found the cAMP/PKA activity uncovered a number of presynaptically silent synapses using both methods, and the increase depended on the size of the RRP. Based on our results, these two fluorescent probes are useful tools to follow the alteration of the presynaptic efficacy and examine the properties of presynaptic sites more precisely at tiny presynaptic terminals.

Disclosures: T. Hikima: None. **G. Arbuthnot:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 214.07/C33

Topic: B.08. Synaptic Plasticity

Support: NIH DA011289

Brown Institute for Brain Science (JAK)

Title: Long-term potentiation of glycinergic synapses triggered by interleukin-1β

Authors: *A. M. CHIRILA[1], T. E. BROWN[3], R. J. STEVENSON[2], R. A. BISHOP[2], J. A. KAUER[1]
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Abstract: While glycine is a major inhibitory neurotransmitter in key areas of the CNS, little is known about the regulation of glycinergic synaptic strength in native tissue. Here we report that in the spinal cord dorsal horn, glycinergic synapses on GAD65-EGFP labeled lamina II inhibitory interneurons exhibit LTP, triggered rapidly after 10 minute exposure to the inflammatory cytokine interleukin-1 beta (IL-1β, 10ng/ml; IPSC amplitudes: 165.0 ± 11% of pre-IL-1β values, n=24, $p<0.0001$). Our data suggest that glycine receptor LTP (GlyR LTP) results from an increase in postsynaptic GlyR number or function. To probe this, we first disrupted membrane fusion reactions in GAD65-EGFP neurons. Blocking postsynaptic SNARE proteins by including N-ethylmaleimide (NEM; 5mM) in the recording pipette prevented GlyR LTP (IPSC amplitudes: 88.9 ± 9.3% of pre-IL-1β values, n=9, n.s.). We next recorded glycinergic IPSCs on GAD65-EGFP cells for a baseline period, followed by application of the high affinity GlyR antagonist strychnine (2μM) to block all surface GlyRs. Slices were then washed for 20 min and either IL-1β or BSA was bath-applied. Recovery from strychnine block was significantly greater in slices treated with IL-1β compared to BSA treated controls (% recovery at 40-46 minutes after washing strychnine: control 8.7 ± 3.7%; IL-1β: 28.8 ± 7.1%, n=5; $p<0.05$). Together our data are consistent with the possibility that IL-1β promotes exocytosis of intracellularly sequestered GlyRs. To test how GlyR LTP correlates with inflammatory hyperalgesia, we examined spinal cord slices from mice with *in vivo* formalin-induced inflammation vs. slices from saline treated controls. GABAergic neurons from saline-treated mice had robust IL-1β induced GlyR LTP, while those from formalin-treated hyperalgesic mice did not (IPSC amplitudes: saline-injected: 176.6 ± 16.7%, n=9; formalin-injected: 104.1 ± 10.5%, n=7; $p<0.005$). Furthermore, glycinergic mIPSCs in cells from formalin-treated mice were significantly larger compared to saline-treated controls (mIPSC amplitudes, formalin-treated, n=8: 148 ± 12% of saline-treated animals, n=5; $p<0.05$), suggesting that these synapses may be potentiated *in vivo* by inflammation-induced release of IL-1β. GlyR LTP is a novel form of plasticity that could disinhibit projection neurons carrying nociceptive information and may contribute to the development of inflammatory pain.

Disclosures: A.M. Chirila: None. **T.E. Brown:** None. **R.J. Stevenson:** None. **R.A. Bishop:** None. **J.A. Kauer:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 214.08/C34

Topic: B.08. Synaptic Plasticity

Support: NIH Grant 5R01MH092926-04

Title: Synaptic cooperativity and postsynaptic polarization jointly determine cortical plasticity during DC stimulation

Authors: *A. RAHMAN, M. BIKSON
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Abstract: Cortical neurons receive synchronous and convergent synaptic input from thousands of afferents that are susceptible to plastic changes. Effective long-term plasticity of the primary motor cortex is, however, contingent upon local disinhibition leading to rapid unmasking of latent intracortical connections. Alternatively, coincident input relieves the requirement for disinhibition to induce long-term potentiation. In rat primary motor cortex, we measured *in vitro* the population excitatory postsynaptic potentials evoked by a brief train of adapting afferent inputs and found that DC stimulation modulates synaptic efficacy through postsynaptic polarization and recruitment of ascending fibers. Acutely, we show that DC stimulation attenuates short-term depression during adaptation to afferent activity and post-adaptation, therefore sustaining higher levels of synaptic efficacy during stimulation. Modeling work based on experimental measurements indicates DC stimulation can enhance cortical potentiation through cooperative plasticity. Our results suggest a mechanism of inducing cortical synaptic plasticity during transcranial direct current stimulation (tDCS) driven by the number of coincident inputs. These results also point toward a prominent role of upstream brain regions from the primary motor cortex that drives motor output during tDCS and transcranial magnetic stimulation (TMS) through corticocortical and thalamocortical connections, particularly from somatosensory and premotor areas.

Disclosures: **A. Rahman:** None. **M. Bikson:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 214.09/C35

Topic: B.08. Synaptic Plasticity

Support: FAPESP Brazil 2012/06123-4

Title: Hippocampo-prefrontal synaptic plasticity and neuronal firing in the mediodorsal thalamus

Authors: *L. S. BUENO, JR, R. N. RUGGIERO, J. P. LEITE
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Abstract: Afferents from dorsal hippocampal CA1 and mediodorsal nucleus of thalamus (MD) share targets in the prefrontal cortex (PFC). MD and PFC are reciprocally connected, while projections from CA1 to the PFC are unidirectional. Noteworthy, there are no known projections from dorsal CA1 to the MD, suggesting that CA1 modulates the MD via PFC. Since MD and CA1 are thought to cooperate in PFC functions, we asked if CA1-induced long-term potentiation (LTP) in the PFC changes its communication with the MD. In urethane-anesthetized rats, we implanted a stimulating electrode into dorsal CA1, and recording microwire arrays into ipsilateral MD and PFC (prelimbic area). Electrical test pulses were applied into CA1 every 15 sec during a 30 min baseline, followed by LTP induction through high-frequency stimulation. Test pulses were then resumed for an additional two-hour recording. According to our initial results, test pulses changed firing in both PFC and MD, demonstrating the CA1-PFC-MD flow of information. High-frequency stimulation expectedly induced LTP of PFC field responses, and firing responses of both PFC and MD were altered by LTP induction. Our preliminary findings indicate that CA1-PFC synaptic plasticity can modulate MD activity. This could signify that MD-PFC excitatory reverberation assimilates information from CA1, especially during executive processes.

Disclosures: L.S. Bueno: None. **R.N. Ruggiero:** None. **J.P. Leite:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 214.10/C36

Topic: B.08. Synaptic Plasticity

Title: Layer-specific developmental profile of visual cortex function in the awake mouse

Authors: *J. L. HOY, C. M. NIELL
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Abstract: Layer-specific developmental profile of visual cortex function in the awake mouse
J.L. Hoy and C.M. Niell Institute of Neuroscience and Department of Biology, University of Oregon The laminar structure and conserved cellular organization of mouse visual cortex provide a useful model to determine the mechanisms that support the development of basic visual system function. However, the normal development of many receptive field properties, as well as the emergence of synchronized network activity, have not yet been thoroughly quantified as a function of cortical layer. Here, we employ multisite electrophysiological recording in the awake mouse across an extended period of development, starting at eye opening, to measure these functional properties in V1. We find that receptive field structure and properties such as response linearity rapidly reach mature-like states over the first few days after eye-opening in nearly all cortical layers. This first phase of development coincides with the maximum change in the peak firing rate of visually evoked activity. In a second distinct phase of development, features such as the tuning width of orientation selective units and peak spatial frequency response remain poor up to 4 days after eye-opening, but then rapidly reach adult levels by 7 days after eye-opening. In contrast, we see a gradual increase in orientation selective units within layers 2-5 between eye-opening and 7 days later. Not all developmental trends are common between layers. Direction selectivity undergoes significant developmental change only in layer 4 and units in layer 6 display more complex developmental progression relative to the other layers. Finally, behavioral state modulation of LFP gamma power also changes significantly over development in a layer specific manner. Unlike many individual receptive field properties, LFP oscillations begin to gradually mature after eye-opening but do not fully reach maturity until approximately 1.5 months of age. On-going studies seek to determine the cell type-specific molecular mechanisms that drive these changes across layers.

Disclosures: J.L. Hoy: None. **C.M. Niell:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 214.11/C37

Topic: B.08. Synaptic Plasticity

Support: CRC889

Title: PSD-95 regulates experience-dependent maturation of excitatory synapses in the visual cortex

Authors: X. HUANG[1], *Y. DONG[2], S. LÖWEL[3], O. SCHLÜTER[1]
[1]European Neurosci. Inst., Göttingen, Germany; [2]Dept Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [3]Bernstein Fokus Neurotechnologie and Johann-Friedrich-Blumenbach Inst. für Zoologie und Anthropol, Univ. of Göttingen, Göttingen, Germany

Abstract: Postsynaptic density-95 (PSD-95) is a postsynaptic signaling scaffold protein. It regulates synaptic transmission in excitatory synapses, presumably via coordinating signaling events in long-term synaptic plasticity. In the visual cortex (VC), the expression level of PSD-95 increases after eye opening and reaches the plateau at adult. Here, we studied the role of PSD-95 in regulating synaptic maturation during the critical period. We performed electrophysiological analysis from acute VC slices of PSD-95 KO and WT mice at different developmental stages. Unlike in WT control animals, where the number of AMPA receptor silent synapses decreases during development, the percentage of silent synapse in PSD-95 KO mice prevailed at high levels. Furthermore, knocking down PSD-95 in adult WT mice restored high level of silent synapse. The development of GABAergic transmission was normal in PSD-95 KO animals, suggesting that PSD-95 does not regulate the maturation of the inhibitory system. Thus PSD-95 is essential for the experience-dependent maturation and maintenance of the matured state of VC excitatory pyramidal cell synapses.

Disclosures: X. Huang: None. **Y. Dong:** None. **S. Löwel:** None. **O. Schlüter:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 214.12/C38

Topic: B.08. Synaptic Plasticity

Title: Keratan sulfate is involved in ocular dominace plasticity in visual cortex

Authors: *T. NATORI[1,2,3,4], K. NAGAI[2], K. KADOMATSU[3], Y. KOMATSU[4]
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Abstract: The major components of the extracellular matrix (ECM) in the central nervous system are proteoglycans (PG), hyarulonan, and tenascins. Especially, ECM around neurons is

called perineuronal nets (PNN). PNN begins to increase after critical period. In young animals, monocular deprivation leads to an ocular dominance shift, whereas in adults after the critical period, such shift can not be observed. However, it has been reported that the digestion of chondroitin sulfate with chondroitinaseABC (ChABC) recovers ocular dominance plasticity in adult rat primary visual cortex. Since there are many keratan sulfate proteoglycans (KSPG) in the central nervous system, KS may also function on the regulation of neuronal plasticity in primary visual cortex. We used slices of visual cortex prepared from adult rats. Before preparing slices, enzymes keratanase II (KSII) or ChABC was repeatedly injected at the same sites of visual cortex everyday for three days. After 4 or 5 days of treatment, we examined long-term potentiation (LTP). LTP was observed in slices from CP rats, but not from adult control rat. Interestingly, LTP was recovered in the slices from adults treated with KSII or ChABC. The magnitude of LTP in KSII or ChABC-treated adult slices was the same as that from CP. Next we examined whether KSII treatment of adult rats facilitates ocular dominance plasticity. Visual acuity was determined with visual evoked potentials. We found that KSII or ChABC treated rats recover ocular dominance plasticity like CP. Regarding the effects of enzyme treatment in brain cells, we surprisingly found that microglia was activated, and inhibition of microglial activation by minocycline prevented the recovery of ocular dominance plasticity. We found that KS digestion in primary visual cortex recovers ocular dominance shift in adult rats via microglial activation. Our data suggest that KS is involved in the regulation of neuronal plasticity and microglial activation contributes to the ocular dominance plasticity.

Disclosures: **T. Natori:** None. **K. Nagai:** None. **K. Kadomatsu:** None. **Y. Komatsu:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 214.13/C39

Topic: B.08. Synaptic Plasticity

Support: NIH

CIHR

FRSQ

IPN

Title: The involvement of Fragile-X related protein-1 (FXR1P) in regulating GluA2 mRNA translation, synaptic plasticity and learning and memory

Authors: *E. NURO[1], D. COOK[1], E. JONES[1], H. ALTIMIMI[1], W. FARMER[1], E. HANNA[1], A. BARBON[2], D. NELSON[3], J. ROCHFORD[4], D. STELLWAGEN[1], J.-C. BÉÏQUE[5], K. MURAI[1]
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Abstract: Fragile X Related Protein 1 (FXR1P) is one of two autosomal homologues of the Fragile X Mental Retardation Protein (FMRP), a protein whose expression is significantly reduced in Fragile-X Syndrome. Like FMRP, FXR1P is an mRNA binding protein that is implicated in regulating the translation of specific target proteins. However, in comparison to FMRP, little is known about the function of FXR1P in the brain. Our lab has recently discovered that FXR1P co-localizes with translational machinery near synapses suggesting that it could play a role in locally controlling the levels of proteins involved in synaptic plasticity and learning and memory. In order to test this, we have generated an FXR1 conditional knockout mouse model where the FXR1 gene is conditionally ablated from neurons in the forebrain, including the hippocampus. Interestingly, we have found that FXR1P is critical for regulating the expression of the AMPA-type glutamate receptor subunit GluA2; a protein known to have a profound role in synaptic plasticity and learning and memory. Moreover, we have also found that FXR1P conditional knock-out mice have significant changes in synaptic plasticity, synaptic morphology, and cognitive function. We are currently investigating the mechanism through which FXR1P regulates GluA2 expression, and in turn understand the implications of this mechanism in synaptic plasticity and learning and memory.

Disclosures: E. Nuro: None. **A. Barbon:** None. **D. Nelson:** None. **J. Rochford:** None. **J. Béïque:** None. **D. Cook:** None. **E. Jones:** None. **H. Altimimi:** None. **W. Farmer:** None. **E. Hanna:** None. **D. Stellwagen:** None. **K. Murai:** None.

Poster

214. LTP: Pre- and Postsynaptic Mechanisms I

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: FAPESP: process 2011/23874-0

FAPESP: process 2012/07522-0

Title: Cortical synaptic plasticity requires the production of reactive oxygen species

Authors: *R. DE PASQUALE, T. F. BECKHAUSER, M. S HERNANDES, L. R. G BRITTO
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Abstract: Reactive oxygen species (ROS) are signaling factors involved in many intracellular transduction pathways. In the nervous system, ROS are thought to modulate various mechanisms of synaptic plasticity. One important source of ROS production in the brain is the Nox2 isoform of the NADPH oxidase complex. Stimulation of NMDA receptors activates Nox2, which provides selective oxidative responses accompanying the induction of synaptic changes. Nox2 activity is known to be important for the induction of LTP in the hippocampus. However, the involvement of Nox2 in cortical plasticity is still unclear. To address this issue, we performed whole cell recordings in brain cortical slices obtained from wild type and knock out mice for the gp91phox subunit of Nox2. We evaluated synaptic plasticity by inducing LTP and LTD in layer 2/3 neurons of the primary visual cortex. We found that genetic ablation of Nox2 suppresses LTP and LTD. Our results also showed that this effect is age-dependent, suggesting that Nox2 is critical to maintain synaptic potentiation even after the maturation of visual cortical circuitry.

Disclosures: R. De Pasquale: None. **T.F. Beckhauser:** None. **M. S Hernandes:** None. **L.R. G Britto:** None.

Poster

215. Oscillations: Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 215.01/C41

Topic: B.09. Network Interactions

Support: NINDS: 1F31 NS083247

DARPA: BAA-09-27

NIH: RO1 NS-050434

Title: Robust inhibition and gamma oscillations in a cortical area that lacks parvalbumin-expressing inhibitory interneurons

Authors: *A. U. SUGDEN[1], S. L. PATRICK[2], R. D. BURWELL[3], B. W. CONNORS[2]
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Abstract: Three protein markers completely define exclusive classes of neocortical inhibitory interneurons in mice; parvalbumin (PV), somatostatin (Som), and a serotonin receptor subtype (5HT3aR). The relative proportions of these classes are consistent across the vast majority of the neocortex. As a group, inhibitory interneurons are vital for preventing seizures, regulating temporal and spatial coding, and generating oscillations and synchrony in pyramidal cells. Yet surprisingly, although PV cells are the most common class of inhibitory interneurons in most areas, PV cells are absent in the ventral region of postrhinal cortex (vPOR), a visuospatial association area. Furthermore, much work has implicated the fast-spiking (FS) PV cells in the generation of gamma oscillations; pulsed optogenetic activation of PV cells drives the network with strong resonance near 40 Hz. The naturally PV-sparse vPOR presents an opportunity for determining whether PV cells are critical for gamma oscillations. We have compared the sources of synaptic inhibition in vPOR to those of the neighboring dorsal POR (dPOR), where PV cells comprise 40% of the interneurons. Spontaneous miniature IPSCs onto excitatory cells were decreased in frequency by ~40% in vPOR, commensurate with the smaller number of inhibitory cells. Optogenetic stimulation of Som cells showed no difference in inhibitory currents between regions, while responses to stimulation of PV cells were lower by an order of magnitude in vPOR. Although the sum of the inhibitory currents from PV and Som cells would suggest a difference in global inhibition between vPOR and dPOR, optogenetic and electrical activation of all sources of inhibition onto excitatory cells yielded equally strong responses. Paired-pulse ratios of IPSCs were also similar in vPOR and dPOR. Furthermore, ramped optogenetic stimulation of excitatory cells induced gamma oscillations of similar power in both regions, even when the vPOR was isolated from neighboring PV-containing cortices. Optogenetic stimulation of inhibition onto inhibitory interneurons showed that inhibitory currents are smaller in vPOR. Reduced disinhibitory circuitry in vPOR enhances the total inhibitory input to its excitatory cells, which matches the dPOR control cortex. And, although Som cells demonstrate a low-threshold spiking phenotype in primary sensory cortex, roughly half of the Som cells of POR displayed a fast-spiking phenotype. Our results suggest that vPOR has adapted its local circuits and intrinsic physiological properties to maintain high levels of inhibitory function in the absence of PV interneurons.

Disclosures: A.U. Sugden: None. **S.L. Patrick:** None. **R.D. Burwell:** None. **B.W. Connors:** None.

Poster

215. Oscillations: Mechanisms

Location: Halls A-C

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Topic: B.09. Network Interactions

Support: NIH P30 EY000785

NIH R01 EY015788

NIH Grant RR19895

NIH Grant RR029676-01

Title: Functional mapping of developing neocortical networks

Authors: *J. B. ACKMAN[1], H. ZENG[2], M. C. CRAIR[1]
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Abstract: The cerebral cortex exhibits spontaneous and sensory evoked patterns of activity during fetal and postnatal development that are crucial for the activity-dependent formation and refinement of neural circuits. Knowing the source and flow of these activity patterns locally and globally is crucial to understanding self-organization in the developing brain. Here we describe a system for imaging patterns of activity throughout the developing isocortex *in vivo* at a 'mesoscopic' level of resolution in mice. Ongoing activity in the neonatal cerebral cortex was characterized by distinct and repetitively active domains measuring hundreds of microns in diameter that were coordinated within and between the hemispheres. These cortical activity patterns gave rise to characteristic network architectures with functional associations between brain areas and across hemispheres that were similar in mice of the same age but changed with development. Our imaging approach offers an unprecedented ability to study functional connectivity within and between the cerebral hemispheres at a scope and scale which bridges the microscopic and macroscopic resolutions offered by traditional neurophysiology and neuroimaging based recordings and will provide a practical non-invasive means to assess cortical connectivity with high spatial resolution across development and in pathophysiological models for autism, epilepsy, and schizophrenia.

Disclosures: J.B. Ackman: None. **H. Zeng:** None. **M.C. Crair:** None.

Poster

215. Oscillations: Mechanisms

Location: Halls A-C

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Program#/Poster#: 215.03/C43

Topic: B.09. Network Interactions

Support: NSF GRFP

NEI R01 EY019179-29

Nelson Fund

Title: Cholinergic modulation of gamma oscillations depends on a specific receptor subclass

Authors: *A. S. BRYANT, C. GODDARD, J. R. HUGUENARD, E. I. KNUDSEN
Stanford Univ., Stanford, CA

Abstract: Changes in gamma power correlate with attentional deficits in psychiatric diseases, and can be induced by cholinergic modulation experimentally in the mammalian neocortex and hippocampus and in the avian optic tectum (superior colliculus). However, the mechanisms by which different acetylcholine receptors modulate the components of the gamma-generating circuitry are not well understood. The midbrain selection circuit includes a local gamma-generating circuit within the optic tectum (composed of both NMDAergic and GABAergic circuit elements) that is reciprocally connected to an evolutionarily conserved cholinergic nucleus (Ipc). Cholinergic feedback powerfully regulates the power of tectal gamma oscillations (Goddard et al 2012); the precise mechanisms underlying this modulation are not known. We investigated these mechanisms; we found that nicotinic acetylcholine receptors (nAChRs) are primarily responsible, acting to enhance the power of gamma oscillations by selectively increasing the inhibitory component of the midbrain gamma generator. We identified the precise site of this enhancement, a population of neurons that are driven via the action of non-a7 subunit-containing nAChRs (non-a7 nAChRs). These neurons are located within a region of the gamma generator that contains parvalbumin-positive interneurons, a type of neuron that is critical for the generation of gamma oscillations within the cortex and hippocampus. Activation of these non-a7 nAChRs is both necessary and sufficient for cholinergic modulation of tectal gamma oscillations. Together, our results suggest a mode of cholinergic modulation whereby acetylcholine is broadly released, but can exert precise effects on a complex circuit through the selective expression of specific postsynaptic receptors on a specific neuronal population.

Disclosures: **A.S. Bryant:** None. **C. Goddard:** None. **J.R. Huguenard:** None. **E.I. Knudsen:** None.

Poster

215. Oscillations: Mechanisms

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Program#/Poster#: 215.04/C44

Topic: B.09. Network Interactions

Support: CIHR

NSERC

Title: Optogenetic activation of glutamatergic medial septum neurons drive activity across the hippocampal network

Authors: J. ROBINSON[1], F. MANSEAU[1], *L. K. SRIVASTAVA[2], S. WILLIAMS[1]
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Abstract: The medial septum diagonal bands of broca (Ms-DBB) area provides important connections to the hippocampus and plays a critical role in theta rhythms associated with learning and memory. Three main neuronal populations have been identified in this region: cholinergic, GABAergic and glutamatergic. While the role of GABAergic and cholinergic neurons connectivity have been explored for the several decades, the influence of glutamatergic neurons is not well understood. To further explore the role of MS-DBB glutamatergic cells we have used optogenetics to specifically target this population of neurons. The first aim of our study has been to determine at the cellular level how glutamate release from MS-DBB neurons can modulate post-synaptic targets in the subiculum, CA1 & CA3 on both principal cells as well as interneurons across the hippocampus. The second aim of the current study has been to determine whether increasing the firing rate of glutamate neurons is sufficient to modulate hippocampal theta oscillations *in vitro*. We have specifically targeted glutamatergic MS-DBB neurons through injection of a Cre- dependent AAV virus containing the light-sensitive protein ChETA construct in a VGLUT2-CRE mice line. Recording techniques include both patch-clamp and field recordings in the *in vitro* septo-hippocampal preparation. By selectively activating this neuronal population, we have observed postsynaptic responses in both neurons within the septum as well as interneurons in the hippocampus. With varying frequencies of light stimulation,

excitatory post-synaptic responses can be observed up to 2.5 mV on interneurons in the str oriens of area CA3. In addition, glutamatergic MS-DBB neurons can modulate hippocampal theta rhythm in the whole septo-hippocampal network *in vitro*, increasing both the power and oscillatory strengths of theta rhythm across subregions of the hippocampus. With periodic stimulation of the medial septum, theta oscillatory power was increased up to 30% from baseline. These experiments may provide insight into of how medial septum glutamate neurons contribute to hippocampal theta activity. Finally, these results will also provide the background necessary to investigate how medial septum glutamatergic input contribute to memory formation.

Disclosures: J. Robinson: None. **L.K. Srivastava:** None. **F. Manseau:** None. **S. Williams:** None.

Poster

215. Oscillations: Mechanisms

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Topic: B.09. Network Interactions

Support: NIH Grant MH060605

NSF Grant DMS-1313861

Title: Current versus voltage clamp measurements of resonance in neuronal systems: lessons from the response of biophysical models to oscillatory inputs

Authors: *F. NADIM[1], H. G. ROTSTEIN[2]
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Abstract: Many neuron types exhibit resonance properties in which the impedance amplitude of the neuron is maximal at a non-zero frequency (fres). Impedance (Z = V / I) can be measured using sinusoidal inputs in current clamp (Iclamp) or voltage clamp (Vclamp) conditions. Experimental studies have reported that similar fres values are measured in the two conditions; however, it is not clear whether these values correspond to identical impedance properties. We address this problem using mathematical analysis of conductance-based neuron models. Although the Iclamp and Vclamp techniques are complementary, there are fundamental differences between the two: in Vclamp, V and therefore dV/dt are controlled, whereas in Iclamp

these factors are free to vary. As a result, the description of the membrane properties in Iclamp requires an additional dynamic variable V and therefore one extra effective dimension. This has important consequences for the degrees of complexity in the two conditions and implies that time-varying signals should produce fundamentally different responses. We use 2D models to explore the impedance (amplitude and phase) properties in response to sinusoidal inputs in Iclamp and Vclamp. For the appropriate parameter values, these models exhibit amplitude and phase resonance (zero phase shift response at a nonzero input freq fphase). In Iclamp, the (sinusoidal) input signal is simple but the response dynamics are 2D. Conversely, in Vclamp, the response dynamics are 1D but there is higher complexity in the input signal which involves both the sinusoidal V and dV/dt. We first examine the resonant properties of linearized models. Despite the difference in effective dimensionality of the underlying systems, surprisingly, in the linear case, the impedance profiles (f-Z) are identical. In contrast, the effects of nonlinearities on the impedance profiles in the full biophysical models are properly captured only in Iclamp conditions. In particular, the amplification of resonance power by resonant and amplifying currents is lost in Vclamp and the two conditions produce different values of fres. Similarly, the two conditions produce different phase shifts and different fphase values. The advantage of Vclamp is that it provides complete instantaneous control over the voltage range and trajectory, thus resulting in a controlled measurement of ionic currents. However, although Vclamp and Iclamp conditions provide identical impedance profiles for linear systems, these methods are not equivalent when comparing the impedance profiles and resonance properties of neurons and that, in general, Iclamp conditions provide a more accurate estimate of the neuron's response.

Disclosures: **F. Nadim:** None. **H.G. Rotstein:** None.

Poster

215. Oscillations: Mechanisms

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Topic: B.09. Network Interactions

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Title: The role of a persistent inward current in shaping membrane resonance properties of different neuron types in an oscillatory network

Authors: *D. M. FOX[1], H.-A. TSENG[3], H. G. ROTSTEIN[4], F. NADIM[2]
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Abstract: Membrane resonance, a maximal voltage response to sinusoidal current input at a non-zero frequency (fres) is a property of many neurons types and is often correlated with the frequency of the networks in which they are embedded. Neuromodulators that act on voltage gated ionic currents that underlie membrane resonance can also change fres. However, resonance often involves the interaction of multiple voltage-gated currents and thus it is unclear how a neuromodulator that targets a single ionic current affects the resonance properties. It is also unknown whether the changes in fres influence network activity. We examine the modulatory effects of changing a persistent inward current in shaping the resonance properties of an oscillatory neuron. In the pyloric network of the crab stomatogastric nervous system, the pacemaker PD neurons show resonance with fres (~ 1Hz) correlated with the network frequency. In contrast, the follower LP neurons show a significantly higher fres (~1.5 Hz). Both cells have an h-current (Ih) and are targets of peptide neuromodulators that activate a voltage-gated non-specific inward current (IMI). We compare the sensitivity of fres, measured in voltage clamp, in the PD and LP neurons to the voltage ranges of oscillation (Vlo to Vhi). Our results show that fres in both cells increases if Vhi is increased. However, only in PD, and not in LP, fres is sensitive to Vlo. Using a multi-objective genetic algorithm approach, we compare single-compartment models that include Ih and IMI to show how the parameters of these two currents can give rise to the different sensitivities. In voltage clamp, fres results from the addition of currents having different amplitudes and phases. To understand why fres is sensitive to Vlo and Vhi we analytically track gating variables of individual ionic currents to see how the amplitudes and phases interact with input frequency. We show how fres, and the amplitude and phase of Ih and IMI change with changes in their dynamics. Moreover, we show how this is affected when Vlo and Vhi are varied. Finally, we explore the dynamics of IMI and Ih activation using realistic voltage waveforms of the PD and LP neurons at different amplitudes and frequencies to examine the role of this modulatory current during ongoing pyloric oscillations. Our results underline the different resonant properties generated by the same ionic currents in different neuron types in a network.

Disclosures: D.M. Fox: None. **H. Tseng:** None. **H.G. Rotstein:** None. **F. Nadim:** None.

Poster

215. Oscillations: Mechanisms

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Title: Dopaminergic modulation of hippocampal sharp wave-ripple *in vitro*

Authors: *T. MIYAWAKI, H. NORIMOTO, T. ISHIKAWA, Y. WATANABE, N. UEMURA, N. MATSUKI, Y. IKEGAYA
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Abstract: Hippocampal sharp wave (SW)/ripple complexes are thought to contribute to memory consolidation. Previous studies suggest that behavioral rewards facilitate SW occurrence *in vivo*. However, little is known about the precise mechanism underlying this enhancement. Here, we examined the effect of dopaminergic neuromodulation on spontaneously occurring SWs in acute hippocampal slices. Local field potentials were recorded from the CA1 region. A brief (1 min) treatment with dopamine led to a persistent increase in the event frequency and the magnitude of SWs. This effect lasted at least for our recording period of 45 min and did not occur in the presence of a dopamine D1/D5 receptor antagonist. Functional multineuron calcium imaging revealed that dopamine-induced SW augmentation was associated with an enriched repertoire of the firing patterns in SW events, whereas the overall tendency of individual neurons to participate in SWs and the mean number of cells participating in a single SW were maintained. Therefore, dopaminergic activation is likely to reorganize cell assemblies during SWs.

Disclosures: T. Miyawaki: None. **H. Norimoto:** None. **T. Ishikawa:** None. **Y. Watanabe:** None. **N. Uemura:** None. **N. Matsuki:** None. **Y. Ikegaya:** None.

Poster

215. Oscillations: Mechanisms

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Topic: B.09. Network Interactions

Support: NSC102-2321-B-010-019-

NHRI-EX103-10105NI

Title: Somatostatin-positive inhibitory interneurons mediate inter-dentate gyrus inhibition

Authors: *T.-Y. YEN, C.-C. LIEN
Inst. of Neurosci., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Somatostatin-expressing (SOM+) GABAergic neurons of the dentate gyrus (DG) were thought to be local-circuit interneurons with their axons primarily restricted to the same side of the DG. Recent studies found a group of SOM+ GABAergic neurons with long-range axonal projections to the contralateral DG. However, the function of these long-range projections remain unknown. In this study, we combined optogenetics and electrophysiology to identify the targets of SOM+ GABAergic long-range projections in the contralateral DG. Our preliminary results showed that SOM+ neurons mediate inter-DG inhibition with target-cell-type-specific efficacy and connectivity. Intriguingly, interneurons receive stronger inhibition compared to granule cells. SOM+ neuron-mediated inhibition may provide an association signal for coordinated function of bilateral DGs and warrants further study.

Disclosures: T. Yen: None. **C. Lien:** None.

Poster

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Topic: B.09. Network Interactions

Support: NSC102-2321-B-010-019-

NSC100-2320-B-010-014-MY3

Title: A synaptic homogeneity principle for parvalbumin-expressing interneuron synapses in the hippocampal dentate gyrus

Authors: *C.-J. CHEN, C.-C. LIEN
Inst. of Neurosci., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The hippocampus is a key brain structure for learning and memory. The dentate gyrus (DG) serves as a primary gate of the hippocampus. Local-circuit GABAergic inhibitory interneurons (INs) in the DG comprise a heterogeneous cell population with distinct molecular, morphological, and electrophysiological properties. Among them, parvalbumin expressing (PV(+)) INs are a striking type of inhibitory IN and play an important role in controlling neuronal activity and therefore mediate neuronal synchronization. PV+ INs are fast-spiking and exhibit extensive axonal arborization within the DG granule cell layer. They innervate granule cells, non-fast-spiking INs, and fast-spiking INs in the granule cell layer with high connection rates. Although anatomical evidence suggests that PV+ INs also innervate hilar neurons, including hilar mossy cells and hilar INs, the functional connections between PV(+) INs and hilar neurons remain unknown. Here, we combined optogenetics with electrophysiology to address this question. Our preliminary results found that granule cells receive strong synaptic input from PV(+) INs compared to hilar mossy cells and hilar INs. However, the temporal dynamics of inhibitory inputs to granule cells, hilar mossy cells, and hilar INs are not significantly different, indicating that synapses between PV+ INs and their target cells display target cell-independent short-term synaptic plasticity.

Disclosures: C. Chen: None. **C. Lien:** None.

Poster

215. Oscillations: Mechanisms

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Topic: B.09. Network Interactions

Support: NSC100-2320-B-010-014-MY3

NHRI-EX103-10105NI

Title: Afferent-specific recruitment of interneurons in the dorsal hippocampal dentate gyrus

Authors: *T. HSU[1], M.-H. TAI[2], C.-C. LIEN[1]
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Abstract: Dentate gyrus (DG) is the primary gate of the hippocampus and receives several excitatory afferents projections from different brain areas. How the principal cells, that is,

granule cells (GCs) and various local-circuit GABAergic interneurons respond to different excitatory inputs remains unclear. Here, we used optogenetics to investigate the target-cell-specific neurotransmission at the hilar commissural pathway-DG and medial perforated pathway (MPP)-DG circuits. We found that monosynaptic excitatory transmission at both hilar commissural pathway-GC and MPP-GC synapses exhibited similar short-term synaptic depression. In striking contrast, hilar commissural pathway- and MPP-recruited GABAergic transmission onto GCs were markedly different. The ratio of inhibitory GABAergic conductance versus excitatory conductance (I/E ratio) in single GCs was monotonically increased during 10 Hz successive stimulation of the hilar commissural pathway, whereas the I/E ratio was decreased during the same stimulation of the MPP pathway. Differential recruitment of interneurons by these two pathways may account for the difference in GABAergic transmission. In agreement with this notion, ML-like and TML-like interneurons received stronger input from the hilar commissural pathway compared to the MPP pathway, and were more reliably recruited by the hilar commissural pathway during 10 Hz successive stimulation. By contrast, the MPP pathway preferentially recruited ML-like interneurons at the onset of stimulation train and did not recruit TML-like interneurons. Our results suggested that the use-dependent shift of the excitation-to-inhibition in the DG GCs is specific to the type of glutamatergic afferent.

Disclosures: **T. Hsu:** None. **M. Tai:** None. **C. Lien:** None.

Poster

215. Oscillations: Mechanisms

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Topic: B.09. Network Interactions

Support: NSC102-2321-B-010-019

NHRI-EX103-10105NI

Title: Inhibitory control of dynamic range of hippocampal dentate granule cell population

Authors: ***C.-T. LEE**, C.-C. LIEN
Inst. of Neuroscience, Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The hippocampus plays an important role in learning and memory. The dentate gyrus (DG), which serves as a gateway to the hippocampus, filters the excitatory afferent inputs from

the cortex and sends the outputs to other hippocampal areas. Little is known, however, about the range of afferent input strengths that DG granule cell (GC) populations can represent. We found that the dynamic range that GCs can represent is much narrower compared to CA1 pyramidal cells. We examined the intrinsic properties, excitatory and inhibitory postsynaptic conductance evoked at threshold input strength of GCs recruited over the range of input strengths and founded that all these factors are not correlated to threshold input strength. However, application of gabazine, a $GABA_A$ receptor antagonist, revealed that feedforward inhibition participates in narrowing the dynamic range of GC populations, which is striking contrast to the CA1 region, the output station of the hippocampus. Examination of threshold input strength and spike delay suggested that this feedforward inhibition is primarily mediated by fast-spiking interneurons and molecular layer interneurons. By using optogenetics, we found that parvalbumin (PV)-expressing interneurons are primarily involved in regulating the dynamic range of GC populations during period of sparse activity. By contrast, GC dynamics during series of incoming activities are differentially regulated by PV- and somatostatin (SST)-expressing interneurons. PV-expressing interneurons control the onset of spike series, whereas SST-expressing selectively regulate the dynamics in the late spike series. Together, our results indicate that GABAergic interneurons constrain the dynamic range of dentate GC populations during different states of neuronal activities.

Disclosures: C. Lee: None. **C. Lien:** None.

Poster

215. Oscillations: Mechanisms

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Topic: B.09. Network Interactions

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Title: Routing information in networks at the edge of synchrony

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Computat. Neurosci., Goettingen, Germany; [3]Max Planck Inst. For Dynamics and Self Organization, Goettingen, Germany; [4]Inst. de Neurosciences des Systèmes, Marseille, France

Abstract: Behavior and cognition require a dynamically reconfigurable communication scheme, flexible in scales much faster than the ones imposed by mechanisms modifying the architecture of the network's structural connectivity. Processes such as attention expose that the brain can actively select specific information-flow channels in very fast time scales. A well established hypothesis known as communication through coherence [Fries, 2005], proposes oscillatory coherence as a possible gating strategy to regulate inter-areal information transmission. Recent studies, however, have challenged this view arguing that synchronous episodes in brain signals are of short duration (100 ms) [Burns, Xing, Shapley,2011] and of a drifting frequency, [Ray, Maunsell, 2010] making oscillatory coordination an unreliable mechanism for information routing. In this work we aim to bring together these views in apparent contradiction. We show that in a model of a local neural circuit of randomly interconnected conductance-based neurons with heterogeneous properties, a robust regime characterized by transient synchrony emerges. When multiple local circuits are connected by long range excitation, short-lived events of coordinated synchronization between areas occur with a self-organized frequency tracking. We show furthermore that, during these events, phase locking also transiently occurs, not necessarily in phase, but favoring phase relations reminiscent of attractors that the connected circuits would develop for stronger synchrony ("ghost attractors"). These phase relations in turn determine preferential directions for the information transmission as measured by Transfer entropy. In particular, effectively unidirectional communication in presence of a bidirectional connectivity can arise at the edge of developing synchrony, and the direction of transmission can be flexibly inverted. In particular, we show that this direction can be actively controlled by applying a weak driving bias, and even by precisely-timed synchronous pulses stimulation. A newly developed time-resolved Transfer Entropy estimator further allows us to show that the resulting changes in the instantaneous phase difference distribution translate into changes of information flow within only a few oscillatory cycles, smaller than the average gamma-burst length.

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Poster

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Title: Contrasting the role of Ih and ICaT currents in post-inhibitory rebound mechanisms of reciprocal-inhibitory networks

Authors: J. DETHIER[1], G. DRION[1], *R. SEPULCHRE[2]
[1]Univ. of Liege, Liege, Belgium; [2]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Models with reciprocal inhibition are ubiquitous in the literature. For instance, common rhythmic motor behaviors produced by central pattern generators (CPGs) involve half-center oscillators. Those oscillators consist of two inhibitory neurons that do not oscillate in isolation, but oscillate when reciprocally connected (Marder & Calabrese 1996). The same mechanism is described in models of the thalamic reticular nucleus to account for thalamocortical spindle oscillations (Wang & Rinzel 1992). Reciprocal inhibition can generate oscillations only if the isolated neurons exhibit the property of post-inhibitory rebound (PIR), a transient depolarization evoked by a prior hyperpolarizing stimulus (Perkel & Mulloney 1974). Two distinct ionic currents have been clearly identified in PIR: a hyperpolarization-activated cation current, I_h (Ansgstad *et al.* 2005), and a low-threshold T-type calcium current, I_{CaT} (Steriade *et al.* 1990). While the two currents cooperate in PIR, we exhibit an important difference between them: I_h produces a transient increase in excitation in the neuron but is slow restorative, that is, it is a source of negative feedback in the slow time scale of the neuron repolarization, whereas I_{CaT} is slow regenerative, that is, is a source of positive feedback. As a consequence if PIR is caused by I_h only, the neuron remains memoryless/monostable at any time. The transient increase in excitation results in a rebound-burst mechanism with restorative firing. In sharp contrast, if I_{CaT} is involved in PIR, the neuron might acquire memory/bistability and produces regenerative firing activity during the rebound. The cellular regenerativity may be undetectable in the time trace of action potentials of the isolated neuron but has a major impact on robustness and modulation at the network level. We compare a network that is purely restorative with a model that includes a slow regenerative current: we investigate how the two networks respond to heterogeneity in the connections (maximal conductances and kinetics) and in the neuron intrinsic properties, as well as how modulation can affect the networks. We also explore the entrainability of the two networks by an external source. Our study shows that the network oscillations are endogenous/robust in the regenerative case and exogenous/entrainable in the restorative case. The properties of I_h and I_{CaT} suggest a drastically different role for the two currents, with I_h more prominent in networks with major sensory feedbacks, to deal with environmental perturbations for instance, and I_{CaT} more prominent in networks with a strong endogenous rhythm, much less responsive to external inputs.

Disclosures: **J. Dethier:** None. **G. Drion:** None. **R. Sepulchre:** None.

Poster

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Support: BNBF CRCNS 01GQ1113

Title: Distinct modulation of low-frequency and high-frequency γ-activity in the barrel cortex by GABAA receptors containing α1- and α2-subunits

Authors: *J. I. HOFMANN[1,2], C. SCHWARZ[1,2], U. RUDOLPH[3], B. ANTKOWIAK[4]
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Abstract: High-frequency electrical activity in the cerebral cortex (>25 Hz) is associated with multiple aspects of cognitive information processing. It is shaped by GABAA receptor-mediated inhibition, but the individual contributions of GABAA receptors containing α1- and α2-subunits remain obscure. To further elucidate this topic, ongoing high-frequency activity in the mouse barrel cortex was modified by systemic administration of diazepam, a drug that binds to GABAA-receptors containing α1,2,3, 5 -subunits and a γ-subunit. Investigations were carried out on two different strains of mutant mice. In α2,3,5-knockin mice, diazepam only enhanced the function of receptors incorporating α1-subunits, whereas in α1,3,5-knockin mice only α2-containing receptors were sensitive to diazepam. In both strains diazepam decreased multi-unit action potential firing to a similar degree. Yet, local field potentials (LFPs) were differently affected by diazepam in the two genotypes. In α1,3,5-knockin mice, the drug significantly enhanced the power in the β- low γ-band (20-50 Hz) whereas high γ-power (60-100 Hz) was attenuated. But in α2,3,5-knockin mice, diazepam decreased the power density in both, the low and high γ-band. We further quantified the correlation between LFPs simultaneously monitored with four different electrodes in the barrel cortex. Correlated activity was enhanced by diazepam in α1,3,5-knockin mice but not in α2,3,5-knockin mice. Taken together, these findings suggest that diazepam-induced potentiation of GABAA receptors harboring α2- but not α1-subunits augments the power in the β- and low γ-band via strengthening cortical synchrony but probably not by increasing the discharge rates of cortical neurons. We hypothesize that α2- mediated

hyper-synchrony underlies the phenomenon of paradoxical EEG-excitation, which is a hallmark of sedative drugs acting via GABAA receptors. Furthermore, the opposing actions of diazepam on low- and high γ-activity are in line with the existence of at least two distinct generator networks.

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Poster

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Title: The role of Na+/K+ ATPase activity in neuronal oscillations and synchrony

Authors: *O. GONZALEZ, G. FILATOV, M. BAZHENOV
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Abstract: Na+/K+ ATPase activity is a major mechanism for maintaining the balance of Na+ and K+ ion concentrations in the brain. This pump is electrogenic and therefore contributes to maintaining the resting state of a neuron. Previous computational and experimental studies suggested that the Na+/K+ ATPase may play an important role in epileptogenesis. It has been shown that applying cardiac glycoside Ouabain (ATPase antagonist) to mouse and rat hippocampal slices in concentration of tens to hundreds of uM leads to the development of epileptiform activity throughout the whole hippocampus. However, the exact mechanism of the effect of the Na+/K+ pump on neural excitability and network dynamics has yet to be established. Here, we studied the effects of sub-micromolar concentrations of the Ouabain analog Strophanthidin (STDN) in the mouse hippocampus *in vitro*. We observed two distinct synchronous firing patterns following bath application of STDN. Bath applications of 100 - 500 nM STDN generated low frequency, high amplitude continuous synchronous activity. However, bath applications of 500nM - 5μM STDN lead to the development of low amplitude, high frequency bursting firing of the hippocampal network. Spatio-temporal analysis revealed that all synchronous network activity was initiated, and had largest amplitudes in the CA3 region of the hippocampus before spreading to the other regions. Overall, we show the existence of distinct

patterns of network synchrony due to differences in pump activity, and that sub-micromolar concentrations of STDN are sufficient for generating synchronous network activity in the mouse hippocampus.

Disclosures: **O. Gonzalez:** None. **G. Filatov:** None. **M. Bazhenov:** None.

Poster

215. Oscillations: Mechanisms

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Univ. Michigan PTSP Fellowship

Title: Mechanisms of fast ripple generation in a computational model

Authors: ***C. G. FINK**[1], S. GLISKE[2], T. ASSAF[3], W. STACEY[2]
[1]Physics, Ohio Wesleyan Univ., Delaware, OH; [2]Neurol., Univ. of Michigan, Ann Arbor, MI; [3]Computer Sci., Univ. of Michigan, ANN ARBOR, MI

Abstract: High frequency oscillations (HFOs) have been associated with both normal and pathological brain activity [1], and show promise as biomarkers for identifying the epileptogenic zone in patients with epilepsy [2]. HFOs may be classified according to peak frequency content as either Ripples (100-250 Hz) or Fast Ripples (>250 Hz). Some studies indicate that Fast Ripples are better biomarkers of the epileptogenic zone than are Ripples [3], while other studies show that frequency content alone is insufficient to identify HFOs as normal or pathological [4]. Gaining a better understanding of the mechanisms which generate HFOs may help in making this clinical distinction. In this study we present a computational model to investigate mechanisms by which a wide range of network activity--from gamma rhythm to Fast Ripples--may be generated in hippocampal cortex, specifically focusing on the network properties necessary to produce Fast Ripples. In this model all activity is elicited from a network of pyramidal and basket cells simply by modulating the mean frequency of afferent synaptic noise. The LFP is obtained by summing the currents from all cellular compartments, thereby reflecting both action potentials (APs) and post-synaptic potentials (PSPs). Oscillations are generated in the network by varying the mean level of synaptic noise, and various network properties are modulated in order to determine their

effect upon Fast Ripple generation. We find that in general, PSP-dominated LFPs are incapable of generating Fast Ripples, while AP-dominated LFPs may generate both Ripples and Fast Ripples. Several factors may facilitate the emergence of Fast Ripples, including loss of inhibitory connections, recurrent connectivity between pyramidal cells, and loss of gap junction connectivity between basket cells. Counterintuitively, AP-dominated Ripples are characterized by asynchronous pyramidal cell spiking. Such Ripple activity is interrupted by brief (~50 ms) periods of Fast Ripple activity associated with transient organization of pyramidal cell spiking into two out-of-phase clusters (similar to the results in [5]). This model suggests that gamma, fast gamma, Ripples, and Fast Ripples, both normal or abnormal, exist on a continuum with specific features related to the underlying network activity and connectivity. [1] JGR Jefferys et. al. Progress in Neurobiology, 2012. [2] J Jacobs et. al. Progress in Neurobiology, 2012. [3] RJ Staba et. al. Annals of Neurology, 2004. [4] J Engel et. al. Epilepsia, 2009. [5] G Foffani et. al. Neuron, 2007.

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Poster

215. Oscillations: Mechanisms

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Topic: B.09. Network Interactions

Title: Circadian oscillations of [Ca2+]i and extracellular glutamate in the suprachiasmatic nucleus reveal a role for neuronal-glial interaction in circadian time-keeping

Authors: *M. BRANCACCIO, M. H. HASTINGS
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Abstract: The hypothalamic suprachiasmatic nucleus (SCN) is responsible for synchronising the circadian clocks present across cells and tissues and aligning them to day/night cycles, thereby establishing appropriate circadian rhythms of physiology and behaviour. SCN cells contain an intra-cellular transcriptional-translational feedback loop (TTFL), whereby Bmal1/Clock dimers activate transcription of *Per* and *Cry* genes, and the loop is completed by Per/Cry auto-repression of the Bmal/Clock activity. The entire process oscillates with a ~24h periodicity. The discovery that such an intra-cellular TTFL operates in virtually all cells has questioned its importance in, alone, conferring the unique properties of robustness and internal synchrony of the SCN. Clearly,

inter-cellular mechanisms within the SCN circuit may also be important. We therefore investigated the role of neuronal-glial interaction in SCN time-keeping. We first searched for potential extra-cellular mediators of such interaction and focused on glutamate, a principal glio-transmitter. We transduced SCN organotypic slices with AAV encoding the extracellular glutamate ($[Glu]_e$) reporter iGluSnFR and performed long-term imaging. A sustained ~24 hours $[Glu]_e$ oscillation was revealed, that persisted in the absence of exogenously provided glutamate. The GABAergic nature of SCN neurons (~95%) and the resistance of the glutamate oscillation to TTX, confirmed its extra-synaptic nature. We then investigated the relationship between $[Glu]_e$ and neuronal $[Ca^{2+}]_i$. We have previously shown that the peak $[Ca^{2+}]_i$ is an early event in the SCN circadian day and precedes TTFL genes activation. We performed long term calcium-glutamate imaging by using iGluSnFR and neuronal RCaMP1h. Surprisingly, we found that $[Glu]_e$ and $[Ca^{2+}]_I$ are perfectly anti-phasic. We hypothesised that the drop in $[Glu]_e$ may be due to increased glutamate uptake. Remarkably, inhibiting glial and neuronal glutamate uptake simultaneously de-synchronised neuronal $[Ca^{2+}]_i$ across the SCN, thus suggesting a role for $[Glu]_e$ in synchronising the circuit. Intersectional and pharmacogenetic approaches further demonstrated the presence of a glial-neuronal dichotomy, necessary to encode circadian time in the SCN.

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Poster

215. Oscillations: Mechanisms

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DARPA-BAA-09-27

Title: Contributions of electrical synapses and interneuron subtypes to correlated inhibition during cortical network activity

Authors: *G. NESKE, B. W. CONNORS
Brown Univ., Providence, RI

Abstract: Synchronous oscillatory activity in the gamma range (30-80 Hz) has been observed during sensation, attention, and working memory tasks. Synchronization of spikes in inhibitory interneurons and of inhibitory inputs to pyramidal cells is especially strong during these processing periods, suggesting that correlated inhibition is crucial for shaping the functional properties of the active neocortex. While interneurons are connected by both chemical and electrical synapses, the relative contribution of these connections to synchronized inhibition is still not well understood. Furthermore, while interneurons are physiologically diverse, the extent and functional roles of synchronized inhibition provided by different interneuron subtypes are not known. We studied the roles of electrical synapses among interneurons and correlated firing among interneuron subtypes during persistent network activation (i.e. Up states) in acute slices of mouse barrel cortex. We found, first, that while both excitatory cells and interneurons fire during Up states, spike synchrony is higher between interneuron pairs than between excitatory cell pairs. We then studied the roles of electrical synapses in correlated inhibition during Up states by comparing connexin36 knockout (Cx36 KO) and wild-type (WT) animals. Gamma-range power of the inhibitory inputs recorded in excitatory cells was slightly higher in WT compared to Cx36 KO. However, when we measured cross-correlations between inhibitory currents recorded in pairs of pyramidal cells, the half-width of the cross-correlogram was slightly, but significantly sharper in the WT, but peak cross-correlations in the WT and Cx36 KO were similar. Additionally, there were no significant differences between the WT and Cx36 KO in the phase relationships between excitatory cell spikes or inhibitory postsynaptic currents and gamma-range LFP activity. To study the roles of different interneuron subtypes, we directly controlled the level of interneuron-subtype-specific correlated inhibition by expressing ChR2 in either of two major interneuron subtypes, parvalbumin (PV) and somatostatin (SOM) cells, and pulsing light during Up states. We will consider the effects of enhancing correlated inhibition provided by these different subtypes on the following properties of excitatory cells during Up states: membrane potential dynamics, spike precision and reliability, and sensitivity or gain. Our data suggest that electrical synapses are not crucial for most aspects of correlated inhibition or regulation of spike timing during cortical network activity.

Disclosures: G. Neske: None. **B.W. Connors:** None.

Poster

215. Oscillations: Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 215.19/C59

Topic: B.09. Network Interactions

Support: CIHR

NSERC

Title: Optogenetic investigation of septal GABAergic modulation of hippocampal theta rhythm

Authors: R. BOYCE[1], S. GLASGOW[1], *S. WILLIAMS[2], A. ADAMANTIDIS[3]
[1]McGill Univ., Montreal, QC, Canada; [2]Dept Psych, McGill Univ., Verdun, QC, Canada; [3]Neurol. Dept., Bern Univ. Hosp., Bern, Switzerland

Abstract: Hippocampal neurons oscillate in synchrony at theta (4-10 Hz) frequencies during periods of wakefulness and rapid-eye-movement (REM) sleep, and evidence suggests that these theta rhythms are required for cognitive processing. The hippocampus receives cholinergic, glutamatergic and inhibitory GABAergic inputs from the medial septum (MS), a brain region required for normal theta rhythm generation *in vivo*. Previous work using lesional, pharmacological or electrical modulation of MS cell activity suggested that septal GABAergic neurons may be important for theta rhythm generation. However, due to the difficulty in achieving both temporal precision in combination with cell-type specificity using these methods, the causality of this neural pathway on hippocampal theta rhythms remains to be clarified. Here, we genetically targeted archaerhodopsin (ArchT), a silencing opsin to GABAergic neurons of the MS. We found that yellow light pulses reliably hyperpolarized ArchT-expressing cells by ~ 40 mV, preventing spiking in transfected neurons completely, in the MS in brain slices *in vitro*. Using a combination of optogenetic and electrophysiological (MS unit recording and dorsal hippocampal CA1 field potential and unit recording) techniques in freely-moving mice, we further found that spiking of canonically GABAergic neurons in the MS was completely and reversibly blocked and hippocampal theta power was significantly (> 60%) and reversibly attenuated when septal GABAergic neurons were optically inhibited during periods of active wakefulness or REM sleep. These results demonstrate that septal GABAergic neurons are critical for normal hippocampal theta rhythm *in vivo*. Additionally, this data may implicate this neuronal population as an important component of cognitive processing mechanisms during wakefulness as well as REM sleep, a concept that is presently being tested experimentally.

Disclosures: R. Boyce: None. **S. Glasgow:** None. **S. Williams:** None. **A. Adamantidis:** None.

Poster

215. Oscillations: Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 215.20/C60

Topic: B.09. Network Interactions

Support: EPSRC/Eli Lilly Case Award Studentship

Title: The mechanisms underlying pyramidal cell participation in hippocampal sharp waves

Authors: *L. A. ATHERTON[1], K. TSANEVA-ATANASOVA[2], J. R. MELLOR[1]
[1]Univ. of Bristol, Bristol, United Kingdom; [2]Univ. of Exeter, Exeter, United Kingdom

Abstract: The discovery of replayed sequences of hippocampal place cell activity during sharp wave ripples (SWRs) has led directly to the fundamental concept that this reactivation underlies the consolidation of memories formed during learning. However it is still unclear what cellular mechanisms govern which hippocampal pyramidal cells fire action potentials (or participate) within a given SWR. Here we addressed this question using a combined *in vitro* and computational modelling approach. We confirmed pharmacologically that both excitation and inhibition are critical for the emergence of spontaneous sharp waves in submerged mouse hippocampal slices. Analysis of the synaptic currents in hippocampal pyramidal cells, during the ongoing sharp waves, revealed that while inhibition dominated CA3 pyramidal cell responses, CA1 pyramidal cells were either dominated by excitation or inhibition. Using a recently developed hippocampal CA3-CA1 computational network model (Taxidis et al., 2012), we then predicted changes in pyramidal cell participation during SWRs resulting from modulation of excitatory or inhibitory synaptic transmission at different synapses within the hippocampal network. These predictions shed light on how network activity during learning may shape participation in subsequent SWR oscillations.

Disclosures: L.A. Atherton: None. **K. Tsaneva-Atanasova:** None. **J.R. Mellor:** None.

Poster

215. Oscillations: Mechanisms

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Program#/Poster#: 215.21/C61

Topic: B.09. Network Interactions

Support: DFG Ha4466/3-1

DFG SFB 936

DFG SPP1665

Title: Glutamatergic and GABAergic synaptic interplay underlies the theta-gamma network oscillations in the prefrontal cortex of neonatal rats

Authors: *S. H. BITZENHOFER, K. SIEBEN, K. SIEBERT, I. L. HANGANU-OPATZ
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Abstract: The emergence of mnemonic abilities critically depends on the oscillatory coupling within neonatal prefrontal-hippocampal networks. Hippocampal theta bursts drive via direct axonal projections the generation of discontinuous oscillatory activity in the prelimbic (PL) subdivision of the prefrontal cortex. While the features of prelimbic activity patterns have been characterized in detail and frequency analysis uncovered both theta- and gamma-band oscillations, the cellular elements contributing to their generation are still unknown. For their elucidation, we combined *in vivo* whole-cell patch-clamp recordings with extracellular multi-site local field potential (LFP) recordings from the PL of anaesthetized neonatal [postnatal day 6-8] rats. Biocytin-stained and morphologically characterized layer V neurons in the PL were classified in pyramidal and multipolar neurons. They had similar passive as well as active membrane properties and showed three classes of spontaneous postsynaptic currents (sPSCs): (i) glutamatergic fast sPSCs with monoexponential decay, (ii) glutamatergic slow sPSCs with biexponential decay, and (iii) GABAergic sPSCs. Both glutamatergic and GABAergic sPSCs occurred in bursts that synchronized in theta (4-12 Hz) and beta-low gamma (15-50 Hz) frequency with the extracellularly recorded discontinuous network oscillations. The coupling of synaptic inputs and outputs with the network activity was assessed by calculating the oscillation-triggered intracellular current average and event-triggered LFP average. Glutamatergic inputs to pyramidal and multipolar neurons were locked to theta or gamma oscillations, respectively, whereas GABAergic inputs to both cell types were locked to theta activity. The firing of multipolar neurons in the PL appeared phase locked to the network activity in theta and gamma frequency, but no locking was found for pyramidal neurons. Moreover, the bursts of glutamatergic inputs to pyramidal and multipolar neurons, as well as the action potential firing of pyramidal neurons were highly correlated to the start of local field activity in theta and gamma frequency. Thus, complex synaptic interactions between pyramidal and multipolar neurons may provide the framework for temporal coding in the neonatal PL.

Disclosures: S.H. Bitzenhofer: None. **K. Sieben:** None. **K. Siebert:** None. **I.L. Hanganu-Opatz:** None.

Poster

215. Oscillations: Mechanisms

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Support: The General Researcher Program (#2013058415) of National Research Foundation of Korea

The Future Systems Healthcare Project of KAIST

Title: Structural irregularity in local neural circuit induces global modulations of neural synchronization

Authors: *P. SAILAMUL, J. JANG, S.-B. PAIK
Bio and Brain Eng. Dept., KAIST, Daejeon, Korea, Republic of

Abstract: Abnormality in neural synchronization is often accompanied with some brain disorders such as schizophrenia and epilepsy. Yet the link between these correlated neural activity patterns and structure of the underlying neural circuit is not completely understood. In this study, we use model simulations of large neural network to better understand the relation between the structural irregularity in local neural circuit and the global synchronization of neural activity. First, we examined how a neural circuit in the cortex can be structured by the interaction between nearby neurons. We developed the mosaics of Excitatory(E) and Inhibitory(I) neurons using our developmental model algorithm: the local repulsive interaction model. By setting different repulsive interaction range, we could generate mosaics of various spatial regularities. Next, to simulate neural activities in the network of various spatial regularity, we created model neural networks with a regular E mosaic and three different I mosaics; irregular, quasi-regular, and regular mosaics. The local cortico-cortical connections were generated by the statistical wiring model, where the probability and the strength of local synaptic connection only depend on the distance between two neurons. Then, using Hodgkin-Huxley model neurons in the NEURON simulator, we examined the correlated neural activities in above three cases. We observed that correlated neural activities -spontaneous oscillation in gamma band frequency- are generated in all three cases when a random Poisson spike train is given as a feedforword input to the network. However, from the statistical analysis of firing rates and inter-spike interval, we found that the irregular mosaic showed the most unevenly distributed local activity among the three cases. More importantly, we also observed that this spatial irregularity in neural activity can make the network unstable, which induces the modulation of global synchronization more readily. When we increase the feedforward input firing rate given to the network, the frequency of spontaneous gamma oscillation in the network also varied proportional to the input rate change, and this variation was most significant in the irregular mosaic network. In addition, irregular mosaic

network often showed a transition of global synchronization pattern from modest-synchronized oscillation to sharp-synchronized wave propagation. Our results show that the structural irregularity in the neural circuit can induce abnormal neural synchronizations, possibly leads to the modulation of global activity pattern that might be important to the normal operation of the brain system.

Disclosures: **P. Sailamul:** None. **J. Jang:** None. **S. Paik:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

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Topic: B.10. Intrinsic Membrane Properties

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Charles A. King Trust

Title: Homeostatic conductance regulation in multicompartment conductance-based model neurons

Authors: ***A. H. WILLIAMS**, T. O'LEARY, E. MARDER
Brandeis Univ., Waltham, MA

Abstract: Neurons possess homeostatic mechanisms that maintain their electrophysiological properties over long time spans despite ion channel turnover and environmental perturbations. Such plasticity mechanisms have been observed at the level of circuits, single cells and within subcellular compartments including the soma [1], dendritic spines [2], the axon initial segment [3], and at pre-synaptic terminals [4]. It remains unclear how these spatially distributed regulatory mechanisms can be orchestrated to regulate the overall properties of a neuron. We extended an existing model of ion channel regulation [5] to a multicompartment model. We accounted for both global and local forms of homeostatic regulation by modeling the production of channel precursors (mRNA) and ion channels in two separate steps. The global regulation rules model the activity-dependent production and removal of ion channel mRNA transcripts; the local regulation rules within each compartment model the translation and insertion of ion channel proteins into the cell membrane. We find that a simple regulatory rule can compensate for

growth of neuronal processes as well as inter-neuronal variability in morphology. Furthermore, tuning the local regulation rules is sufficient to produce cell compartments with unique ion channel densities and electrical properties. For example, specific parameter combinations can establish active and passive dendritic compartments as well as a spiking axonal segment within the same multicompartment cell. The model predicts that channel densities will be spatially correlated, for example, a channel present in the soma and a distal dendritic branch may be expressed at a consistent ratio, even if the expression is variable from cell to cell at each of these locations. Finally, we explore the requirement for local vs global activity dependent regulation. If the global homeostatic mechanism (i.e. mRNA production) is activity-dependent, the model can compensate for perturbations that alter activity levels at the soma. However, perturbations that alter neural activity in distal cell compartments may not be sensed by the global mechanism. More fine-grained control can be implemented by making the local regulation rules (i.e. channel insertion rates) activity-dependent. [1] Swensen & Bean (2005). J Neurosci. 25(14):3509-20. [2] Kirov & Harris (1998). Nat Neurosci. 2(10):878-83. [3] Grubb & Burrone (2010). Nature. 456(7301):1070-4. [4] Frank et al. (2006). Neuron. 52:663-77. [5] O'Leary et al. (in press). Neuron.

Disclosures: **A.H. Williams:** None. **T. O'Leary:** None. **E. Marder:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

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Topic: B.10. Intrinsic Membrane Properties

Support: Fondecyt 1080630 (MS)

Mideplan ICM-PO5-001-F (JB)

Conicyt PhD Fellowship (JV)

Title: Modulation of frequency preference by changes in input resistance

Authors: *J. A. VERA[1], U. PEREIRA[2], B. REYNAERT[1], J. BACIGALUPO[1], M. SANHUEZA[1]
[1]Univ. De Chile, Santiago, Chile; [2]Dept. of Statistics, The Univ. of Chicago, Chicago, IL

Abstract: Neurons from different mammalian brain regions (including neocortex, hippocampus and amygdala) display subthreshold frequency preference in the theta range (4-12 Hz) when stimulated with oscillatory current injection, a phenomenon called neuronal resonance. It is speculated that this property may contribute to tune and to stabilize oscillatory activity in neuronal networks. Frequency preference results from the high-pass filter effect produced by the slow activation of a hyperpolarization-activated current in addition to the low-pass filtering of voltage fluctuations due to the passive membrane properties (resistance and capacitance). Together, these properties determine the impedance profile of neurons, characterized by the resonance frequency (frequency at impedance peak) and resonance strength (ratio between peak impedance and impedance at 0.5 Hz). In active neuronal networks membrane input resistance (Rin) varies in a wide range depending on synaptic bombardment (showing a 0-80% decrease from its value in a silent network). We investigated the relationship between Rin and frequency preference in CA1 pyramidal and cortical amygdala neurons in rat brain slices, using dynamic clamp and the ZAP protocol (injection of an oscillatory current of constant amplitude and linearly increasing frequency; 0-20 Hz). Our results show that a 50% decrease in Rin generated by the addition of a virtual leak conductance (16.6 ± 2.9 nS) shifted the resonance frequency from 6.3 ± 0.4 to 7.3 ± 0.4 Hz ($p<0.0005$, $n=10$), while decreasing resonance strength by 9% (from 1.23 ± 0.03 to 1.11 ± 0.02; $p<0.005$). On the other hand, a 50% increase in Rin by the addition of a negative virtual leak conductance of -6.1 ± 0.8 nS produced the opposite effect, increasing resonance strength from 1.23 ± 0.03 to 1.34 ± 0.05 ($p<0.005$, $n=10$) and decreasing resonance frequency from 6.5 ± 0.4 to 5.2 ± 0.3 Hz ($p<0.0005$, $n=10$). To test the modulation of frequency preference during spiking we used a similar stimulation protocol on cortical amygdala neurons. We showed that selective spiking at 2-4 Hz recorded in control conditions was shifted to 4-10 Hz after a 30% reduction in Rin, in agreement with our observations in hippocampal neurons. These results show that frequency preference of resonant neurons is modulated by changes in Rin and suggest that in the intact brain this property may constitute a tuning mechanism to adjust frequency preference of single neurons in interplay with network activity levels.

Disclosures: J.A. Vera: None. **U. Pereira:** None. **B. Reynaert:** None. **J. Bacigalupo:** None. **M. Sanhueza:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

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Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant R01MH085074

NIH Grant R01EB016407

Title: Membrane potential-dependent synaptic integration in entorhinal stellate neurons

Authors: *J. J. MARTINEZ, M. ECONOMO, J. A. WHITE
Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: Stellate cells (SCs) of the medial entorhinal cortex exhibit robust spontaneous membrane-potential oscillations (MPOs) in the theta (4-12 Hz) frequency band as well as theta-frequency resonance in their membrane impedance spectra. Past experimental and modeling work suggests that these features may contribute to the phase-locking of SCs to the entorhinal theta rhythm and may be important for forming the hexagonally tiled grid cell place fields exhibited by these neurons *in vivo*. Among the major biophysical mechanisms contributing to MPOs is a population of persistent (non-inactivating) sodium channels. Because the resulting persistent sodium conductance (GNaP) gives rise to an inward current with steep voltage dependence near spike threshold, these channels endow SCs with a prominent decrease in synaptic current as membrane potential approaches spike threshold, resulting in a negative slope conductance. In this study, we used dynamic clamp to test the hypothesis that this negative slope conductance gives rise to voltage-dependent, and thus MPO phase-dependent, changes in the amplitude of excitatory and inhibitory post-synaptic potential (PSP) amplitudes. We find that PSP amplitude depends on membrane potential, exhibiting a 4-6% increase in amplitude per mV depolarization. The effect is larger than_and sums quasi-linearly with_the effect of the synaptic driving force, V - Esyn. As SC MPOs 10 mV in amplitude are commonly observed *in vivo*, this voltage- and phase-dependent synaptic gain is large enough to modulate PSP amplitude by over 50% during theta-frequency MPOs. Phase-dependent synaptic gain may therefore impact the

phase locking of grid cells *in vivo* to ongoing network

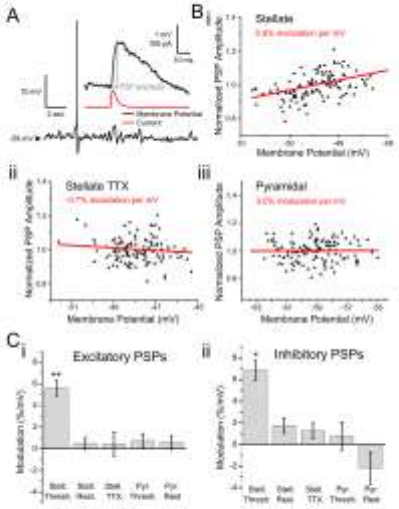

Figure 3

oscillations.

Disclosures: **J.J. Martinez:** A. Employment/Salary (full or part-time):; University of Utah. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R01MH085074, NIH R01EB016407. **M. Economo:** None. **J.A. White:** A. Employment/Salary (full or part-time):; University of Utah. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH R01MH085074, NIH R01EB016407.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

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Topic: B.10. Intrinsic Membrane Properties

Support: HDR-0932339

EF-11137897

Title: Intrinsic plasticity accompanies synaptic LTD in Purkinje cells

Authors: *Z. YANG, F. SANTAMARIA
UTSA, San Antonio, TX

Abstract: Cerebellar Purkinje cells show activity dependent changes of intrinsic excitability in both behavioral learning and *in vitro* slice recordings. It is still unknown whether long term depression (LTD) of parallel fiber-Purkinje cell (PF-PC) synapse is associated with modulation of intrinsic membrane properties. We performed whole-cell patch-clamp recordings in slice from mice 16-23 days old. PF-PC LTD was induced in voltage clamp mode by pairing parallel fiber stimulation and somatic depolarization. This protocol generated robust reduction of EPSC of about 40%. Intrinsic properties were recorded in current clamp mode before and after synaptic LTD induction. We found an increase of overall excitability in somatic recording and a leftward shift of the input-output curve after LTD induction. There was also a parallel decrease of sag ratio and an increase of input resistance at more hyperpolarized potential, indicating a reduction of h current. We did not observe significant changes in afterhyperpolarization amplitude following action potential trains. Since there are two components in our LTD induction protocol, we further tested the excitability change by somatic depolarization or parallel fiber stimulation only, in order to elucidate the stimulation pattern required for this intrinsic plasticity. We found that neither stimulation protocol alone can lead to overall excitability change, indicating the requirement of concomitant somatic depolarization and dendritic stimulation for intrinsic plasticity. This non-synaptic plasticity may serve as a homeostatic mechanism and could affect future information processing by Purkinje cells.

Disclosures: Z. Yang: None. **F. Santamaria:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

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Program#/Poster#: 216.05/C67

Topic: B.10. Intrinsic Membrane Properties

Title: Long lasting depression of intrinsic excitability in fast spiking interneurons decreases inhibition in putative granule cells of the dentate gyrus

Authors: *D. DASGUPTA, S. K. SIKDAR
Mol. Biophysics Unit, Indian Inst. of Sci., Bangalore, India

Abstract: The local fast spiking interneurons (FSINs) of the Dentate Gyrus (DG) are post-synaptically connected to the granule cells (GCs), which in turn make perisomatic inhibitory synapses onto the GCs, thus configuring a strong feedback circuit. Local FSINs and their feedback control are critical for neuronal network oscillations and have been associated with various behavioural paradigms. However, the prolonged effects of gamma frequency synaptic activity on the FSINs have not been explored previously. We used whole-cell current clamp patch recordings on acute hippocampal slices to address this question and observed a sustained decrease in intrinsic excitability in the FSINs following repetitive 30 Hz synaptic stimulations of the mossy fibers (gamma bursts). Interestingly, when the gamma bursts were paired with FSIN membrane hyperpolarization, the decrease in excitability was accentuated to ~ 35% from the baseline, while pairing with membrane depolarization significantly attenuated the plasticity of intrinsic excitability. Paired pulse ratio measurement of the synaptic responses did not show significant changes during the experiments. The induction protocols were observed to be associated with increase in post-synaptic calcium, with the maximum increase occurring during the pairing of gamma bursts with membrane hyperpolarization and a minimal increase during pairing with membrane depolarization. Bath perfusion of 1-Naphthylacetyl spermine (NASPM) significantly attenuated the calcium rise, suggesting the involvement of calcium-permeable AMPA receptors in the observed phenomenon. Chelation of post-synaptic calcium with intracellular BAPTA blocked the expression of plasticity. The calcium-permeable AMPA receptors and postsynaptic HCN channel conductance were found to be involved in this form of plasticity in the FSINs. Simultaneous dual patch recordings from synaptically connected FSIN and putative GC, confirmed that a decrease in intrinsic excitability of the FSINs was accompanied by decreased inhibitory post synaptic potentials in the GCs. Experimentally constrained network simulations using NEURON showed increased spiking in the post-synaptic GC owing to decreased input resistance in the FSIN. We hypothesize that the plasticity in the FSINs induced by local network activity may serve to increase information throughput into the downstream hippocampal subfields besides providing neuroprotection to the FSINs. This form of plasticity may have implications in epilepsy which is associated with high network activity and significant death of interneurons in neuronal microcircuits.

Disclosures: D. Dasgupta: None. **S.K. Sikdar:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

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Topic: B.10. Intrinsic Membrane Properties

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NIH Grant 1P01NS079419

Charles A. King Trust

Title: Temperature robust neuronal function from activity-dependent ion channel regulation

Authors: *T. O'LEARY, E. MARDER
Volen Ctr. For Complex Systems, Brandeis Univ., Waltham, MA

Abstract: Animals that cannot regulate their body temperature (poikilotherms) far outnumber animals that do (homeotherms) in terms of biomass and number of species, yet the principles underlying temperature-robustness in poikilothermic nervous systems are not understood. Neuronal function depends on the kinetic properties of ion channels and other enzymes expressed in neurons, and this dependence can be very sensitive to small changes in parameters. The exponential temperature dependence of ion channel kinetics (the 'Q10') differs several-fold between ion channel types expressed in poikilotherms such as crustaceans. Nonetheless, important circuit properties - such as phase relations in central pattern generating circuits - are robust to temperature changes of more than 10 degrees Celsius [1, 2]. Modeling work has shown that such robust function does not come for free because membrane conductances with realistic Q10s generically become de-tuned and disrupt neuronal function as temperature changes by a few degrees [3]. How is temperature robust neural activity achieved in spite of this potential de-tuning? One possibility is that activity-dependent mechanisms [4] that maintain neuronal properties are constrained in cold-blooded animals by the requirement to produce temperature-robust output. We developed a model of activity-dependent ion channel regulation that can produce stable neuronal activity in spite of underlying differences in the temperature dependence and density of conductances in model neurons. We find that the constraints that temperature-robustness imposes on self-regulating models can be met due to the large number of parameter sets that map to specific activity patterns. As a consequence, neurons in poikilotherms are

predicted to co-regulate conductances in specific ways that make important neuronal properties temperature-robust. References 1. Tang LS, Goeritz ML, Caplan JS, Taylor AL, Fisek M & Marder E (2010) PLoS biology 8(8). 2. Tang LS, Taylor AL, Rinberg A, & Marder E (2012) J Neurosci. 32(29) 3. Caplan JS, Williams AH, Marder E (2014) J Neurosci. 34(14) 4. O'Leary T, Williams AH, Franci A, Marder E (2014) Neuron (in press)

Disclosures: T. O'Leary: None. **E. Marder:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

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Program#/Poster#: 216.07/C69

Topic: B.10. Intrinsic Membrane Properties

Support: GM097433

Title: Calcium-sensing receptor signaling mediates increases in neocortical neuron excitability following decreased external calcium

Authors: B. J. KNIGHT[1,2], C. L. WILLIAMS[1,2], N. P. VYLETA[3], *S. M. SMITH[1,2]
[1]PCCM, Portland VA Med. Ctr., PORTLAND, OR; [2]Oregon Hlth. & Sci. Univ., Portland, OR; [3]Inst. of Sci. and Technol., Klosterneuburg, Austria

Abstract: Reduction in extracellular [Ca2+] ([Ca2+]o) decreases action potential threshold in neurons, increasing excitability. Classically, this effect has been attributed to a loss of screening by Ca2+ of the negatively charged plasma membrane surface, reducing the electrical field within the membrane and thereby shifting the gating voltage-sensitive channels in a hyperpolarizing direction. Surprisingly, it was recently shown that [Ca 2+]o-dependent changes in excitability in hippocampal neurons are mediated by a pathway consisting of a cation channel (NALCN), and two intracellular proteins, UNC-79 and UNC-80. Furthermore, it was hypothesized that changes in [Ca2+]o were detected by the calcium-sensing receptor (CaSR), which is upstream of UNC-79, UNC-80, and NALCN. Using patch-clamp techniques we examined the role of CaSR in regulating excitability in mouse neocortical neurons. We found a large reversible increase in action potential firing (885 ± 161 mean ±SEM action potentials/150 sec; n=19) in pharmacologically isolated wild-type neurons (block of glutamatergic and GABAergic transmission) when external [Ca2+] and [Mg2+] were reduced from physiological values

(1.1/1.1 mM) to 0.2/0.2 mM. However, in recordings from CaSR-/- neurons, the response to the same decrease in [Ca2+]o/[Mg2+]o was substantially attenuated (246 ± 131, p=0.004; n=16). The CaSR-/- mice were kindly provided by Dr Wenhan Chang, UCSF and San Francisco VAMC. In wild-type neocortical neurons, voltage-clamped at -70 mV, the leak current was 11 ± 5 pA when [Ca2+]o/[Mg2+]o was 1.1/1.1 mM but shifted by -14 ± 4 pA (p=0.006, n=16 neurons) following reduction of [Ca2+]/[Mg2+] to 0.2/0.5 mM. This inward current was associated with a 26 ± 3% reduction in input resistance (p=0.0003), indicating channel activation. In contrast, in age-matched neurons (16.1 ± 0.4 vs 17.2 ± 0.5 days in culture) from CaSR-/- mice, leak current at -70 mV was 9 ± 6 pA when [Ca2+]o/[Mg2+]o was 1.1/1.1 mM and unaffected (9 ± 8 pA, p=0.9, n=15) by the same decrease in [Ca2+]o/[Mg2+]o. As well, null mutant neurons had lower basal input resistance (320 ± 32 MΩ, p=0.035) that decreased less with lowered [Ca2+]o/[Mg2+]o than wild-type (12 ± 4%, p=0.007). Taken together, our data are consistent with [Ca2+]o-dependent changes in intrinsic neocortical neuron excitability being mediated by CaSR and not entirely due to surface charge screening.

Disclosures: **B.J. Knight:** None. **N.P. Vyleta:** None. **S.M. Smith:** None. **C.L. Williams:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 216.08/C70

Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant R01-NS082761-01

Title: Arx expression is necessary for normal maturation of intrinsic and synaptic properties of parvalbumin expressing inhibitory interneurons

Authors: ***D. J. JOSEPH**[1], A. J. MCCOY[1], E. D. MARSH[1,2]
[1]Pediatrics Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Perelman Sch. of Med. at the Univ. of Pennsylvania, Philadelphia, PA

Abstract: Transcription factors combinatorially specify the identity of interneurons during development. Some of these transcription factors remain expressed in the mature interneuron pool, suggesting a different, yet unknown, function. We found that postnatal conditional ablation of one transcription factor, the Aristeless Related homeobox gene (Arx), in parvalbumin positive

interneurons resulted in a loss of parvalbumin basket cell markers such as Kv3.1 and the Cat-315 component of the perineuronal net. These neurochemical changes were associated with hippocampal based learning deficits and electrographic seizures in some animals. Therefore, we studied the effect of Arx loss on both the intrinsic and synaptic properties of parvalbumin interneurons with postnatal loss of Arx. Arx was conditionally knocked out of Parvalbumin (Parv) interneurons by crossing a floxed Arx mouse (Arxfl/fl) with a Parv-Tomato Cre-recombinase (Parv-TomCre) mouse. Hippocampal slices were generated from postnatal day 35-40 mice. Whole-cell recordings were made from Arx+/+- or Arx-/- Parv-Tom positive neurons visually identified with fluorescence and patched using IR-DIC videomicroscopy in the hippocampal CA1 region. Membrane properties were characterized in response to rectangular current pulses. Excitatory and inhibitory synaptic events were recorded in each cell. Preliminary results revealed Parv+ interneurons with loss of Arx are marginally larger and displayed lower input resistance than control interneurons. Active membrane properties such as AP threshold, AP amplitude, and half-width duration are reduced in Arx-/- Parv-Tom positive interneurons compared controls. In addition, Arx-/- Parv-Tom cells require higher current intensity (Rheobase) to reach depolarization threshold. Current-frequency curves show that Arx-/- Parv-Tom cells fire fewer action potentials than controls with increasing current injection. Synaptic recordings of spontaneous and miniature excitatory show that synaptic events on Arx-/- Parv-Tom cells are generally of lower amplitudes and occur at lower frequency than controls. Interestingly, the amplitude and frequency of inhibitory synaptic events are reduced in Arx-/- Parv cells as well. Our preliminary study demonstrates that postnatal loss of Arx alters both passive and active intrinsic properties of Parv interneurons in the hippocampus. In addition, both inhibitory and excitatory synaptic transmissions onto PV+ cells were negatively modulated by loss of Arx. These findings suggest that active expression of Arx may be a key player in the maturation of electrophysiological properties of inhibitory interneurons.

Disclosures: **D.J. Joseph:** None. **A.J. McCoy:** None. **E.D. Marsh:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

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Program#/Poster#: 216.09/C71

Topic: B.10. Intrinsic Membrane Properties

Support: Wellcome Trust Grant WT087363MA

BBSRC Grant BB/L000679/1

Title: Cholinergic receptor induced long-lasting plasticity of hippocampal axonal KV7 channels

Authors: K. MARTINELLO, D. A. BROWN, *M. M. SHAH
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Abstract: Kv7 channels underlie a slowly-activated and non-inactivating voltage-dependent K+ current that modulates action potential (AP) firing and synaptic integration. This current is fully blocked by muscarinic receptor activation, hence the name M-current (1). KV7 channels are expressed in both somata and axons. In hippocampal dentate gyrus granule cells, though, our findings suggested that the M-current is present preferentially in axons where it influences the action potential threshold (2). In support, immunohistochemistry showed that KV7 subunits are predominantly located in axons. Whether these axonal KV7 channels are modulated by muscarinic receptors remains to be elucidated. To investigate this, we performed electrophysiological recordings from granule cell somata in slices obtained from P22-28 rats and either stimulated cholinergic fibers or applied the muscarinic receptor agonist, Oxotremorine-M. Activation of muscarinic receptors by these methods led to a sustained reduction in the action potential threshold and persistently enhanced granule cell firing. This effect was due to long-term inhibition of axonal KV7 channels as pre-treatment with XE991 prevented it. This was confirmed using voltage-clamp recordings from granule cell neurons. Thus, our results show that muscarinic receptor activation causes a long-lasting inhibition of axonal KV7 channels, resulting in a decrease in action potential threshold and enhanced granule cell activity. 1. Brown, D.A. & Passmore, G.M., Br. J. Pharmacol., 156, 1185-95, 2009. 2. Martinello et al., Soc Neurosci. Abstracts, 2013.

Disclosures: K. Martinello: None. **M.M. Shah:** None. **D.A. Brown:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

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Program#/Poster#: 216.10/C72

Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant NS048476

NIH Grant DK084052

Title: Low-threshold calcium channel critical for activity oscillations of dopamine neurons in mouse arcuate nucleus

Authors: *X. ZHANG, A. N. VAN DEN POL
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Abstract: Bursting activity is important for neurons to increase efficacy of neuropeptide/neuromodulator release and neurotransmission. By inhibiting pituitary prolactin release, dopamine neurons in the arcuate nucleus play an important role in lactation. A previous study (Lyons et al., 2010) showed that dopamine neurons in the rat arcuate nucleus display rhythmic oscillations in action potentials with cells showing a burst of spikes, followed by a period of spike absence; the oscillations appeared to be dependent on gap junction coupling. Here we used transgenic mice expressing a fluorescent reporter gene driven by the tyrosine hydroxylase promoter in arcuate dopamine neurons to study electrophysiological characteristics with whole-cell patch-clamp recording. The resting membrane potential (RMP) for bursting dopamine cells was -74.1 ± 1.9 mV (n=30) which was negative to the RMP for POMC (-54.5±1.1mV, n=33, $p<0.01$) or NPY (55.4 ± 0.8 mV, n=25, $p<0.01$) neurons in the arcuate nucleus. The oscillation frequency was 0.068 ± 0.004 Hz (n=39) in whole-cell current-clamp. We did not observe any current oscillations in voltage-clamp configuration. When action potentials were blocked by TTX, the membrane potential oscillation was intact with a frequency of 0.072 ± 0.008 Hz (n=29). This is consistent with a report that other hypothalamic neurons also display oscillations in the presence of TTX (Chu et al., 2012). The oscillation was abolished by nonselective voltage-gated calcium channel and by selective T-type calcium channel blockers. These data together suggest that activation of T-type calcium channels is necessary for the regular bursting of mouse arcuate dopamine neurons. Short (2 ms) optogenetic stimulation of arcuate dopamine neurons expressing ChIEF-dtTomato induced a single action potential and after-depolarization potential of 15.1 ± 1.5 mV (n=6). Longer (5 ms) optogenetic stimulation induced a brief burst in dopamine neurons. Our results suggest that T-type calcium channel may play an important role in burst firing of arcuate dopamine neurons.

Disclosures: X. Zhang: None. **A.N. van den Pol:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 216.11/D1

Topic: B.10. Intrinsic Membrane Properties

Support: DFG Grant TR-SFB134

Cologne Graduate School for Ageing Research (CGSAR)

Title: Cellular mechanisms of firing patterns in synaptically isolated, midbrain dopaminergic neurons

Authors: U. COLLIENNE[1,3], S. POPOVYCH[1,4], S. HESS[1,3], M. E. HESS[3,5], J. C. BRÜNING[3,5,6,7], S. DAUN-GRUHN[1,4], *P. KLOPPENBURG[2,3]
[1]Biocenter, Dept. of Zoology, [2]Univ. of Cologne, Cologne, Germany; [3]Excellence Cluster on Cell. Stress Responses in Aging-Associated Dis. (CECAD), Cologne, Germany; [4]Res. Group of Computat. Biol. (DFG-Heisenberg Programme), Cologne, Germany; [5]Max Planck Inst. for Neurolog. Res., Cologne, Germany; [6]Ctr. for Mol. Med. (CMMC), Cologne, Germany; [7]Dept. I of Intrnl. Medicine, Ctr. for Endocrinology, Diabetology and Preventive Med., Univ. Hosp. of Cologne, Cologne, Germany

Abstract: The midbrain dopaminergic (DA) neurons in the substantia nigra (SN) and ventral tegmental area (VTA) are an essential part of the reward/hedonic system. Therefore, we are interested in the metabolic modulation of the intrinsic electrophysiological properties of these neurons (Cell Metab. 2011; 13:720-728 and Nat Neurosci. 2013; 16(8):1042-8). For this purpose we require a solid baseline from which the modulatory actions can be studied. *In vivo* studies, which combined electrophysiological recordings with behavioral test, revealed that DA neurons possess three firing patterns that are correlated with the prediction and detection of rewards: (1) a single spiking pattern, (2) a burst firing pattern and (3) a hyperpolarized state in which the cells remain silent. In contrast, *in vitro* DA neurons have frequently been described to produce a characteristic, highly regular pacemaker firing pattern (Brain Res Rev. 2008; 58(2), 314-21). Notably, this pacemaker firing persists even in complete synaptic isolation. However, in the SN of rodent brain slice preparations a subpopulation of DA neurons was identified that did not show pacemaker firing, but an irregular firing pattern (J Physiol. 2010; 588(Pt 10), 1719-35). This irregular, non-pacemaker firing pattern is within the same low frequency range as previously described for regularly firing, pacemaker DA neurons. To analyze the cellular mechanisms that lead to this irregular firing pattern, we performed perforated patch clamp recordings on acute brain slice preparations of adult mice. Here, we found that the small calcium-dependent (SK) potassium current was significantly decreased in non-pacemaking DA neurons. Additionally, the amplitude of the hyperpolarization activated (H) current is decreased compared to pacemaker DA neurons. However, single pharmacological blockage or reduction of either the SK potassium current or the H current in DA neurons with regular pacemaking did not lead to an irregular firing pattern. Currently, we are defining the exact role of the aforementioned currents in the generation of pacemaker patterns in DA neurons. Therefore we simultaneously

reduce the amplitude of the SK potassium current and the H current in DA neurons with regular pacemaking. The experimental data will be used to build computational models to verify whether the observed changes in intrinsic currents are sufficient to explain the different firing patterns recorded in DA neurons.

Disclosures: **U. Collienne:** None. **S. Popovych:** None. **S. Hess:** None. **M.E. Hess:** None. **J.C. Brüning:** None. **S. Daun-Gruhn:** None. **P. Kloppenburg:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 216.12/D2

Topic: B.10. Intrinsic Membrane Properties

Title: Distinct roles of the soma and proximal dendrites in spontaneous firing activity of the midbrain dopamine neuron

Authors: ***J. JANG**, M. PARK
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Abstract: In spontaneously firing midbrain dopamine neurons, although action potentials are generated at the proximal dendrite from which an axon develops, we previously reported that pacemaker activity is determined by electrical balance between the soma and proximal dendrites. However, at the moment, it is not clear how the soma and proximal dendrites formulate pacemaker activities. A brief pulse of glutamate can generates two distinct excitatory and inhibitory responses on dopamine neuron firing; the initial high-frequency firing and the subsequent Ca2+-dependent pause. When we stimulated a series of small areas along a dendrite with caged-glutamate photolysis, excitatory and inhibitory firing responses were different distinctively according to the stimulation sites of a dendrite. The high-frequency firing was generated equally within the proximal dendritic region, but not in the distal dendritic regions. The postfiring pause was rapidly decayed with distance from the soma and no response from the distal dendritic regions. Local dendritic Ca2+-uncaging experiments revealed that Ca2+-induced suppression of spontaneous firing purely depended on the amplitude of the Ca2+ spikes or closeness to the soma. Firing induced Ca2+ dynamics was slow in the soma, whereas it was highly faster in the dendrite due to the different surface area/volume ratios. In addition, continuous suppression of local proximal dendritic regions with Ca2+ uncaging failed to

suppress spontaneous firing, permanently. All these data suggest the generator-counteract balancer model in which the proximal dendrites act as a leading zone for pacemaker potential generation and the soma plays as a counteract balancer.

Disclosures: J. Jang: None. **M. Park:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 216.13/D3

Topic: B.10. Intrinsic Membrane Properties

Support: RCMI NIMHD 8G12-MD007600

COBRE Center for Neuroplasticity, acknowledge: NIH NIGMS 1P20GM103642

Title: Caffeine effects on the intrinsic properties of lumbar ventral horn neurons

Authors: *M. S. RIVERA OLIVER[1], Y. ALVAREZ-BAGNAROL[2], M. DIAZ-RIOS[3,4]
[1]Univ. of Puerto Rico-Institute of Neurobio., San Juan, PR; [2]Biol., Univ. of Puerto Rico- Rio Piedras campus, San Juan, PR; [3]Anat. and Neurobio., Univ. of Puerto Rico-Medical Sci. campus, San Juan, PR; [4]Inst. of Neurobio., San Juan, PR

Abstract: Caffeine is the most consumed psychoactive drug in the world, with about 90% of the population (including children) in the United States regularly consuming caffeine-containing beverages or foods. It produces similar behavioral effects as other classical psychostimulants, such as cocaine and amphetamines, mainly motor activation, arousal, and reinforcing effects related to neural reward systems. Caffeine is known to be a non-selective adenosine receptor antagonist. Most of the studies assessing the effects of caffeine and/or adenosine receptor agonists and antagonists on locomotor behavior have been performed on freely behaving rodents using systemic administration of these drugs that can activate multiple neural pathways making extremely difficult to elucidate specific mechanisms of action. We propose to elucidate the cellular mechanisms by which caffeine modulates the intrinsic membrane properties of spinal neurons through bath perfusion onto spinal cord slices, which contain significant components of the spinal network for controlling locomotion. Preliminary data on extracellular recordings from spinal lumbar nerves in the presence of NMDA, dopamine (DA) and serotonin (5-HT) which is

known to elicit a fictive locomotor pattern, shows that caffeine modulates motor activity by enhancing the bursting properties of motorneurons producing the recorded motor output (Acevedo, et al. SfN abstract). We studied the effects of neuromodulation by caffeine and other adenosine receptor antagonists on the intrinsic properties of spinal neurons using pharmacological blockade and patch clamp recordings in current clamp mode. The application of caffeine in the presence of blockers for glutamatergic, glycinergic, GABAergic and cholinergic neurotransmission produce no significant effects on the membrane potential and action potential (AP) properties of most neurons recorded. The application of caffeine in the presence of locomotor inducing drugs (5-HT/NMDA/DA) had a dramatic excitatory effect by depolarizing the membrane potential, hyperpolarizing the threshold for AP elicitation and decreasing the AP afterhyperpolarization (AHP) of most neurons recorded. These results suggest that caffeine excites spinal neurons through the modulation of dopamine and possibly glutamate neurotransmission, as previously shown in the brain, in the spinal cord through its blockade of adenosine receptors.

Disclosures: **M.S. Rivera Oliver:** None. **Y. Alvarez-Bagnarol:** None. **M. Diaz-Rios:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 216.14/D4

Topic: B.10. Intrinsic Membrane Properties

Title: Energy constraints on neuronal activity

Authors: ***Z. GREGURIC FERENCEK**, M. L. GERTZ, Z. OBAIDA, J. R. CRESSMAN
George Mason Univ., Fairfax, VA

Abstract: Neuronal systems support a wide range of complex functions performed by networks of neuronal cells. Communication through these networks is mediated by the generation and transmission of action potentials, synaptic transduction, and dendritic summation. These processes dissipate energy stored in the electrochemical gradients of the cell, and reduction of these gradients can significantly alter neuronal signaling. These electrochemical gradients are continually reestablished through the conversion of ATP to ADP by the Sodium/Potassium pump. These dynamics are investigated using a conductance based model that incorporates ionic dynamics for the neuronal, glial, and vascular compartments. In addition, a simplified model of

aerobic and anaerobic glucose metabolism is used to determine ATP production. We report on the limits of sustained and transient neuronal computation as determined by the constraints of glucose metabolism, and ionic regulation.

Disclosures: Z. Greguric Ferencek: None. **M.L. Gertz:** None. **Z. Obaida:** None. **J.R. Cressman:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 216.15/D5

Topic: B.10. Intrinsic Membrane Properties

Support: NSF GRFP 2013165292

Title: Stimulation-induced ionic effects on neuronal transmission

Authors: *M. L. GERTZ, Z. GREGURIC FERENCEK, J. R. CRESSMAN
George Mason Univ., Fairfax, VA

Abstract: Changes in ionic concentration gradients have profound effects on neuronal transmission and play an important role in neuronal activity. Under modest activity, ionic concentrations exist in a nearly steady state as the neuron re-establishes activity-induced fluctuations in its ionic gradients very rapidly. However, this system can be removed from its dynamic steady state by excessive activity, causing ionic concentrations to dissipate towards equilibrium leading to a number of effects that can dominate neuronal dynamics. Oxygen data show that the recovery from a short stimulating burst is on the order of minutes, and stimulating faster than this interval does not permit a recovery to baseline. These changes in oxygen can be linked to the sodium/potassium pump rate to help elucidate the ionic composition inside and outside the cells. Simultaneous potassium measurements show much faster recovery suggesting sodium accumulation is responsible for the prolonged metabolic stress reflected in the oxygen data. By taking simultaneous measurements of extracellular oxygen and potassium we have access to the flow of energy in and out of the tissue.

Disclosures: M.L. Gertz: None. **Z. Greguric Ferencek:** None. **J.R. Cressman:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 216.16/D6

Topic: B.10. Intrinsic Membrane Properties

Support: NSERC Discovery Grant 386601

Title: Estimates of persistent inward current in human motor neurons during postural sway

Authors: R. C. A. FOLEY, *J. M. KALMAR
Dept. of Kinesiology, Wilfrid Laurier Univ., Waterloo, ON, Canada

Abstract: Persistent inward current (PIC) is a membrane property critical for increasing gain of motor neuron output. In humans, most estimates of PIC are made from plantarflexor or dorsiflexor motor units with the participant in a seated position with the knee flexed. This seated and static posture neglects the task-dependent nature of the monoaminergic drive that modulates PIC activation. It is well documented that there is an increase in descending serotonergic neuron activity during tasks requiring greater muscle activation or at increasing speeds of locomotion. Estimates of PIC made during isometric ramp contractions are technically less challenging; however these seated estimates may drastically underestimate the amount of PIC that occurs in human motor neurons during functional movement. The current study estimated PIC using the conventional paired motor unit technique which uses the difference between reference unit firing frequency at test unit recruitment and reference unit firing frequency at test unit de-recruitment (ΔF) during triangular-shaped, isometric increases in force as an estimate of PIC. Estimates of PIC and reciprocal inhibition were also collected during a standing anterior postural sway that elicited a ramped increase and decrease in soleus motor unit activation. For each paired motor unit, ΔF estimates of PIC made during conventional isometric ramps made during the seated posture were compared to those made during standing postural sway. Reciprocal inhibition was also measured in each posture using the post-stimulus time histogram (PSTH) technique. Inhibition was measured to ensure that any change in ΔF was due to neuromodulation and not the result of confounding factors associated with the change in joint angle during postural sway. It was hypothesized that an increase in ΔF would be seen during standing compared to sitting due to greater neuromodulatory input. We found that ΔF estimates during standing postural sway were an average of 1.06pps (SE±0.32pps) higher than PIC estimated in the isometric seated task for the same motor unit. Within participants, standing ΔF estimates averaged 214% (SE±25.9%) that of seated estimates. Instantaneous firing frequency at test unit onset was not significantly

different during postural sway (mean=9.41pps, SE±0.50pps) compared to seated isometric torque ramps (mean=10.41, SE±0.33pps). Standing ΔF measures met all validation criteria outlined for paired-motor unit estimates and are an effective functional estimate of PIC in humans. These results demonstrate that conventional seated PIC estimates should not be generalized to functional movement as a large component of PIC is task-dependent.

Disclosures: R.C.A. Foley: None. **J.M. Kalmar:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 216.17/D7

Topic: B.10. Intrinsic Membrane Properties

Title: The effect of focused ultrasonic stimulation on the activity of hippocampal neurons in multi-channel electrode

Authors: *J. B. CHOI, K. CHO, D. JANG
Dept. of Biomed. Engineering, Hanyang Univ., Hanyang Univ., Seoul, Korea, Republic of

Abstract: The neuromodulating methods for diagnostic and therapeutic tools in neurophysiology have been investigated in the last decade. Especially noninvasive methods, for example transcranial direct current stimulation and transcranial magnetic stimulation, have received attention for alternative tools to invasive ones with unavoidable risks. These noninvasive methods, however, showed a low spatial resolution and limited penetration depth. Recently some studies have shown that focused ultrasonic mechanical energy has modulatory effect on the nervous system. Mechanical waves generated from an ultrasonic transducer that can be focused in small spots provide high spatial resolution and depth of penetration than other noninvasive tools. Many experiments have investigated the neuromodulatory effects of ultrasonic stimulation, yet there are only a few researches investigating the effect of ultrasonic stimulation on associated neuronal cultures. The motivation for the present study was to investigate the modulatory effect of low intensity, low frequency ultrasonic stimulation on hippocampal neurons. In this study, the changes in neural network activities during ultrasonic stimulation were recorded using a multi-electrode array. Neuronal cells easily form monolayer on multi-electrode platform, providing neuronal network model *in vitro* that can be imaged, and stimulated. In this study, the ultrasonic stimulation changed the neuronal network activities, especially at specific parameters. In the

result, the relative change of neuronal activities shows great descent with pulse duration of 50 ms and pulse repetition frequency of 10 Hz. This change also had an effect on post exposure period. This result suggests that there is a possibility that mechanical stimulation can affect neuronal activities for a while. It is interesting that the timing of neuronal activity and stimulus seemed unsynchronized which suggests that the mechanical stimuli may not have a direct effect on neuronal activities but somehow help neuronal cells to be in a more excitable state, so the overall neuronal network activities were decreased. There is an assumption that ultrasonic stimulation promotes the secretion of substances from neuronal tissues, which effects the occurrence of neuronal network activities. Similar to previous study, sonication with specific parameters changed neuronal activities while other intensities had no modulatory effect on neuronal cultures. This observation might indicate that acoustic intensity is major key for stimulating neuronal activity. But further studies with various drugs such as channel blockers will be required.

Disclosures: **J.B. Choi:** None. **K. Cho:** None. **D. Jang:** None.

Poster

216. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Halls A-C

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Program#/Poster#: 216.18/D8

Topic: B.10. Intrinsic Membrane Properties

Support: NIH R01 EY019578

NSF IOS-0746558

Title: A computational model of collision detection in the optic tectum in Xenopus tadpoles

Authors: E. V. JANG[1], *A. S. KHAKHALIN[1,2], C. M. CIARLEGLIO[1], C. D. AIZENMAN[1]
[1]Neurosci., Brown Univ., Providence, RI; [2]Biol., Bard Col., Annandale-on-Hudson, NY

Abstract: Neural circuits in the optic tectum of Xenopus tadpoles are selectively responsive to visual stimuli that represent objects approaching the animal at a collision trajectory (looming stimuli). This stimulus selectivity is known to underlie an adaptive collision avoidance behavior in this species. While we recently showed that the balance of excitation and inhibition has a crucial role in enabling stimulus selectivity in the tectum, it is still unclear how the balance

between the recurrent network activity and the newly arriving sensory flow is achieved in this structure. More generally, it is still unknown how the looming stimuli are encoded and detected by the tectal circuits, and also, despite the clear indication for the presence of strong recurrent excitation in the tectum, the exact topology of these recurrent feedback circuits remains elusive. Recently we completed a comprehensive census of tectal cell electrophysiological properties, by measuring and analyzing 30+ synaptic and intrinsic excitability parameters in each of 200+ cells from 80+ experimental animals from different developmental stages and during homeostatic plasticity. When combined with over a decade's worth of electrophysiological analysis from our and other laboratories, we have an incredibly rich data set describing the development of tectal cell physiology during key developmental time periods. In this work we use this data set to build a high fidelity spiking network model of the tectum with the goal of generating predictions about the topology of recurrent connections within the tectum, as well as the dynamics of this system. After tuning and calibrating this model on experimental data, we compare different patterns of recurrent network connectivity, and different levels of balance between recurrent activity and sensory flow, to identify the range of parameters in which the network exhibits selectivity for looming stimuli. This allows us to make predictions about the topology of recurrent connections in the biological optic tectum that can then be tested experimentally. We also show how intrinsic excitability of individual tectal cells affects the selectivity of the network as a whole, and describe how homeostatic modulation of intrinsic properties can change selectivity thresholds in this model, thus affecting the behavior of the animal.

Disclosures: **E.V. Jang:** None. **A.S. Khakhalin:** None. **C.M. Ciarleglio:** None. **C.D. Aizenman:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 217.01/D9

Topic: C.05. Aging

Support: NIH-AG031158

Title: Characterization of pattern recognition receptor expression in cerebellar granule cells and investigating their role in synapse development and maintenance

Authors: *N. W. DEKORVER[1], J. ARIKKATH[2], S. J. BONASERA[3]
[2]Developmental Neurosci. Department, Munroe-Meyer Inst., [3]Intrnl. Medicine, Geriatrics, [1]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Within the brain, aging can present with various phenotypes including loss of coordination, mobility, metabolic regulation, and as cognitive impairments. In order to find potential therapies to combat the effects of aging in the brain, it is crucial to investigate biological mechanisms underlying behavioral changes in regions of the brain corresponding to these deficits. We have demonstrated that the C57BL6 mouse strain present with a clear mobility phenotype and pronounced changes in excitatory synaptic organization within the cerebellar internal granule cell layer (iGCL) associated with aging. Our previous studies have also shown an increase in pattern recognition receptors (PRRs) and immune component expression that is not localizable to microglia in the aging brain. The roles of many PRRs have been well described within the immune system; however their role in nervous system, particularly in synapse formation and maintenance has yet to be fully described. To further investigate the role of PRRs and immune component proteins in synaptic formation, maintenance, and function, we have developed a purified cerebellar neuron culture from post natal day 3, 5, 7, and 9 C57Bl6 mice consisting of less than 2.0% astrocytes and 1.0% microglia, roughly 80% $NeuN^+$ cells and a 10-20% level of apoptosis measured by activated Caspase-3 staining at D6 *in vivo*. Primary granule cell cultures also stained positive for Tau and Map2, as well as for synaptic markers Vglut1 and PSD95 further confirming the neuronal phenotype. Neuronal activity was verified by calcium imaging of spontaneous action potentials and potassium chloride induced depolarization. Characterization of PRR transcript expression from purified cerebellar granule cell cultures at day 0, 3, 6 and 9 shows temporal increases in transcript expression of TLR2, TLR4, Trem2, Clec7a, Lilrb3, Fcer1g, and complement protein C3 closely following the *in vitro* time course of synaptic network development. These results further justify the validity of using primary cerebellar granule cell cultures to study granule cell physiology, including molecular mechanisms and cell signaling pathways underlying synapse development, maintenance, and cerebellar granule cell excitability.

Disclosures: N.W. Dekorver: None. **S.J. Bonasera:** None. **J. Arikkath:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 217.02/D10

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: RO1NS070159

Title: Elucidating the functions of gad1 in cranial development and neurotransmission within larval zebrafish

Authors: *L. JOHNSTON[1], A. VANLEUVEN[1], R. BALL[1], M. O'CONNOR[2], T. DORE[2], J. LAUDERDALE[1]
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Abstract: γ-Aminobutyric Acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system of all vertebrates. GABA is synthesized from glutamate by the enzymatic activity of glutamic acid decarboxylase (GAD). There are two GAD isoforms which are encoded by the gad1 and gad2 genes, and they produce a 67kD form called GAD67 and a 65kD form called GAD65, respectively. Our work is focused on characterizing the function of the gad1 gene in larval zebrafish. Previous work in mice has shown that gad2 mutants are phenotypically indistinguishable from their wild type littermates, yet they exhibit stress-induced seizures. Gad1 mutants, on the other hand, are not viable past birth due to a severe cleft palate. This defect has prevented further study of gad1's role in early development. Based upon this, the GAD-1 enzyme seems to be involved in both early cranial development and later in neurotransmission. In our study, translation blocking morpholinos against the gad1 and gad2 genes were used to alter GAD expression within the larval zebrafish. While the GAD-2 morphants look phenotypically normal at 24hpf, GAD-1 morphants exhibited altered cranial structures. These craniofacial deformities arise from abnormally developed cranial chondrocytes, leading to smaller and misshapen cranial cartilages. This phenotype can be rescued by injecting GAD-1 mRNA, which supports that this phenotype is specific to gad1 knockdown. Also, through the use of caged-morpholinos, these craniofacial deformities could be bypassed when uncaging was carried out at 1dpf. This has allowed us to focus on the neurological aspects of GAD-1 reduction. Electrophysiological recordings have shown increased and abnormal brain activity for the GAD-1 morphants, when compared to wild type animals. Together, these findings support the idea that the GAD-1 enzyme exhibits a novel function in craniofacial development, independent of its activity in GABA synthesis.

Disclosures: **L. Johnston:** None. **A. VanLeuven:** None. **R. Ball:** None. **M. O'Connor:** None. **T. Dore:** None. **J. Lauderdale:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 217.03/D11

Topic: B.11. Glial Mechanisms

Support: NIH Grant DA031833

NIH Grant 2T32NS51156-6

NIH Grant 1F32NS083283-01A1

Title: Spines with multiple excitatory contacts are sites for axonal competition during synaptic development

Authors: *W. C. RISHER[1], S. PATEL[2], J. SINGH ALVARADO[3], O. Y. CALHAN[3], C. EROGLU[1]
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Abstract: A dendritic spine is thought to receive a single excitatory synaptic input. This "one spine: one synapse" notion is a key assumption underlying many studies of brain connectivity. Using serial section electron microscopy (ssEM) in the mouse primary visual cortex (V1), we discovered that up to 25% of all excitatory synapses in this region are made onto spines with multiple excitatory contacts (SMECs) during early postnatal development (P14). This fraction decreases with age, revealing SMECs as an important transitory structure in synaptic maturation. Surprisingly, in the cortices of mice that lack the astrocyte-secreted synaptogenic protein hevin, SMECs persist into later ages (P25). Further immunohistochemical analyses revealed that, in the cortices of hevin KO mice, thalamocortical synapses are significantly reduced compared to WT. By contrast, intracortical synapses trended towards an increase. These findings show that astrocyte-secreted hevin is required for the establishment and maintenance of thalamocortical synapses in the developing cortex. In the absence of hevin, thalamocortical synaptic contacts are not stabilized, resulting in "takeover" of spines by the more numerous intracortical axons. Therefore, lack of hevin results in prolonged competition between axons for spines, thus the SMECs persist. Taken together, these findings reveal that spines can form excitatory synaptic connections with multiple presynaptic partners, challenging the "one spine: one synapse" assumption of connectivity. In addition, SMECs provide sites for competition between different axonal inputs during development, and astrocytes control this process via the release of hevin. Future studies may determine whether impaired resolution of axonal competition may underlie the structural deficiencies observed in many types of neurodevelopmental disorders.

Disclosures: W.C. Risher: None. **S. Patel:** None. **J. Singh Alvarado:** None. **O.Y. Calhan:** None. **C. Eroglu:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 217.04/D12

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Profiling hippocampal neurons after early preconditioning with certain excitatory amino acids reveals distinct signaling pathways that promote neuronal survival

Authors: *L. K. FRIEDMAN, A. M. SLOMKO, S. HU, W. WAN
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Abstract: Immature neurons not only resist neurotoxicity produced by experimental epileptic seizures or early *in vitro* application of certain excitatory amino acids (EAAs), but also generate substantial neuroprotective/ preconditioning effects to subsequent seizures or certain EAAs. Our prior *in vivo* microarrays suggest that increases in anti-apoptotic genes, reduction in inflammation, and increased signal transduction pathways are induced by early excitatory events to promote cell survival. In order to gain better understanding of which genes and pathways are responsible for specific adaptive effects, we used our protocol to expose immature hippocampal neurons to high doses of glutamate (250μM), NMDA, (100μM), or kainate (KA) (300μM) for 48 hrs (5-7 DIV) followed by washout. Transcriptome profiling was performed 7 days later, just prior to a "second hit", a time when cultured neurons mature (14 DIV). Despite the delay, many genes were up- and down- regulated. Glutamate treatment resulted in the highest number of total upregulated (892) and uniquely upregulated genes (521). KA treatment had extensive gene overlap with glutamate and presented the lowest number of uniquely upregulated genes (17). NMDA treatment revealed large numbers of uniquely upregulated genes (328), and downregulated genes (234) compared to glutamate (10) or KA (18). Many commonly upregulated (211) and downregulated (228) genes were also observed. Similar to early life conditioning seizures, Ca2+-binding proteins, heat shock, oxidative stress, and certain anti-apoptotic Bcl-2 gene members significantly increased after glutamate or NMDA but not after KA. Presynaptic Ca2+ sensors, signal transduction, G-coupled proteins, certain growth factors, synaptic vesicle docking and neurotransmitter regulation predominated after NMDA exposure, whereas regulation of voltage gated Ca2+ and K+ channels, anti-apoptotic interleukins, presynaptic mGluR3 and mGluR7, KA and nicotinic cholinergic receptors, and phospholipase and phosphodiesterases predominated after glutamate treatment. Certain voltage gated Ca2+ channels were exclusively downregulated after NMDA treatment. Protective gene candidates expressed only after glutamate included neuropeptide Y, neurotensin R1, and neurotrophic

tyrosine kinase R2. KA was the only EAA that resulted in sustained opioid receptor (μ and δ) upregulation consistent with *in vivo* reports. However, except for overexpression of a Bcl-2 like gene, protective gene candidates were not observed after KA suggesting that sustained adaptation in early life is predominantly mediated via NMDA and other fast and slow glutamatergic related signaling pathways.

Disclosures: L.K. Friedman: None. **A.M. Slomko:** None. **S. Hu:** None. **W. Wan:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

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Program#/Poster#: 217.05/D13

Topic: B.11. Glial Mechanisms

Support: FP7-PEOPLE-2012-IOF Grant 8272 "Neuron-Astro-Nets"

ABCDE ERCIM Alain Bensoussan Fellowship

Title: Mechanism for astrocyte-mediated persistent activity

Authors: *M. DE PITTÀ[1,2], E. BEN-JACOB[3,4], H. BERRY[2]
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Abstract: Persistent activity is marked by the increase of neuronal firing rates compared to baseline, in response to specific stimulatory cues. In primates performing delayed-response tasks, persistent activity emerges during the delay period and is regarded as the neural correlate of working memory - that is, the ability to transiently hold and manipulate goal-related information to forthcoming actions. Several different mechanisms have been suggested for persistent activity, yet it remains unknown whether it stems from inherent properties of neurons or the plasticity of their synaptic connections or both. Astrocytes, the main type of glial cells in the cortex, have recently emerged as potential active players in synaptic plasticity due to their proposed ability to regulate synaptic neurotransmitter release in response to neuronal activity. Because available models of persistent activity do not take into account astrocytes, we used a biophysical model to investigate the possible role of astrocyte regulation of synaptic plasticity in the emergence of persistent neuronal firing. Our study suggests that selective persistent activity could indeed

emerge from astrocyte-mediated short-term synaptic facilitation triggered by the brief presentation of a stimulatory cue. The remarkable time scale separation between synaptic dynamics and its modulation by astrocyte-released gliotransmitters indeed makes possible the coexistence of multiple states of synaptic release. In this fashion, astrocyte-mediated facilitation can outlast the cue and switch to a self-sustained mode where it is maintained by the ongoing synaptic activity. This form of astrocyte-mediated presynaptic long-term potentiation could be responsible for the emergence of persistent increased neuronal firing, thus providing the tripartite synapse with a persistent trace of the past presentation of the cue. Taken together, these results suggest a novel astrocyte-based mechanism for persistent activity, providing experimentally testable hypotheses for the possible involvement of astrocytes in cognitive tasks related to working memory.

Disclosures: **M. De Pittà:** None. **H. Berry:** None. **E. Ben-Jacob:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 217.06/D14

Topic: B.11. Glial Mechanisms

Support: NIH Grant NS20480

Title: Schwann cell-mediated remodeling of the mouse neuromuscular synapse

Authors: ***Y. LEE**[1], I. W. SMITH[1], M. MIKESH[2], K.-A. NAVE[3], M. SCHWAB[3], W. J. THOMPSON[1]
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Abstract: The mouse neuromuscular junction (NMJ), the cholinergic synapse between a motor neuron and a skeletal muscle fiber, has contiguous “gutters” of high-density acetylcholine receptor (AChR) aggregates precisely apposed by terminal arbors of motor axons. This structure is normally highly stable. In contrast, mice whose motor axons overexpress a membrane-tethered form of neuregulin1 (NRG1-III) the AChR are found in non-contiguous “islands” innervated by nerve terminal varicosities. Such morphological change, commonly referred to as “fragmentation,” is observed frequently in muscles of aged or dystrophic muscles, likely from damage and regeneration of synaptic segment of muscle fibers. We found no evidence, however,

of increased necrosis/regeneration of muscle fibers in NRG1-III transgenic animals as these muscles had no change in centrally nucleated myofibers. While a diminished ability to induce and/or maintain NMJs could also result in fragmentation, agrin is concentrated at NRG1-III transgenic NMJs and its downstream signaling in muscle appear to be unaffected as indicated by AChR β subunit phosphorylation - a phenomenon closely associated with agrin-induced AChR aggregation. In addition, there was no evidence of denervation in muscles of transgenic mice. Although the components of the synaptic basal lamina - laminin β2 and acetylcholinesterase - are altered in their distribution to mirror the postsynaptic changes, evidence suggests their modification is subsequent to changes in postsynaptic AChR. Thus, none of the well known pre- or postsynaptic mechanisms causing fragmentation of NMJs appears responsible for the altered synaptic morphology of NRG1-III transgenic NMJs. However, the glial components of NMJs, terminal Schwann cells (tSCs), appear activated by the excess axonal NRG1: they increase in number at the synapse and extend fine processes. Ultrastructural examination of the transgenic NMJs revealed evidence of tSC activity normally seen only at neonatal NMJs and implicated in maturation and remodeling of developing NMJs: encroachment by tSC processes into synaptic cleft, despite the presence of a SC repulsive factor laminin β2 and increased phagocytic activity directed against presynaptic nerve terminals. These finding strongly suggest that the fragmentation of NMJs in NRG1-III mice is actively mediated by behaviors of SCs that normally aid in maturation of the peripheral synapse and are regulated by levels of axo-glial NRG1 signaling.

Disclosures: **Y. Lee:** None. **I.W. Smith:** None. **M. Mikesh:** None. **K. Nave:** None. **M. Schwab:** None. **W.J. Thompson:** None.

Poster

217. Synapse Development, Maintenance, and Aging

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Program#/Poster#: 217.07/D15

Topic: B.11. Glial Mechanisms

Support: NIMH 7K01MH097957

Title: Sonic hedgehog signaling in astrocytes regulates synaptic connectivity

Authors: *A. GARCIA[1,2], A. AHARON[2], Y. ZUO[2]
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Abstract: The secreted molecule, Sonic hedgehog (Shh), plays critical and well characterized roles during nervous system development, including patterning of the dorsal/ventral axis and regulation of cell proliferation and specification. In the postnatal cortex, Shh signaling between Layer 5 and Layer 2/3 neurons is required for the proper establishment of cortical microcircuits. Our previous study identified distinct populations of astrocytes in the adult forebrain that express the transcription factor, Gli1, indicating that Shh signaling persists well past development, and into adulthood. We identified neurons as the source of Shh, but the precise role of Shh signaling between neurons and astrocytes in the forebrain is unknown. In this study, we examine the hypothesis that Shh signaling in astrocytes is required for the proper establishment and long term maintenance of cortical synapses *in vivo*. We crossed GFAP-Cre;Smofl/fl with transgenic mice expressing green fluorescent protein (GFP) under control of the Thy1 promoter (Thy-GFPM). GFAP-Cre;Smofl/fl;Thy1-GFPM mice express GFP in a subset of Layer 5 cortical neurons in a Smo conditional knock out background (Smo CKO). We performed morphometric analysis of dendrites and spines in the somatosensory cortex. Smo CKO mice exhibit an increase in spine density in the apical dendrites of Layer 5 cortical neurons, and a concomitant increase in expression of the astrocytic glutamate transporter, GLT1. Interestingly, spine density in young animals (2- 4 weeks old) shows no difference between mutants and controls, suggesting that Shh signaling between neurons and astrocytes is required for developmental pruning of synapses. These results suggest that neuron-astrocyte communication through Shh signaling plays an important role in circuit formation. Ongoing studies are examining the dynamic turnover of dendritic spines by chronic, *in vivo* imaging.

Disclosures: **A. Garcia:** None. **A. Aharon:** None. **Y. Zuo:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 217.08/D16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT CB2011-169023

Title: Study of the effect of the peptide Aβ25-35 in the neurogenesis and long-term memory

Authors: *E. RAMIREZ[1], A. SANCHEZ[2], A. PATRICIO[2], D. LIMON[2]
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Abstract: The amiloid-β plays an important role in the neurodegeneration process of Alzheimer´s disease (AD), but its neurotoxic mechanisms is not entirely clear [5]. The Aβ (25-35) fraction mimics the toxic effects of the complete peptide Aβ (1-42) because this decapeptide is able to cause memory impairment and neurodegenerative events [1,4]. The Aβ (25-35) increase oxidative stress, cause neuronal damage and inflammation [6]. Recent evidences have shown that the injection of Aβ (25-35) into the temporal cortex (TCx) of rats increases the inflammatory response [3]; however, it is unclear how the inflammatory process could be involved in the progression of Aβ (25-35) toxicity and abnormal neurogenesis. This has led to a hypothesis which that impairment in memory in AD by inflammation can involve in generating an inappropriate environment for the new neurons to mature [2]. The objective of this study was determined if the administration of aggregated Aβ25-35 into the CA1 region of the rat hippocampus impairs the long-term memory and decrease the neurogenesis in the dentate gyrus (DG). The results showed that in the group Aβ 25-35 decreased the learning and memory in Morris water maze and decrease in the number of dendritic spines. Similarly a change was observed in the neurogenesis indicating that the effect of peptide Aβ 25-35 is chronic progressive. [1] Butterfield et al., TRENDS in molecular medicine. 2001; 7 (12): 548-554. [2] Deng et al., Nat Rev Neurosci. 2010; 11(5): 339-350. [3] Diaz et al., J Alzheimers Dis. 2012; 30(3): 505-22. [4] Limón et al., Neursc. Res. 2009; 63: 129-137. [5] Maccioni et al., Arch. Med. Res. 2001; 32: 367-381. [6] Zussy et al., The American journal of pathology. 2011. 179 (1); 315-334.

Disclosures: **E. Ramirez:** None. **A. Sanchez:** None. **A. Patricio:** None. **D. Limon:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 217.09/D17

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH

Title: Dnmt1 is required for progenitor maintenance during spinal cord development of zebrafish

Authors: ***S. PANAHI**, R. I. DORSKY
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Abstract: Neural progenitor cells must be maintained during development to produce the full complement of neuronal and glial derivatives, and DNA methylation is an important epigenetic mechanism for regulating cell lineage differentiation. Previous studies have shown that genetic deletion of DNA methyltransferase I (Dnmt1) in neuronal progenitor cells results in DNA hypomethylation and premature glial differentiation, but the specific role of this gene in progenitor maintenance is poorly understood. Here we analyzed progenitor maintenance in the spinal cord of zebrafish dnmt1 mutants. We found that spinal cord progenitor markers are progressively lost in embryos lacking Dnmt1 and that the number of proliferative progenitors decreases accordingly. Our preliminary data suggest that Dnmt1 likely regulates methylation of specific developmental genes during spinal cord development. These results allow us to hypothesize that the targets of CpG methylation may be pluripotency genes, and in the absence of methylation progenitor cells may fail to differentiate as neurons. Therefore, loss of Dnmt1 should decrease neurogenesis by up-regulating pluripotency genes and misexpression of Dnmt1 should result in premature differentiation.

Disclosures: S. Panahi: None. **R.I. Dorsky:** None.

Poster

217. Synapse Development, Maintenance, and Aging

Location: Halls A-C

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Program#/Poster#: 217.10/D18

Topic: C.05. Aging

Support: NIH grant 5K99NS080913

NIH grant 2R37NS047344

Title: Mechanisms underlying endogenous adult neural stem cell behavior during aging

Authors: *M. A. BONAGUIDI[1], R. STADEL[2], D. BERG[1], J. PARK[2], E. PU[2], M. OGAWA[2], S. PARK[2], M. BARADARAN-SHORAKA[2], G.-L. MING[1], H. SONG[1]
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Abstract: Aging, simply the act of living longer, is a major risk factor for cognitive decline and the development of neurodegenerative disorders. Neurogenesis - the ongoing production of new cells - declines with age at a time that coincides with increasing incidence of brain vulnerability to injury and disease. Yet, the origins of this decline in structural plasticity remain unknown.

Here, we performed clonal lineage-tracing of radial glia-like neural stem cells (RGLs) marked by Nestin-CreERT2, Gli1-CreERT2 and Ascl1-CreERT2. Time course analysis with computational studies suggest that Gli1# marks the stochastic, multipotent nestin#-RGLs population at the pre-activation state, whereas Ascl1#-RGLs represent a discrete and more proliferative, primed neurogenic population. In young animals, depletion of individual stem cells within dual stem cell populations is compensated by expansion of others in a homeostatic process yielding population asymmetry. With age, overall stem cell number declines as the rate of expansion diminishes due to an increase in the quiescence of both multipotential and more active neurogenic RGLs. As the aged brain retains the capacity to dynamically regulate neurogenesis in response to macroenvironmental changes, remaining endogenous neural stem cells may preserve their capacity to repopulate brain regions - even in older individuals. Understanding the effect of age on neurogenesis and determining the extent of plasticity remaining within the aging neurogenic niche will be of considerable importance for defining age-related cognitive deficits and provide potential therapeutic candidates.

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Poster

217. Synapse Development, Maintenance, and Aging

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Program#/Poster#: 217.11/D19

Topic: A.02. Neurogenesis and Gliogenesis

Support: Swedish Research Council for Medicine and Health

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Swedish Brain Foundation

Wallenberg Scholar

Söderberg Foundation

ERC advanced grant

Title: Parasympathetic neurons originate from nerve-associated peripheral glial precursors cells

Authors: A. FURLAN[1], V. DYACHUK[1], M. KHATIBI SHAHIDI[1], M. GIOVINCO[1], N. KAUKUA[1], C. KONSTANTINIDOU[2], V. PACHNIS[2], F. MEMIC[1], U. MARKLUND[1], T. MÜLLER[3], C. BIRCHMEIER[3], K. FRIED[1], *P. ERNFORS[1], I. ADAMEYKO[1]
[1]Karolinska Institutet, Stockholm, Sweden; [2]MRC Natl. Inst. for Med. Res., London, United Kingdom; [3]The Max Delbrück Ctr. for Mol. Med., Berlin, Germany

Abstract: The peripheral autonomic nervous system reaches far throughout the body and includes neurons with diverse functions, such as sympathetic and parasympathetic. We show that the parasympathetic system in mice, including trunk ganglia and the cranial ciliary, pterygopalatine, lingual, submandibular and otic ganglia, do not arise from neural crest cells, as previously believed. Instead, the parasympathetic fate is induced in nerve-associated glial precursor cells at distal peripheral sites. These bi-potent progenitors generate both glia and neurons, and give rise to most of the neurons in these ganglia. The novel nerve origin places cellular elements for generating parasympathetic neurons in diverse tissues and organs which may enable wiring of the developing parasympathetic nervous system.

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Poster

217. Synapse Development, Maintenance, and Aging

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 217.12/D20

Topic: B.11. Glial Mechanisms

Support: P20 GM103554-02

Title: Measuring synaptic and perisynaptic activity at the developing, neonatal and adult mouse neuromuscular junction using electrophysiological and optical methods

Authors: *D. HEREDIA, G. HENNIG, A. SCURRY, T. GOULD
Univ. of Nevada Reno, Sch. of Med., Reno, NV

Abstract: During embryogenesis, neuromuscular synapses form between presynaptic motor axons and postsynaptic skeletal muscle cells. Perisynaptic or terminal Schwann cells are a

subtype of Schwann cell found at this neuromuscular junction (NMJ). Embryonic NMJs form normally at embryonic day 14.25 (E14.25) but degenerate shortly thereafter in mutant mice lacking Schwann cells. Since genetic forms of activity blockade rescue this degeneration, we reasoned that embryonic Schwann cells regulate synaptic transmission, similar to the role of perisynaptic Schwann cells at the adult NMJ. However, analyzing synaptic activity in embryonic preparations has been difficult because the targets of drugs which block contractility and therefore allow recording are not expressed at these ages. In this study we measured neuromuscular activity using intracellular voltage recording of postsynaptic muscle cells in the E14.25 mouse diaphragm using a drug that selectively blocks skeletal muscle contractility. Similar to adult NMJs, embryonic NMJs exhibit pronounced synaptic depression in response to high-frequency stimulation of the phrenic nerve, although the duration and frequency of stimulation required to induce this form of synaptic plasticity were markedly different. At the embryonic NMJ, stimulation at 10Hz initiates a (50%) decrease of action potential (AP) amplitude within 10 seconds. Higher rates of stimulation, 20 and 40Hz, result in a reduction of AP amplitude (90%) within seconds. In contrast, newborn animals failed to exhibit depressed responses to 10Hz stimulation for 1 minute, but displayed progressively greater decreases in AP amplitude in response to 20 and 40Hz stimulation. Adult animals displayed no rundown in response to 1 minute of 10 and 20Hz stimulation, whereas 40Hz exhibited an AP reduction of ~10% after 1 minute. Measuring these changes both electrically and with fluorescence in mice expressing GCaMP3 in skeletal muscle, we obtained a correlation coefficient of 0.80. We also observed robust increases in intracellular calcium in GCaMP3-expressing Schwann cells in response to high-frequency stimulation at E14.25. Our observations suggest that similar to the adult, Schwann cells at the developing NMJ respond to synaptic activity, and thus may regulate synaptic maintenance by modulating neural activity.

Disclosures: **D. Heredia:** None. **G. Hennig:** None. **A. Scurry:** None. **T. Gould:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.01/D21

Topic: C.03. Parkinson’s Disease

Support: NIH/NIDA Grant R03 DA027111

Title: Neuroprotective mechanisms of the Parkinson’s disease-related protein DJ-1

Authors: *V. MISHRA, S. L. ROY, F. LIU, J.-C. ROCHET
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by a loss of dopaminergic neurons in the *substantia nigra* region of the brain. Dysfunction of the neuroprotective protein DJ-1 is thought to play a role in familial and sporadic cases of PD. Previously we showed that human wild-type DJ-1 protects against different PD stresses (rotenone, proteasome inhibitor, aSyn) by inducing different pro-survival mechanisms, potentially because differences in the subcellular localization of DJ-1 could result in the activation of different neuroprotective responses. Here, we describe the results of studies aimed at testing neuroprotective effects of DJ-1 against another PD-related insult, methamphetamine (METH), which acts via a different mechanism. METH is a widely abused drug that triggers preferential toxicity to dopaminergic neurons by increasing cytosolic dopamine (DA) levels via inhibition of vesicular monoamine transporter-2 (VMAT-2) causing a buildup of cytosolic DA leads to the formation of DA quinone adducts that trigger oxidative stress. We found that METH exposure caused dopaminergic neurite retraction in primary midbrain cultures, and this METH-dependent neurite loss was less pronounced in cultures transduced with adenovirus encoding human WT DJ-1. In addition, we showed that DJ-1 levels are increased in SH-SY5Y and PC12 neuronal cell lines exposed to METH, suggesting that DJ-1 up-regulation is a cellular defense mechanism against METH neurotoxicity. To test the effect of subcellular localization of DJ-1 on neurite retraction, we overexpressed WT DJ-1and DJ-1 variants localized to mitochondria (MLS-DJ-1) or nucleus (NLS-DJ-1) in METH-treated cultures. We found that NLS-DJ-1 had a greater protective effect against METH-induced neurite retraction compared to MLS-DJ-1, suggesting a critical role for nuclear DJ-1 in neurite length regulation. Preliminary results also show a reduction in VMAT-2 mRNA levels in primary midbrain cultures depleted of DJ-1 via shRNA, suggesting that DJ-1 could protect against METH neurotoxicity by modulating VMAT-2 levels. Current efforts are focused on understanding (i) the interplay of DJ-1, VMAT-2 and Nurr-1, a transcription factor that controls the expression of the VMAT-2 gene, in METH treated primary cultures; and (ii) the role of DJ-1 expression in glia and/or neurons in protection against neurotoxicity elicited by METH and other PD-related insults. The results of these studies provide insight into mechanisms by which DJ-1 may alleviate neurodegeneration in the brains of PD patients, and they could stimulate the development of new strategies to treat PD or the neurotoxic effects of METH abuse.

Disclosures: V. Mishra: None. **S.L. Roy:** None. **F. Liu:** None. **J. Rochet:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS065063

Title: rAAV-mediated knockdown of GD3 synthase protects against MPTP-induced neurodegeneration and executive dysfunction

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Abstract: Parkinson's disease is characterized by gradual degeneration of dopaminergic neurons in substantia nigra. More than half of the patients exhibit fronto-striatally-mediated executive dysfunctions such as deficits in attention, planning, judgment, and impulse control. We previously demonstrated that deletion of GD3 synthase (GD3S) is neuroprotective and improves cognitive function in a mouse model of Alzheimer's disease, and that GD3S knock-down protects against MPTP-induced neurodegeneration and motor deficits. The objective of the present study is to determine whether knockdown of GD3S can protect dopaminergic cells and prevent motor and cognitive dysfunction in mice lesioned with subchronic 1-methy-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP; 25 mg/kg x 5 days). Male C57BL/6N wild-type mice were trained on a 3-hole serial reaction-time (SRT) task, which included measures of sustained attention and impulsive behavior, and a battery of sensorimotor tasks. On baseline, each SRT trial included a variable pre-cue period ranging from 3-8 s, and a cue duration of 1 s. Correct responses to the cue resulted in delivery of a 14-mg sweet pellet to the food-restricted subjects. Sustained attention was measured by response accuracy and reaction time. Impulsive behavior was measured by premature responding in the response holes or the food well during the pre-cue period. After reaching stable performance mice were given bilateral striatal injections of a recombinant adeno-associated viral (rAAV) vector expressing a short-hairpin RNA (shRNA) construct targeting GD3S, or a scrambled-construct control. After 3 weeks, mice were received five daily injections of MPTP, followed by a 5-day quarantine period, and were re-trained on the SRT and sensorimotor tasks. GD3S knock-down protected dopaminergic neurons from MPTP-induced neurotoxicity, and prevented the executive and motor dysfunctions. Our data suggest that strategic modification of complex brain gangliosides warrants further investigation as a novel therapeutic strategy for Parkinson's disease.

Disclosures: P. Maiti: None. **M.P. McDonald:** None. **T.S. Rex:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Program#/Poster#: 218.03/D23

Topic: C.03. Parkinson's Disease

Support: NRF-2012R1A1A1012435

SBRI, SMX1132521

Title: Inhibition of PARIS(ZNF746) by CSU-1806 in model of Parkinson's disease

Authors: ***A. JO**[1], R. KHANG[1], H. KANG[1], J.-H. SHIN[1,2,3], T. DAWSON[4,5,3,6], Y.-I. LEE[7], B. LEE[8], G. JEONG[8], V. DAWSON[4,9,10,11,6], H. KANG[12,11,13], Y. LEE[4,11,6], S. KARUPPAGOUNDER[4,11,6], H. JIANG[4,11,6], S.-U. KANG[4,11,6]

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Abstract: [91,4,5991,4,51,4,571,4,51,4,6781,2,3,4,51,2,4,51,4,9 123456_789]We previously observed that parkin regulates **PAR**kin **I**nteracting **S**ubstrate (PARIS,ZNF746) via ubiquitination and accumulated PARIS plays a role as transcriptional repressor on peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) in Parkinson's disease (PD). The significant reduction of PGC-1α associated with dopaminergic neuronal death and the restoration of PGC-1α is considered as a therapeutic target. Here, we identified CSU-1806 as a transcriptional inducer of PGC-1α by high-throughput screening. CUS-1806 restores the reduction of PGC-1α in the models of overexpression of PARIS or parkin deficit. Consistent with increase of PGC-1α by CSU-1806, it also significantly increases the level of nuclear respiratory factor (NRF-1) that is a pathogenic

target gene of PGC-1α on PD. Furthermore, increased protein level of PGC-1α in mouse olfactory bulb, hippocampus, and substantia nigra was observed in CSU-1806-fed mouse. CSU-1806 shows the protective efficacy against MPTP-mediated dopaminergic neuronal death in mice. Taken together, PARIS- PGC-1α pathway is a potential therapeutic target pathway and CSU-1806 may be beneficial to modulate patho-pathway in PD.

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Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Topic: C.03. Parkinson’s Disease

Support: The Morris K. Udall Center of Excellence for Parkinson’s disease Research at Michigan State University (TJC, JWL, NMK)

The Edwin A. Brophy Fund (TJC)

Title: Small proline-rich repeat 1a protein protects nigrostriatal axons from degeneration in the 6-hydroxydopamine lesion rat model of Parkinson’s disease

Authors: ***N. M. KANAAN**, T. GRABINSKI, B. COMBS, A. KNYENSBERG, A. COLE-STRAUSS, Z. MATTINGLY, F. P. MANFREDSSON, T. J. COLLIER, J. W. LIPTON
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Abstract: Multiple lines of evidence suggest Parkinson’s disease (PD) is a dying-back axonopathy and axonal degeneration is quickly becoming recognized as a critical early event in PD pathogenesis. A recent study demonstrated that patients lose their striatal dopamine (DA) projections early in the disease, well before significant somatic loss occurs. This observation explains why DA somatic preservation strategies have not been successful in clinical trials: They do not prevent the dying-back of axonal DA projections in PD. To address the problem of early axonal degeneration in PD, we examined the progressive gene expression changes in the rat ventral midbrain from an intrastriatal 6-hydroxydopamine (OHDA) lesion over 16 weeks. We hypothesized that patterns of gene expression within the substantia nigra (SN) characterized by

early, highly upregulated expression would be indicative of potential protective responses to a striatal 6-OHDA insult. The largest group of genes fitting this expression pattern was the regeneration-associated gene (RAG) family. The most highly upregulated RAG was small proline-rich repeat 1a (Sprr1a), which has been shown to facilitate post-injury axonal regeneration in peripheral nerves likely through stabilizing the cytoskeleton at the growth cone. We then confirmed that Sprr1a is specifically upregulated (using RNAscope *in situ* hybridization) in degenerating SN DA (tyrosine hydroxylase, TH; immunohistochemistry) neurons. We then overexpressed Sprr1a or green fluorescent protein (GFP) with rAAV constructs in the SN followed 1 month later with a striatal 6-OHDA lesion. Animals receiving rAAV-Sprr1a exhibited significant protection of nigrostriatal axons in the striatum as compared to rAAV-GFP as measured by TH densitometry. The protective effect of Sprr1a was further confirmed by both stereological measurements of TH fiber density and volumetric assessments of striatal zones exhibiting severe, intermediate, or no lesion. Animals treated with rAAV-Sprr1a had significantly higher TH+ axon density in the striatum and significantly smaller zones of severe denervation as compared to GFP controls. These data show that Sprr1a is part of the DA neuron's natural armamentarium to resist axonal degeneration and that it can be manipulated to mitigate the loss of striatal DAergic fibers in a rat 6-OHDA model.

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Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Support: Austrian Science Fund (F44020, W11)

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Title: Pyrimidine 2,4,6-triones are a new class of voltage-gated l-type ca^{2+} channel activators

Authors: ***N. J. ORTNER**[1], G. BOCK[1], D. H. F. VANDAEL[3], R. MAUERSBERGER[2], H. J. DRAHEIM[4], R. GUST[2], E. CARBONE[3], P. TULUC[1], J. STRIESSNIG[1]
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Austria; [3]Dept. of Drug Sci., Univ. of Torino, Torino, Italy; [4]CNS Res., Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany

Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative disorders worldwide and until now no cure or treatment to stop its progression are known. The characteristic cell death of dopamine producing neurons in the substantia nigra pars compacta is thought to be caused by increased mitochondrial oxidative stress due to the activity of L-type Ca^{2+} channels (LTCCs) during autonomous pacemaking. Known LTCC blockers inhibit both main brain LTCC isoforms, Cav1.2 and Cav1.3, with similar potency. Therefore therapy-limiting cardiovascular side effects arise from Cav1.2 channel block in the cardiovascular system. Cav1.3-selective blockers without these effects appear as attractive neuroprotective agents in PD. Here we studied the pharmacological properties of a pyrimidine 2,4,6-trione derivative (Cp8; Kang *et al.*, Nat.Comm. 2012) recently reported as the first highly selective Cav1.3 blocker. Pharmacological modulation of I_{Ba} (10 or 15mM) or I_{Ca} (15mM) through Cav1.3 (rat or human long splice variant, $rCav1.3_L$, $hCav1.3_L$) and Cav1.2 (rabbit long or short C-terminus, $rbCav1.2_L$, rbCav1.2s) expressed together with β3 and α2δ1 subunits in tsA-201 cells or using mouse chromaffin cells (MCCs; 2mM Ca^{2+}) was measured using the patch-clamp technique. With Ca^{2+} as charge carrier and using different protocols and channel constructs, Cp8 reproducibly caused a change in current kinetics characterized by a slowing of activation and inactivation accompanied by a prolongation of tail currents. This modulation closely resembled the actions of the LTCC activator FPL64176 and was also observed in MCCs in which non-L-type currents were unaffected. Moreover, Cp8 significantly increased the spontaneous firing frequency of MCCs, accompanied by a reduced after-hyperpolarization of action potentials. Evidence for a weak and non-selective inhibition of Cav1.3 as well as Cav1.2 currents was only observed in a minority of cells using Ba^{2+} as charge carrier. In conclusion, neither potent nor Cav1.3-selective inhibition by Cp8 could be observed under our experimental conditions. Instead, Cp8-induced changes in channel kinetics resemble the activity of known LTCC activators such as FPL64176. Therefore, our data suggest that pyrimidine 2,4,6-triones can also act as Ca^{2+} channel activators.

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Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Topic: C.03. Parkinson's Disease

Support: KIST grant, Korea

Title: Optogenetic rescue of Parkinson's disease symptoms

Authors: *D. LEE, S. J. VARGA, C. QI
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Abstract: A key pathological feature of Parkinson's disease (PD) is the progressive loss of dopaminergic (DA) neurons in the substantia nigra which regulates motor function. Familial forms of PD are known to be directly associated with mutations of certain genes (e.g., α-Syn). In addition, neurotoxin-based models (e.g., rotenone) have been used to study DA neurodegeneration induced by non-genetic, environmental factors. All of these indicate that a combination of genetic and environmental factors contributes to the pathogenesis of PD. Currently, no cure exists. Although several treatments (e.g., L-dopa) are used to alleviate symptoms, none can slow or reverse the progression of the disease. This warrants the development of a new therapeutic method that can rescue both motor symptoms and molecular and cellular damage. An increasingly popular technique, optogenetics can be used in PD models to uncover the effects of stimulation on the DA neuronal circuits damaged by PD causing factors. The fruit fly *Drosophila* has long been used as a model organism for human diseases, primarily due to the genetic similarity with mammals and the sophisticate genetic tools available to study disease mechanisms as well as potential therapies. In this study, we developed a new model of PD using *Drosophila* larvae to study optogenetic rescue of PD symptoms. α-Syn and rotenone were chosen as a genetic factor and an environmental factor, respectively. *Drosophila* larvae (3^{rd} instar) expressing mutant human α-Syn showed age-dependent decline in locomotion and also a significant loss of DA neurons in the brain. Similarly, larvae chronically exposed to rotenone (10μM) showed significant reduction in locomotion accompanied by loss of DA neurons in the brain. Using these two PD models, we showed that application of blue light (470nm) to A53T mutant larvae expressing channelrhodopsin 2 in DA neurons is able to fully rescue their mutation-induced decrease in locomotor speed for both PD models. After one hour of blue light stimulation, the locomotion scores were indistinguishable from those of control larvae. The long-term exposure (24 hours) also rescued locomotor deficits in α-Syn larvae. Currently, we are examining to determine an optimal lighting duration and pattern, and also to test whether the optogenetic rescue of locomotor deficits is related to structural (e.g., number of DA neurons, mitochondria and synapses) and/or functional rescue (e.g. DA neuronal activity). Our study showed that optogenetic stimulation of the larval PD models can alleviate PD-like symptoms, and thus will help to develop better strategies for slowing or reversing the progression of the disease.

Disclosures: D. Lee: None. **S.J. Varga:** None. **C. Qi:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Program#/Poster#: 218.07/D27

Topic: C.03. Parkinson's Disease

Title: The neuroprotective potential of intranasal dnsp-11 on nigral dopamine neurons against intrastriatal 6-hydroxydopamine neurotoxicity

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Abstract: Using both *in vitro* and *in vivo* studies, glial cell line-derived neurotrophic factor (GDNF) became known as one of the more promising neurotrophic factors in its ability to protect dopamine neurons against neurotoxic insult in animal models of Parkinson's disease (PD). The proprotein version of GDNF has been post-translationally processed into a dopamine neuron stimulating peptide, known as DNSP-11. DNSP-11 has been shown to be neuroprotective against TaClo, MPP+ and an intranigral 6-hydroxydopamine (6-OHDA) lesion in rat models of PD. This research project used a different approach to introducing DNSP-11 into the animal model prior to the lesion. An intranasal DNSP-11 technique was used to assess the protection of nigral dopamine neurons against the more progressive intrastriatal lesion of 6-OHDA. Twenty Fisher 344 rats were divided into the following groups: citrate + 6-OHDA and DNSP-11 + 6-OHDA. Citrate or DNSP-11was delivered intranasally prior to the 6-OHDA lesion. The foot fault and cylinder tests were performed to assess behavior improvements following treatment. Brain tissue was processed for tyrosine hydroxylase immunocytochemistry and dopamine cell survival was quantified via stereology.

Disclosures: C.M. Fox: None. **A. Ghoweri:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Topic: C.03. Parkinson's Disease

Support: National Natural Science Foundation of China (81171212)

Title: Nrf2 signaling contributes to the neuroprotection of urate to dopaminergic cells

Authors: *L.-F. HU
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Abstract: Mounting evidence shows that urate may become a biomarker of Parkinson's disease (PD) diagnosis and prognosis and a neuroprotectant candidate for PD therapy. However, the cellular and molecular mechanisms underlying its neuroprotective actions remain poorly understood. In this study, we showed that urate pretreatment protected dopaminergic cell line (SH-SY5Y and MES23.5) against 6-hydroxydopamine (6-OHDA)- and hydrogen peroxide-induced cell damage. Urate was found to be accumulated into SH-SY5Y cells after 30 min treatment. Moreover, urate induced NF-E2-related factor 2 (Nrf2) accumulation by inhibiting its ubiquitinationa and degradation, and also promoted its nuclear translocation; however, it did not modulate Nrf2 mRNA level or Kelch-like ECH-associated protein 1 (Keap1) expression. In addition, urate markedly up-regulated the transcription and protein expression of γ-glutamate-cysteine ligase catalytic subunit (γ-GCLC) and heme oxygenase-1 (HO-1), both of which are controlled by Nrf2 activity. Furthermore, Nrf2 knockdown by siRNA abolished the intracellular glutathione augmentation and the protection exerted by urate pretreatment. In sum, our findings demonstrated that urate treatment may result in Nrf2-targeted anti-oxidant genes transcription and expression by reducing Nrf2 ubiquitination and degradation and promoting its nuclear translocation, and thus offer neuroprotection on dopaminergic cells against oxidative stresses.

Disclosures: L. Hu: None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Topic: C.03. Parkinson's Disease

Title: Sesamol has capacity to reverse the effect of rotenone-induced Parkinson's disease

Authors: *P. KUMAR[1], S. ANGELINE[3], R. K. AMBASTA[2]
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Abstract: In the previous report (Sonia Angeline et al., 2012), we showed an altered expression of protective proteins in rotenone-induced Parkinson's disease (PD)-like rat model. This model exhibited a marked attenuation in the expression of parkin, C terminus Hsp70 interacting protein (CHIP) and PARK 7 protein (DJ1) while enhanced levels of caspases and ubiquitin were seen. Herein, we confirmed the neuroprotective role of sesamol on rotenone-induced rodent model of PD. Rotenone administration was given for 11 days to generate the PD model (Sonia Angeline et al., 2012). From 11th day onward individual doses of sesamol (15 mg/kg) and drugs were given orally to the rotenone PD rat model for 10 consecutive days. The impact of drugs markedly improved the motor skills, body weight, expression of parkin, DJ1, tyrosine hydroxylase and CHIP compared to the group treated with rotenone alone in the striatum and substantia nigra. These results were correlated with the reduction in caspase and ubiquitin levels by immunostaining and immunoblotting. Moreover, improved morphology and survivability of neurons were seen upon sesamol and naringenin treatment in the same rat PD model. Further we confirmed the efficacy of neuroprotective biomolecule administration on muscle from the above PD model and observed the restoration in muscle morphology, elevated level of parkin, DJ1, differential expression of heat shock proteins and reduced cell death. To conclude, for the first time we are demonstrating the comprehensive role of sesamol (rotenone-induced PD model) in neuro and myoprotection that would have great clinical significance.

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Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS034239

Title: Neuroprotective activities of VPAC receptor agonists in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxicated mice

Authors: *K. OLSON[1], L. KOSLOSKI-BILEK[1], R. MOSLEY[1], J. GLEDHILL[2], S. SHANDLER[2], H. GENDELMAN[1]
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Abstract: Interplay between the immune system and the brain is associated with the pathobiology of Parkinson's disease (PD). Our laboratory has shown that impaired innate and adaptive immunity herald rapid and progressive nigrostriatal degeneration in PD. Based on a large volume of previous work from our laboratory and others, we demonstrate that neurodestructive autoimmunity speeds neuronal injury while regulatory T cell (Treg) responses are protective against neural damage. Clinical investigations are currently ongoing to evaluate this therapeutic strategy in humans (NCT01882010). The study addresses the neuroprotective role of an established immune modulator, vasoactive intestinal peptide (VIP) in MPTP-induced nigrostriatal degeneration. VIP mediates a pleotropic range of biological activities via two related receptor subtypes, VIP receptor 1 and 2 ($VPAC_1$ and $VPAC_2$). One such biological activity has been shown to augment Treg-mediated control over neurodegenerative processes. As a therapeutic agent, the clinical utility of native VIP is limited by rapid metabolism and clearance from blood as well as the lack of receptor selectivity and significant untoward effects. Metabolically stable and selective VPAC agonists were developed with improved pharmacokinetic and pharmacodynamic properties compared to VIP. The selective $VPAC_1$ and $VPAC_2$ agonists were used to investigate their ability to prevent MPTP nigrostriatal injury and their role in lymphocyte transformation and adoptive transfer of lymphocytes to attenuate neuronal injuries. Stereological analysis of tyrosine hydroxylase (TH^+) neurons in the substantia nigra after treatment with MPTP and the novel $VPAC_1$ and $VPAC_2$ agonists showed 50% and 79% protection compared to MPTP controls. Densometric analysis indicated striatal termini in MPTP mice were, in part, spared after treatment with VPAC agonists. Immunohistochemical analysis of Mac-1^+ microglia showed a VPAC-mediated reduction in microglial reactivity following MPTP-intoxication. Furthermore, VPAC agonists led to significant downregulation of innate neuroinflammatory responses. Cytokines measured by cytometric bead analysis, demonstrated that either VPAC agonist decreased levels of Th1 and Th17 specific cytokines, including IL-17A, IL-6, and IFN-γ. These results suggest that stable VPAC agonists transform T cell phenotypes to neuroprotective Th2 or Treg phenotypes. The downregulation of pro-inflammatory cytokines implies a phenotypic shift of T cell subsets. Thus, the immune modulatory and neuroprotective properties of VPAC agonists provide evidence to support their development as potential therapeutic agents for PD.

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Poster

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CARNOT Institute

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Title: Effects of intra-cerebral near infra-red illumination on mptp monkeys

Authors: F. DARLOT[1], *C. MORO[1], N. TORRES-MARTINEZ[1], C. CHABROL[1], F. REINHART[1], D. AGAY[1], T. COSTECALDE[1], J. MITROFANIS[2], A. BENABID[1]
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Abstract: Parkinson's disease (PD) symptoms arise after a substantial loss of dopaminergic (DA) cells, mainly in the substancia nigra pars compacta (SNc) of the midbrain, with evidence of mitochondrial dysfunction. All treatments are symptomatic, none of them curative or preventive. Previous studies have shown that substances such as CoQ10 and melatonin help neuroprotecting DA cells in the SNc against neurotoxin induced degeneration in animal models of PD which induce mitochondrial dysfunction. Recent results have reported neuroprotective properties of low intensity light therapy (or photobiomodulation by Near Infra-Red (NIR) light) through activation of cytochrome C oxidase and ATP synthesis in the damaged cells. Preliminary experiments demonstrated *in vivo* protection of DA cells in the SNc of acute and chronic MPTP mice and 6-OHDA rats, associated to improvement of locomotor activity. Here is presented the continuation of the study in MPTP primates treated by NIR (wavelength 670nm) using a prototype of a fully implantable chronic illuminator delivering deep brain NIR treatment of DA cells in the SNc, using a fiber the tip of which is at mid distance between left and right SNc nuclei. The power out of the implant is 10 mW and the regimen of light administration is 5sec ON / 60sec OFF. Thermic effects of the device were previously shown to be limited to +1°C maximum. Acute and chronic control groups (5 monkeys each) received MPTP (0.3 mg/kg/day) during 5 days for the acute group, plus 1 injection per week during 2 additional weeks for the chronic group. NIR treated groups (5 monkeys each, implanted with NIR device) were also submitted to acute or chronic MPTP regimens. They received a NIR treatment, either NIR illumination restricted to

24h around each MPTP injection, or continuously for 3 weeks since the beginning of MPTP injections. Clinical score and motor activity were evaluated daily for 1 week before and 3 weeks after the onset of MPTP injections. Animals were sacrificed 3 weeks after the onset of injections, and the brains processed for histology (TH and Nissl staining, alphasynuclein), for quantification of the neuroprotective effect of NIR treatment. Further experiments are required to study better the dose-effect relationships before clinical application to early PD patients. This study is the first step towards a human clinical trial aimed at evaluating NIR neuroprotective potential for PD patients.

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Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Michigan State University Neuroscience Program T32NS44928 (CLS).

Title: Attenuation of alpha-synuclein-induced neuroinflammation and microgliosis via rho-kinase inhibition: A possible mechanism behind fasudil-mediated neuroprotection

Authors: *M. F. DUFFY[1], J. P. MACKEIGAN[2], F. P. MANFREDSSON[1], S. G. LAMPE[1], N. KUHN[1], C. J. KEMP[1], C. E. SORTWELL[1]
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Abstract: No treatments exist to halt or slow the progression of nigrostriatal degeneration in Parkinson's disease (PD), and many existing treatments exacerbate dyskinesias after prolonged use. The approach of repurposing drugs with known safety profiles in humans can accelerate new developments for PD treatment. Previous studies in our lab have shown that fasudil, a rho-kinase (ROCK) inhibitor, provides neuroprotection from recombinant adeno-associated virus (rAAV) α-synuclein-mediated toxicity. However, the mechanisms behind fasudil-mediated

neuroprotection remain unknown. Recent studies have shown α-synuclein to be a direct mediator of neuroinflammation via upregulation of phagocytic microglia. ROCK regulates microglial polarization and motility. In the present study we examined whether fasudil treatment to rats attenuated neuroinflammation associated with intranigral injection of rAAV α-synuclein. We hypothesized that neuroprotective fasudil treatment would be associated with attenuation of microglial polarization and motility via ROCK inhibition. Nigral tissue sections from rAAV α-syn injected animals treated orally with 1) neuroprotective high dose fasudil chow (25 mg/kg/day), 2) low dose fasudil chow (10 mg/kg/day, non-neuroprotective) or 3) control chow were utilized. Sections were double-labeled for tyrosine hydroxylase (TH, dopamine neurons) and Iba-1 (microglia) immunofluorescence and analyzed using near infrared imaging to quantify Iba-1 signal intensity. Stereological quantification of phagocytic marker CD68 was also performed. While there was a dramatic increase in CD68 immunoreactive cells ipsilateral to rAAV α-syn injection, there were no differences in CD68 immunoreactive cells between treatment groups. rAAV α-syn was associated with a marked increase Iba-1 immunoreactivity. High dose fasudil resulted in a significant decrease in Iba-1 immunoreactivity in the rAAV α-syn substantia nigra (SN), intact SN, and tectum (used as a control) suggesting that fasudil attenuates α-syn-mediated microgliosis. These findings, along with previous findings from our lab, demonstrate that fasudil may protect SN dopamine neurons against α-syn-mediated inflammation via inhibition of ROCK, ultimately attenuating microgliosis. Given that orally administered fasudil has an established safety profile in humans and is, to our knowledge, the first orally available drug to provide neuroprotection in the rAAV α-syn model of PD, it demonstrates potential for development as an effective therapeutic agent to slow progressive nigrostriatal degeneration in Parkinson's disease.

Disclosures: **M.F. Duffy:** None. **J.P. MacKeigan:** None. **F.P. Manfredsson:** None. **S.G. Lampe:** None. **N. Kuhn:** None. **C.J. Kemp:** None. **C.E. Sortwell:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.13/D33

Topic: C.03. Parkinson's Disease

Title: Depletion of proinflammatory monocytes/macrophages is neuroprotective in the myenteric plexus but not in the basal ganglia in a mptp mouse model of parkinson's disease

Authors: *M. COTE[1], C. F. LAVALLÉE[1], A.-A. POIRIER[1], B. AUBÉ[1], S. LACROIX[2], D. SOULET[3]
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Abstract: Patients suffering from Parkinson's disease (PD) frequently display non-motor symptoms like delayed gastric emptying, constipations and defecatory dysfunctions. These gastrointestinal impairments are associated with the degeneration of dopaminergic neurons in the myenteric nervous system. Furthermore, a growing body of evidence supports a critical role for inflammation in the dysfunction of neurons in the central nervous system (CNS) and the enteric nervous systems (ENS) during acute and chronic insults. Since studies suggest that peripheral inflammation originating from the gut may have a major impact in both the initiation and progression of PD, we investigated the role of the innate immune response in neurodegeneration occurring in the CNS and ENS secondary to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioning. Proinflammatory monocytes/macrophages (also known as M1) were depleted by intraperitoneal injections of clodronate-encapsulated liposomes in MPTP and saline-treated animals. We examined the profile of immune cells in their blood and measured the immunoreactivity of several neuronal markers in their striatum and myenteric plexus. Our results show that mice depleted in proinflammatory M1 monocytes by clodronate-encapsulated liposomes treatments were protected against the MPTP-induced loss of tyrosine hydroxylase (TH) expression in the ENS. Furthermore, a strong immune response was observed in undepleted mice treated with MPTP, as demonstrated by the prominent presence of proinflammatory M1 monocytes and the production of IL-1β and IL-6 in all the segments of the gut. However, in the CNS mice were subjected to 25% of striatal TH signal loss following MPTP administration regardless of whether they were depleted or not in proinflammatory M1 monocytes. Moreover, the MPTP treatment elicited a strong microglial activation in the striatum in depleted and undepleted animals. Taken together, our results demonstrate a critical role for proinflammatory M1 monocytes/macrophages in the gastrointestinal dopaminergic dysfunction in the MPTP model of Parkinson's disease. In sharp contrast, the immune response in the CNS was not impaired by the clodronate liposome treatment as circulating monocytes did not cross the blood-brain barrier to reach the brain parenchyma, nor did they contribute to the MPTP-induced toxicity as observed by confocal microscopy.

Disclosures: **M. Cote:** None. **C. F. Lavallée:** None. **A. Poirier:** None. **B. Aubé:** None. **S. Lacroix:** None. **D. Soulet:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.14/D34

Topic: C.03. Parkinson's Disease

Support: NINDS

Bumpus Foundation

Parkinson's Disease Foundation

Title: Targeting dephosphorylation of eukaryotic initiation factor-2a to treat parkinson's disease

Authors: *X. SUN[1], P. AIME[1], J. CRARY[1], L. GREENE[1], O. LEVY[2]
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Abstract: A critical unmet need in the treatment of Parkinson's disease (PD) is protecting vulnerable neurons from degenerating. The study of pathogenic mechanisms in PD-relevant cellular systems may provide targets for neuroprotective therapies. Multiple PD-relevant stressors lead to the phosphorylation of the eukaryotic translation initiation factor-2a (eIF2a), resulting in overall translational attenuation. However, certain transcripts, including the mRNA encoding the transcription factor ATF4, undergo a paradoxical increase in their translation. We have previously found that ATF4 promotes survival in multiple cellular PD models via up-regulation of parkin. These findings imply that phosphorylation of eIF2a would favor neuronal survival by increasing levels of ATF4 and its downstream effector parkin. Interestingly, eIF2a phosphorylation can either promote or reduce neuronal survival, depending on the paradigm studied. Phospho-eIF2a levels are increased in PD brain tissue, and indirect evidence suggests that eIF2a phosphorylation is protective in PD models. Taken together, these studies suggest that eIF2a phosphorylation may regulate neuronal survival in PD, and, further, that modulating eIF2a phosphorylation may be a therapeutic approach to slow neuronal loss. Phosphorylation of eIF2a is regulated by multiple kinase and phosphatase complexes. The regulatory subunit GADD34 binds to the phosphatase catalytic subunit PP1c and directs its dephosphorylation of eIF2a. The small molecule guanabenz acetate (GA) binds to GADD34, preventing its association with PP1c and increasing phospho-eIF2a levels. We have previously found that GA promotes survival of PC12 cells and primary ventral midbrain dopaminergic neurons treated with 6-OHDA through an ATF4-Parkin pathway. Here, we extend our previous findings by demonstrating that GA prolongs eIF2a phosphorylation after 6-OHDA treatment, validating the notion that GA blocks GADD34 and eIF2a dephosphorylation. In addition, GADD34 mRNA and protein are up-regulated in PC12 cells treated with 6-OHDA. We also assessed GADD34 expression in the midbrain of autopsy tissue from PD patients and controls using immunohistochemistry. GADD34 immunopositivity is detected in neuromelanin-containing, i.e. dopaminergic, neurons

of the substantia nigra pars compacta. PD patients display a coarse granular GADD34 label in neuronal perikarya and proximal dendrites compared to a fine granular label present in controls. Direct assessment of the role of GADD34 in PD cellular models is ongoing. In summary, our data suggest that eIF2a phosphorylation could be a therapeutic target for slowing disease progression in PD.

Disclosures: X. Sun: None. **P. Aime:** None. **J. Crary:** None. **L. Greene:** None. **O. Levy:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.15/D35

Topic: C.03. Parkinson's Disease

Support: NIH-COBRE-5P20GM103653-02 (KIM: PI)

Title: Developing combination therapies of lithium/L-Dopa and novel AurimMed Compounds for Parkinson's Disease

Authors: C. A. LAZZARA[1], A. PESYAN[2], J. K. ANDERSEN[3], *Y.-H. KIM[1]
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Abstract: Something Old: Lithium has been suggested to have neuroprotective effects in several models of neurodegenerative disease including Parkinson's disease (PD). Levodopa (L-Dopa) replacement therapy remains the most common and effective treatment for PD, although it induces the complication of L-Dopa induced dyskinesia (LID) after years of use. Here we examined the potential use of lithium in combination with L-Dopa/Carbidopa for both reducing abnormal involuntary movements (AIMs) as well as protecting against cell death in MPTP-lesioned mice and MPP+ damaged N27 dopaminergic neurons. Chronic lithium administration (0.127% LiCl in the feed) in the presence of daily L-Dopa/Carbidopa injection for a period of 2 months was sufficient to effectively reduce AIMs in MPTP-lesioned mice. Mechanistically, lithium was found to suppress MPTP-induced calpain activities *in vitro* and *in vivo* coinciding with down-regulation of calpain-1 but not calpain-2 expression in both the striatum (ST) and the brain stem (BS). Calpain inhibition has previously been associated with increased levels of the rate-limiting enzyme in dopamine synthesis, tyrosine hydroxylase (TH), which is probably

mediated by the up-regulation of the transcription factors MEF-2A and 2D. Lithium was found to induce up-regulation of TH expression in the ST and the BS as well as increasing histone acetyltransferase (HAT) expression, likely resulting in the observed cytoprotection and reduced AIMs in MPTP-lesioned mice. These results suggest the potential use of lithium in combination with L-Dopa/Carbidopa not only as a neuroprotectant, but also for reducing LID and alleviating potential side-effects associated with current treatment for PD. Something New: Based on our *in vitro* cell viability studies, novel AurimMed compounds demonstrated their neuroprotective effects against H_2O_2 or MPP+ induced oxidative stress, in addition to their recovery effect from oxidative stress-induced insults. Currently we are optimizing the most effective combination therapy for preventing dopaminergic neurodegeneration as well as revitalizing damaged dopaminergic neurons in PD.

Disclosures: C.A. Lazzara: None. **A. Pesyan:** None. **J.K. Andersen:** None. **Y. Kim:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson’s Disease

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Title: The beneficial effects of spargel in a *Drosophila* model of Parkinson’s disease

Authors: *K. WU[1], Z. QIAN[2], Y. KE[1]
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Abstract: Parkinson's disease (PD), which is caused by loss of dopaminergic neurons in the substantia nigra, is one of the most common neurodegenerative diseases affecting millions of people world-wide. Abnormal α-synuclein aggregation is the central hallmark of PD and many studies have already demonstrated the deleterious effects of oligomeric and aggregated forms of α-synuclein to dopaminergic neurons. Overexpression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), the master regulator of mitochondrial biogenesis and cellular energy metabolism downregulated in PD, has resulted in contradictory outcomes in neurotoxin-induced PD models. In this study, we overexpressed spargel, the PGC-1α fly homolog, in the well-established A53T α-synuclein fly model to examine how spargel modulates α-synuclein toxicity and the underlying mechanisms. Our data indicated that spargel overexpression did not affect the reduced survival rate of A53T α-synuclein flies but significantly improved motor deficits 30 days post-eclosion, as revealed by negative geotaxis. Whole-mount immunohistochemistry results indicated that dopaminergic neurons in PPL1 and PPM3 cluster were rescued in 30 day-old A53T α-synuclein flies. To investigate the protective mechanism of spargel against α-synuclein, we first checked whether spargel overexpression could modify α-synuclein level and solubility. Our western blot results showed that spargel overexpression did not significantly affect SDS-soluble α-synuclein level when compared with age-matched A53T α-synuclein flies, but significantly reduced urea-soluble forms of α-synuclein, which may represent the toxic insoluble forms of α-synuclein aggregates. To conclude, our findings support that manipulation of endogenous PGC-1α in dopaminergic neurons, which is possible via a number of FDA-approved drugs, can be a promising and readily translatable treatment strategy against α-synuclein-induced toxicity in PD.

Disclosures: K. Wu: None. **Z. Qian:** None. **Y. Ke:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.17/D37

Topic: C.03. Parkinson's Disease

Title: Anti-high mobility group box 1 antibody has neuroprotective effects against Parkinson's disease model of rats

Authors: *T. SASAKI[1], T. AGARI[2], H. TAKEUCHI[2], A. TOYOSHIMA[2], S. SASADA[2], A. SHINKO[2], A. KONDO[2], M. KAMEDA[2], T. YASUHARA[2], K. LIU[3], M. NISHIBORI[3], I.

DATE[2]
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Abstract: Objectives: The involvement of inflammation in the pathogenesis of Parkinson's disease is well known in the field of basic research as well as in the clinical setting. Intranuclear high mobility group box 1 (HMGB1) moves towards extracellular space and acts as an inflammatory factor when the cells are damaged. In this study, we investigated the involvement of HMGB1 and the neuroprotective effects of anti-HMGB1 antibody for Parkinson's disease model of rats. Methods: Adult female Sprague-Dawley rats (200-250g) were used in this study. 6-OHDA (20 μg) was injected into the right striatum and then anti-HMGB1 antibody (1mg/kg), control IgG or saline was intravenously administered immediately, 6 and 24 hours after 6-OHDA injection. Cylinder test and amphetamine-induced rotation test were performed as behavioral assessment at 1 and 2 weeks after surgery. Tyrosine hydroxylase (TH) staining was performed at 2 weeks for evaluation of dopaminergic neurons. Iba1 which is a marker of microglia, HMGB1, MAP2 and GFAP staining were performed at 24 hours, 1 week and 2 weeks for histological evaluation. Results: Behavioral tests revealed that the number of amphetamine-induced rotations at 2 weeks decreased and contralateral bias of cylinder test at 1 and 2 weeks improved in anti-HMGB1 groups. Immunohistochemically, TH-positive fibers in the striatum and TH-positive neurons in the substantia nigra pars compacta were significantly preserved in anti-HMGB1 groups. Iba1-positive cells were decreased at 1 and 2 weeks in anti-HMGB1 groups. In control IgG group, cytoplasmic HMGB1 expressed in GFAP-positive cells at 1 week after surgery. Conclusions: These results suggested that HMGB1 released from GFAP-positive astrocytes was at least partly involved in the mechanism and pathway of degeneration of dopaminergic neurons induced by 6-OHDA exposure. Intravenous administration of anti-HMGB1 antibody suppressed microglial activity and preserved dopaminergic neurons. Anti-HMGB1 antibody exerts anti-inflammatory effects and with subsequent neuroprotective effects.

Disclosures: **T. Sasaki:** None. **T. Agari:** None. **H. Takeuchi:** None. **A. Toyoshima:** None. **S. Sasada:** None. **A. Shinko:** None. **A. Kondo:** None. **M. Kameda:** None. **T. Yasuhara:** None. **K. Liu:** None. **M. Nishibori:** None. **I. Date:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

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NRF Grant 2011-0030028

NRF Grant 2011-0030775

NRF Grant 2012-003338

Title: 7-OH-DPAT induces neuroprotection and attenuate motor deficit in LRRK2 R1441G mice

Authors: *J. KIM, J. KIM, S. KANG, J. JANG, H. SEO
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder that induces degeneration of dopaminergic neurons in substantia nigra pars compacta (SNpc). Several studies suggest that dopamine D3 receptor has relation to motor and cognitive function in PD. D3 receptor is a D2-like family that inhibits adenylyl cyclase through G-proteins. In this study, we determined the effects of D3R agonist, (±)-7-Hydroxy-2-(di-n-propylamino) tetralin hydrobromide (7-OH-DPAT), in *in vivo* model of PD. We found that LRRK2 R1441G mutant (MT) mice showed significantly decreased latency to fall compared to litermate mice (LM). 7-OH-DPAT treatment significantly increased the latency to fall in LRRK2 R1441G MT mice. In open field test, D3R agonist administered LRRK2 R1441G MT mice showed significant increase in the counts of rearing and grooming compared to vehicle control group. Also, the counts of wall rearing and cross were significantly decreased compared to vehicle control group. 7-OH-DPAT administration significantly increased the number of tyrosine hydroxylase (TH)-immunoreactive neurons in SNpc of MT mice. Our results suggest that D3R agonist could induce neuroprotection and attenuate motor deficit in PD model.

Disclosures: J. Kim: None. **J. Kim:** None. **S. Kang:** None. **J. Jang:** None. **H. Seo:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.19/D39

Topic: C.03. Parkinson's Disease

Support: MJFF Research 2014 Grant

Title: mGluR3 PAM: Novel neuroprotective therapeutic strategy for Parkinson disease - *in vitro* neuroprotection and neurotrophic factor production

Authors: *S. SCHANN[1], S. MAYER[1], C. FRANCHET[1], M. FRAULI[1], S. SCHEFFLER[1], T. PILLOT[2], B. MANTEAU[1], P. NEUVILLE[1]
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Abstract: mGluR3 is a novel target that could lead to neuroprotection through production of GDNF and TGF-beta in striatal neurons. This potential was previously demonstrated with a non-selective mGluR2/3 agonist and KO mice. At Domain Therapeutics, novel NCEs with selective mGluR3 positive allosteric modulator (PAM) property were recently discovered. These molecules were subject to chemical optimization and characterized *in vitro* in both neuroprotection and neurotrophic factor production models. Members of these mGluR3 PAMs showed protective activity on primary striatal and cortical neurons and neurotrophic factor production in an mGluR3-dependant manner. The present poster describes the full characterization of the best analogs of the series.

Disclosures: **S. Schann:** A. Employment/Salary (full or part-time):; Domain Therapeutics, SynAging SAS. **S. Mayer:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **C. Franchet:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **M. Frauli:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **S. Scheffler:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **T. Pillot:** A. Employment/Salary (full or part-time):; SynAging SAS. **B. Manteau:** A. Employment/Salary (full or part-time):; Domain Therapeutics. **P. Neuville:** A. Employment/Salary (full or part-time):; Domain Therapeutics.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.20/D40

Topic: C.03. Parkinson's Disease

Support: CHIR Grant MOP-114916

Studentship from FER of the Faculty of Pharmacy, Université Laval

Title: Neuroprotective effect of plasmalogen precursor analogs in a mouse model of Parkinson's disease

Authors: *E. MIVILLE-GODBOUT[1,2], M. MORISSETTE[1], M. BOURQUE[1,2], S. AL SWEIDI[1], T. SMITH[3], A. MOCHIZUKI[3], V. SENANAYAKE[3], D. GOODENOWE[3], T. DI PAOLO[1,2]

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Abstract: Objective: This study investigated neuroprotection with ethanolamine plasmalogens (PlsEtn). Plasmalogens are a unique class of glycerophospholipids that contain a vinyl ether alcohol at the sn-1 position. They are promising because of the roles they play in membrane structure mediated functions such as vesicular release of neurotransmitters and membrane protein activity, free radical scavenging, and as a storage of depot of neuroprotective polyunsaturated fatty acids (docosahexaenoic acid (DHA)). Methods: Male mice were treated for 10 days with a daily oral administration of either the DHA-plasmalogen precursor PPI-1011 or the oleic acid-plasmalogen precursor PPI-1025 (10, 50 or 200 mg/kg). On day 5 the mice received 4 injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The mice were killed on day 11 and striatal biogenic amine concentrations were measured by HPLC. Autoradiography of brain membrane (DAT) and vesicular (VMAT2) dopamine transporters were made and serum plasmalogens concentrations mesured. Results: PPI-1011 (10 and 50 mg/kg) and PPI-1025 (10 mg/kg) completely prevented the decrease of dopamine concentration induced by MPTP, the PPI-1025 prevention at 50 mg/kg was partial. PPI-1011 and PPI-1025 at 10 and 50 mg/kg prevented the MPTP induced decrease of DOPAC and HVA concentrations. We found significant correlations between the levels of DA and DAT or VMAT2 transporters, demonstrating that the precursors of plasmalogens help to protect the terminals of dopaminergic neurons. Results obtained with VMAT2 and DAT transporter demonstrate that a dose of 50 mg protect dopaminergic neurons in the striatum of MPTP toxicity. MPTP also induced a peripheral (serum) depletion in PlsEtn levels. Pre-treatment with plasmalogen precursors that bypass peroxisomal metabolism prevented all serum MPTP-induced effects. In conclusion, the neuroprotective effect of plasmalogen precursor analogs appears to have a bell-curve shape and was dose-dependent. The similar activity of oleic (PPI-1025) and DHA (PPI-1011) plasmalogen precursors suggests that the observed neuroprotection is due to the plasmalogen backbone rather than formation of DHA. The decrease of several plasmalogens observed in the striatum of MPTP mice is in agreement with the results obtained by Fabelo et al. (Mol Med. 2011;17 :1107) that observed a reduction of plasmalogens in cortex of parkinsonian patients. This research was

supported by a CHIR grant to TDP. EMG holds a studentship from FER of the Faculty of Pharmacy, Laval University.

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Poster

218. Parkinson's Disease: Neuroprotective Therapies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.21/D41

Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS78247

NIH Grant NS74443

Title: Prokineticin-2 gene therapy strategy shows anti-inflammatory and anti-apoptotic effects in preclinical cell culture and animal models of Parkinson’s disease

Authors: M. NEAL, D. LUO, D. HARISCHANDRA, R. GORDON, H. JIN, V. ANANTHARAM, *A. G. KANTHASAMY, A. KANTHASAMY
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Abstract: Prokineticin-2 (PK2) is a small secretory peptide with diverse biological functions. We recently identified that dopaminergic neurons upregulate PK2 in response to neurotoxic stress. To better understand the functional role of PK2 upregulation, we created stable PK2-overexpressing dopaminergic cells by transfecting the PK2 gene into mouse dopaminergic MN9D cells. Interestingly, PK2-overexpressing cells exposed to the Parkinsonian neurotoxicant MPP+ showed significant protection against neurotoxicity relative to vector control cells, suggesting a neuroprotective role for PK2 in dopaminergic neuronal cells. Furthermore, the PK2 receptor blocker PC7 attenuated the PK2-induced neuroprotective effects in PK2-overexpressing cells. The neuroprotection mediated by PK2 against MPP+-induced toxicity was associated with increased Bcl2 gene expression and reduced apoptotic cell death. To extend the results of cell culture studies to animal models, we adopted adeno-associated viral (AAV2) vector technology to develop a PK2-GFP gene construct delivery system, and we tested the efficacy of this new

viral vector in mouse models. Stereotaxic injections of the PK2 AAV and a control virus (GFP only) demonstrated that the virus could infect cells in the striatum and substantia nigra as determined by fluorescence microscopy and Western blotting. Neurobehavioral analyses revealed that the PK2 AAV injection does not alter basal motor behavior relative to control mice. PK2 AAV gene delivery in the striatum significantly reduced basal gene expression of inflammatory cytokines, including TNFα, IL-1β and IL-6, indicating an anti-inflammatory function for PK2 in the striatum. Also, iNOS gene expression was reduced following PK2 expression in the striatum. In contrast, PK2 AAV expression significantly upregulated Bcl2 and Pink1, suggesting that PK2 can upregulate cell survival signaling pathways. Initial results show that PK2 AAV expression attenuates MPTP-induced behavioral deficits and TH depletion in the C57 black mouse model. Taken together, these results demonstrate for the first time, that PK2 overexpression can protect dopaminergic neurons in both cell culture and animal models by attenuating the glial cell-mediated inflammatory response as well as by upregulating Bcl2 and Pink-1 protective signaling. Further characterization of the efficacy of PK2 gene therapy in preclinical models of Parkinson's disease may provide a viable neuroprotective strategy for the chronic neurodegenerative disease

Disclosures: **M. Neal:** None. **D. Luo:** None. **A.G. Kanthasamy:** None. **D. Harischandra:** None. **R. Gordon:** None. **H. Jin:** None. **V. Anantharam:** None. **A. Kanthasamy:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Program#/Poster#: 218.22/D42

Topic: C.03. Parkinson's Disease

Support: Department of Science and Technology, India

Title: Neuroprotective and neurotrophic effects of Apigenin in MPTP induced Parkinson's disease in mice

Authors: *S. P. PATIL, SR, P. JAIN, S. SATHAYE
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Abstract: Apigenin, a natural flavonoid, has potential biological effects, including antiproliferative, and anticancer activities. In the present study, our aim was to investigate the neuroprotective and neurotrophic effects of apigenin and also to explore the underlying

mechanisms with respect to Parkinson's disease (PD). MPTP (25mg/kg) along with Probenecid (250mg/kg) was administrated for five consecutive days to induce PD in mice. Apigenin (5, 10 and 20 mg/kg) was administrated orally for 26 days including 5 days of pretreatment. Bromocriptine (10mg/kg) was used as a standard drug. Motor co-ordination and locomotor activities were evaluated by rotarod and open field tests. Oxidative stress was evaluated specifically in the mid brain by assessing various antioxidant enzymes activities. Tyrosine hydroxylase (TH), glial fibrillary acidic protein (GFAP) and brain derived neurotrophic factor (BDNF) were evaluated in substantia nigra (SN) region of the brain by immunostaining. TNF-α was estimated using ELISA technique. Our results demonstrate that apigenin treatment improved the locomotor and muscular activities in MPTP treated mice. Apigenin treatment has significantly improved the antioxidant activity in comparison to MPTP treated mice. TH-positive cells decreased upto 7% in MPTP group as compared to normal mice ($p<0.001$) and were found to be protected from degeneration in apigenin (69%) treated mice ($p<0.001$). Levels of GFAP were also found to be decreased in the SN of the brain due to apigenin treatment as compared to MPTP mice ($p<0.001$). Additionally, BDNF levels were elevated significantly in apigenin treatment group when compared with MPTP treatment mice. In conclusion, apigenin protected the dopaminergic neurons probably through its antioxidant, anti-inflammatory and neurotrophic potential which could be the possible reason for amelioration of the disease process. The above results provide preclinical support for therapeutic potential of this compound in the treatment of PD.

Disclosures: **S.P. Patil:** None. **P. Jain:** None. **S. Sathaye:** None.

Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 218.23/D43

Topic: C.03. Parkinson's Disease

Support: CAPES

CNPq

Title: Neuroprotective effect of diphenyl diselenide on a rat model of Parkinson's disease induced by 6-hydroxydopamine

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Abstract: Aim: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by degeneration of dopaminergic neurons in the nigrostriatal pathway. The antioxidant and neuroprotective properties of diphenyl diselenide [(PhSe)2], together with its effectiveness in inhibiting MAO-B activity, suggest a potential effect of (PhSe)2 on motor dysfunction induced by dopaminergic depletion. Therefore, the purpose of the present study was to investigate whether (PhSe)2 reverses motor and neurochemical impairments in a model of PD induced by 6-hydroxydopamine (6-OHDA) in rats. Methods: Wistar rats (250-300 g) received 20 µg/3 µl of 6-OHDA or 3 µl of vehicle into the right striatum. Three weeks later, animals were subjected to rotational behavioral test induced by D-amphetamine and divided into four groups: Sham; (PhSe)2; 6-OHDA and 6-OHDA+(PhSe)2. Rats were treated with (PhSe)2 (1 mg/kg/day; orally) or vehicle (canola oil) during 30 days. At the end of treatment, cylinder, stepping, bridge, and open-field tests were performed and brain was collected for the measure of brain-derived neurotrophic factor (BDNF), proBDNF, and tyrosine hydroxylase (TH) in the nigrostriatal region by immunoblotting assay. Data were analyzed using a two-way ANOVA followed by the Duncan's test, when appropriate (significant when $p<0.05$). Results: 6-OHDA infusion triggered a considerable increase in D-amphetamine-induced rotational behavior demonstrating the effectiveness of 6-OHDA infusion before starting the (PhSe)2 treatment. Subsequently, 6-OHDA-injected rats decreased the number of wall contacts with contralateral forelimb in the cylinder test. 6-OHDA also decreased the number of responses with contralateral forelimb in rats evaluated in the stepping test. (PhSe)2 treatment restored the normal behavior in 6-OHDA-infused rats in both tests. In the bridge test, (PhSe)2 treatment reverted the altered equilibrium function induced by 6-OHDA infusion in rats. Besides, 6-OHDA infusion and/or (PhSe)2 treatment did not alter the number of crossing and rearing in the open-field test. In addition, the expression of BDNF, proBDNF, and TH in the nigrostriatal region of 6-OHDA-lesioned rats was reduced. Interestingly, (PhSe)2 treatment restored proBDNF and TH expression in the nigrostriatal region of 6-OHDA-infused rats. Conclusion: Results demonstrated that (PhSe)2 treatment reversed motor impairment in a model of PD in rats, which was mediated, at least in part, by restoring proBDNF and TH expression. Thus, the current study suggests a potential neuroprotective role for (PhSe)2 in PD.

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Poster

218. Parkinson's Disease: Neuroprotective Therapies

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Topic: C.03. Parkinson's Disease

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Botany in Action fellowship

Title: Nepalese and Native American traditional medicine: What can we learn to treat Parkinson's disease?

Authors: A. DE RUS JACQUET[1], R. SUBEDI[2], S. K. GHIMIRE[2], *J.-C. ROCHET[1]
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Abstract: Parkinson's disease (PD) is an age-related neurodegenerative disorder characterized by the death of dopaminergic neurons from the substantia nigra. Five million people are affected by Parkinson's disease (PD) worldwide. Current therapies fail to slow the underlying neurodegenerative disease and can only temporarily relieve symptoms. On the other hand, Nepalese and Native American societies have a long tradition of herbal medicine. Although these societies evolved in different sociological and cultural contexts, herbal medicine is a central component of their identity. Our central hypothesis is that plant species used to treat PD-like symptoms in both Nepalese and Native American traditional medicines have a high potential to alleviate neurodegeneration. From May to July 2012, we conducted a total of 36 interviews in Nepal, and we identified 46 potentially active plant species. Subsequent interviews with Native Americans from the Lumbee and Blackfeet tribes led to the identification of additional plants with potential activity against PD-related symptoms. Plant extracts are being screened for protective activity in neuronal cultures exposed to environmental poisons that have been linked epidemiologically to elevated PD risk, including the pesticide rotenone and the herbicide paraquat. Our most promising plant candidates are being tested in fluorescence-based assays that monitor the ability to activate various pro-survival mechanisms, including induction of autophagy, rescue of mitochondrial function, and activation of cellular antioxidant responses. Tinospora sinensis, a climber used to treat paralysis in Nepal, shows exceptional neuroprotective activities in our cell culture models of PD. The ethanolic extract rescues neuronal death triggered by rotenone in a chronic exposure model and by paraquat and rotenone in primary midbrain cultures. In addition, the extract induces up-regulation of the Nrf-2 antioxidant pathway.

Tinospora sinensis is a promising medicinal plant for the design of therapies that not only are readily available to traditional communities but also have a high potential to be developed into a modern Western medicine. Our findings are relevant to the identification of therapeutic targets in PD and suggest public health strategies for indigenous and Western populations.

Disclosures: A. de RUS Jacquet: None. **R. Subedi:** None. **S.K. Ghimire:** None. **J. Rochet:** None.

Poster

220. Epilepsy: Circuits

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Program#/Poster#: 220.01/D45

Topic: C.07. Epilepsy

Title: Functional analysis of the p.A294V hotspot mutation in KCNQ2 reveals that the consequence on IM current is not predictive of disease severity in early onset encephalopathies

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Abstract: Mutations in the *KCNQ2* gene, which encodes a subunit of the voltage-dependent potassium the Kv7.2 subunit of the voltage-dependent potassium channel responsible for the M current, have been identified in early onset epileptic encephalopathies (EOEE) and in benign familial neonatal seizures (BFNS). The prognosis for patients is very heterogeneous from benign forms (BFNS) with normal neurological development to severe forms (EOEE) with strong deterioration of motor and cognitive function. So far, the phenotypic variability has mostly been

explained by the functional impact of mutations on the M current measured in non-neuronal heterologous expressing system (decreased by ~25 % in BFNS and > 50% in EOEE, Miceli et al. 2013; Ohran et al. 2014). We have previously reported 16 de novo *KCNQ2* mutations in EOEE patients, without any cortical malformations or metabolic disease (Milh et al. 2013). Here, we investigated the functional consequence of the p.A294V hotspot mutation located in the pore domain and identified in 9 EOEE patients (7 in our cohort and 2 additional patients reported by Kato et al. 2013). For this purpose KCNQ current was recorded using the whole cell patch-clamp method in CHO cells transfected with a plasmid expressing $KCNQ2^{A294V}$ alone or together with plasmids expressing $KCNQ2^{WT}$ or KCNQ3. We report that the homomeric $KCNQ2^{A294V}$ is not functional. The association of $KCNQ2^{A294V}$ with KCNQ3 reduces the current by 83%, and by 30% when $KCNQ2^{WT}$ was added, an association that mimics the situation in cells of EOEE patients. Other parameters such as the kinetics and the conductance-voltage relationships for heteromeric mutant channels were not different from wild-type heteromeric channels. Surface biotinylation of CHO cell and western blot analysis of lysates show that the mutation decreased the total amount of KCNQ2 and reduced the surface expression of the subunit. We suggest that the p.A294V mutation accelerates KCNQ2 degradation and on the other hand, induces defects in the gating of KCNQ2 mutant channels that are successfully addressed to the membrane. In cultured hippocampal neurons, the presence $KCNQ2^{A294V}$ induces a strong redistribution of the heteromeric mutant channels from the axon initial segment to the somatodendritic compartment. This lack of KCNQ channels is susceptible to induce abnormal excitability by various mechanisms. From these data, we propose that (i) Ala-294 although located in the pore domain is essential for proper channel formation and expression to the plasma membrane; (ii) the degree of KCNQ current inhibition in non-neuronal heterologous system cannot predict the neurological outcome.

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Poster

220. Epilepsy: Circuits

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Topic: C.07. Epilepsy

Support: Israel Science Foundation

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Title: Reorganization of the CA1 hippocampal network in the chronic kainate model of epilepsy

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Abstract: Epilepsy is a common neurological disorder characterized by recurrent unprovoked seizures caused by various pathologic processes in the brain. One of the best characterized chronic models of temporal lobe epilepsy is the kainate model. A potential mechanism that may underlie development of epilepsy (epileptogenesis) is reorganization of the network and change in the network topology, that in turn causes an increased tendency of the network to hyper-activate and hyper-synchronize.In this study we investigated this possibility by comparing the functional network characteristics and network topology in control and chronic epileptic rats. We simultaneously recorded action potential firing from multiple neurons from the CA1 region of the hippocampus using multi-tetrode single-unit recordings from control rats (Sprague Dawley) and rats with chronic kainate induced temporal lobe epilepsy, in which spontaneous seizures develop after a kainite induced status epilepticus (SE). Recordings were performed 5-10 weeks after the kainite induced SE. We used the multi-tetrode single-unit recordings, and especially the pair-wise cross correlations between recorded neurons to compare network synchronization, functional network properties and topology in normal and epileptic rats. Multi-unit single-unit recordings from the CA1 region of normal and epileptic rats revealed a significantly higher degree of synchronization in epileptic rats (as assessed by the pair-wise cross correlation function) compared to control. There were no significant differences in auto correlation between unit pairs which means that the difference in synchronization was not a result of increased bursting in the epileptic rats. With respect to network properties the network tended to show higher connectivity as shown by a significantly higher local cluster coefficient in the epileptic group compared to control. We found no significant difference in path length between the two groups. Our results show that the epileptic network undergoes reorganization with a higher functional connectivity and increased synchronization compared to control animals. These network changes may underlie the tendency to generate seizures in these rats.

Disclosures: I. Khoury: None. **Y. Schiller:** None.

Poster

220. Epilepsy: Circuits

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Topic: C.07. Epilepsy

Support: NIH Grant R44NS062477

Title: High-throughput assay of seizurogenic activity using multi-well microelectrode array technology

Authors: D. MILLARD[1], A. NICOLINI[1], S. CHVATAL[1], M. BROCK[1], K. COOK[1], *J. ROSS[2]
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Abstract: The lack of advancement in anti-epileptic drugs (AEDs) over the last 30 years, along with the continued need for improved proconvulsant screening in drug safety, motivates the need for new assays of seizurogenic neural activity. An *in vitro* approach for detecting and quantifying seizurogenic activity could provide a predictive and high-throughput avenue for the evaluation of the efficacy of AEDs and the proconvulsant risk of other compounds. Towards this end, microelectrode arrays (MEA) have been used extensively for characterizing and quantifying neural activity *in vitro* and have recently been scaled to a multi-well format that facilitates high throughput electrophysiological analysis. Indeed, MEAs have shown promise in predicting the toxicity of neuro-active compounds by measuring changes in the population mean firing rate of a neuronal network. However, further consideration of how recorded spikes are grouped in space and time across neural populations may lead to improved sensitivity for *in vitro* seizurogenic assays. Here, we present an assay of seizurogenic activity based upon the Axion BioSystems Maestro multi-well MEA system. We used previously published metrics for the detection of burst spiking events and the quantification of synchronization across a neural population. Data are included from both rat cryopreserved cortical cultures and human induced pluripotent stem cell derived populations, in response to pharmacological manipulation with AEDs (i.e. Carbamazepine, Valproic Acid) and reference compounds with known proconvulsant risk (i.e. 4-Aminopyradine, Strychnine, Pentylenetetrazole). Our results support the use of multi-well MEA technology for the high throughput evaluation of complex neuronal networks *in vitro* to inform the development of AEDs, while also quantifying the proconvulsant risk of candidate pharmaceuticals in a pre-clinical setting.

Disclosures: **D. Millard:** A. Employment/Salary (full or part-time):; Axion Biosystems. **J. Ross:** A. Employment/Salary (full or part-time):; Axion Biosystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axion Biosystems. **A. Nicolini:** A. Employment/Salary (full or part-time):; Axion Biosystems. **S. Chvatal:** A. Employment/Salary (full or part-time):; Axion Biosystems. **M. Brock:** A. Employment/Salary (full or part-time):; Axion Biosystems. **K. Cook:** A. Employment/Salary (full or part-time):; Axion Biosystems.

Poster

220. Epilepsy: Circuits

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Title: Circuit changes underlying network synchronization during post-traumatic epileptogenesis

Authors: *K. P. LILLIS[1], Z. WANG[1], G. Q. ZHAO[2], B. J. BACSKAI[1], K. J. STALEY[1]
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Abstract: In secondary epilepsy, a seizure-prone neural circuit forms following an injury to the brain (e.g. trauma). The nature of both the epileptogenic changes that take place during the latent period and the continuing evolution that occurs following the onset of seizures remain relatively unknown. We have used both whole-cell patch clamp electrophysiology and serial two-photon calcium imaging to characterize network changes occurring in the hippocampal organotypic slice culture model of post-traumatic epileptogenesis. The ratio of mIPSC to mEPSC charge transfer rate did not significantly change throughout early epileptogenesis, suggesting that the network alterations underlying the propensity for spontaneous seizures are more complicated than a simple dearth of inhibition. To characterize these network alterations during the first week *in vitro*, CA1 pyramidal neurons were imaged periodically using two-photon targeted path scan calcium imaging. Over the first 24 hours, as interictal population bursts emerged, there was a rapid increase in the proportion of neurons participating in the bursts and in the synchrony of each burst. Ictal activity typically began during the second or third DIV, and evolved more gradually than the interictal activity. Over the first 8 DIV, seizure onset became progressively more synchronous across the population of neurons imaged. Cross-correlation-based network analyses quantitatively confirmed that the earliest spontaneous seizures were characterized by a slow buildup of predominantly local, correlated activity preceding seizure onset. The onset of later seizures was comprised of an abrupt transition from unmeasurable to fully synchronous ictal calcium activity, corresponding to a sharp, spatially uniform increase in correlated activity. Concomitant with the decrease in pre-ictal buildup of activity, was also a significant decrease in

measurable, non-ictal calcium transients, suggesting that, in well-developed epilepsy, very moderate bursts of synchronous activity are sufficient to initiate seizures. Together, these data support the concept that epilepsy after brain injury is a consequence of changes in the synaptic circuitry of neural networks, rather than the proportion of excitatory to inhibitory synapses, and that this circuitry continues to evolve after the latent period and onset of seizure activity.

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Poster

220. Epilepsy: Circuits

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MIUR FIRB (RBAP11X42L)

Title: Activation of parvalbumin interneurons at the epileptogenic focus facilitates focal seizures generation in a mouse model of temporal lobe epilepsy

Authors: I. MARCON[1], M. SESSOLO[1], S. BOVETTI[2], G. LOSI[1], L. MARIOTTI[1], T. FELLIN[2], *G. CARMIGNOTO[1]
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Abstract: The potentiation of GABAergic inhibition is commonly used in clinical medicine to control seizures. Recent studies reported that optogenetic activation of a subset of GABAergic

interneurons, i.e., the Parvalbumin (Pv) fast-spiking interneuons, can interrupt ongoing seizures. Optogenetic targeting of this specific interneuron class has been thus proposed as a new, potentially effective strategy to control seizures. Such an approach might be particular relevant in focal temporal lobe epilepsy, where seizures initiate from a restricted brain region (i.e., the epileptogenic focus). At this region, Pv interneurons could be specifically targeted by current fiber optic-mediated strategies and their selective activation could, in principle, prevent seizure generation. However, a direct experimental proof of this hypothesis has not been provided yet. To clarify this issue, we used optogenetics in combination with intra- and extra-cellular electrophysiological recordings from layer V in a mouse cortical slice model of temporal lobe epilepsy in which evoked seizure events closely mimic those recorded *in vivo*. In this model focal seizure-like discharges could be evoked by local NMDA applications in the presence of 4-aminopyridine and low Mg2+. The fact that in the model we can predict in advance where and when a seizure will be generated, offers us the unique opportunity to investigate if and how Pv interneurons contribute to the early events that cause neural circuits to generate a focal seizure. We here provide evidence that a pulse light stimulation (473 nm) restricted to ChR2-expressing Pv interneurons at the epileptogenic focus before the application of the ictogenic stimulus (NMDA pulse): i) lowers the threshold of focal seizure generation (n=23) and ii) prolongs the overall duration of the epileptic event (duration of seizures in absence and presence of light stimulation were, respectively: 144 ± 17 s vs 194 ± 18 s n=16). In contrast, a similar light activation restricted to Pv interneurons of a region distant from the focus (i.e., the penumbra region) opposes seizure propagation (n=6). Our study call for a careful reconsideration of the optogenetic approach that aims to control seizures by targeting Pv interneurons.

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Poster

220. Epilepsy: Circuits

Location: Halls A-C

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Topic: C.07. Epilepsy

Title: Neural field theory of generalized seizures

Authors: *X. ZHAO, P. A. ROBINSON
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Abstract: The mechanisms underlying generalized seizures are explored with neural field theory. A corticothalamic neural field model that has accounted for multiple physiological states observed in human EEG is extended with bursting dynamics and used to explore changes leading to pathological seizures. It is confirmed that generalized seizures arise from instabilities in the system and replicate experimental studies observed in numerous animal models and clinical studies. **Methods** We extend a well known neural field model by incorporating bursting dynamics in the reticular nucleus. Using previously published results, we show how bursting behavior can be expressed in terms of rate dynamics and incorporated into the firing activity of the reticular nucleus in our neural field model. The bursting dynamics in the reticular thalamus interacts with the thalamic relay nucleus to enhance network oscillations at frequencies near the bursting frequency. We first show that implementing the bursting dynamics preserves previous results in normal arousal states then proceed to examine the model's pathways to generalized seizures. **Results** (i) The neural field model extended with bursting dynamics is able to reproduce previously studied arousal states such as wake, spindles in light sleep, deep sleep, and seizure activity. (ii) We explore three new mechanisms through which generalized seizures arise in the extended neural field model. Firstly, increasing bursting dynamics in the reticular nucleus. Secondly, a shift in the differential activation of GABA receptors towards the slower GABA-B receptor also results in seizure activity. Thirdly, increasing corticothalamic coupling strength replicates seizure activity discovered in our original neural field model. Our dynamic spectra of the generalized seizures show that there is a strong $2-4$ Hz component during the generalized seizures, which is in agreement with clinical studies for generalized absence seizures. (iii) We provide a potential framework for explaining the pharmacological action of drugs used in the study absence seizures with a phase diagram describing the model's seizure activity. **Conclusion** We have used a neural field model with bursting dynamics in the reticular nucleus to study different types of instabilities which give rise to generalized absence seizure dynamics. We have shown that it can account for physiological arousal states and explain the onset of generalized seizures. Furthermore, we have provided a useful framework for understanding the pharmacology of seizure drugs in terms of the model's phase diagram.

Disclosures: **X. Zhao:** None. **P.A. Robinson:** None.

Poster

220. Epilepsy: Circuits

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Topic: C.07. Epilepsy

Support: NIH R00NS065130

Title: Thalamic circuit modulation by ATP-sensitive potassium channels

Authors: *K. A. SALVATI, D. R. WYSKIEL, R. P. GAYKEMA, M. P. BEENHAKKER
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Abstract: Absence epilepsy is a non-convulsive generalized seizure disorder characterized by a sudden arrest of consciousness and 3-5Hz spike-and-wave discharges (SWD) in electroencephalography (EEG) recordings (Crunelli and Leresche, 2002). Absence seizure generation involves the cortico-thalamo-cortical network comprised of the reticular thalamic nucleus (RT), thalamocortical neurons (TC) and cortical neurons. Hypoglycemia increases blood flow to the thalamus in humans (Arbelàez et al., 2012), suggestive of increased neuronal activity, and increases SWDs in rodents (Reid et al. 2011). Acute hypoglycemia can switch the body from a primarily glucose-dependent metabolic state to one dependent on the metabolism of the ketone bodies (KBs), β-hydroxybutyrate and acetoacetate, and may recruit ATP-sensitive potassium (K_{ATP}) channels. Indeed, K_{ATP} channels are highly sensitive to cellular metabolic states and can regulate neuronal excitability (Ma et al. 2007), and depress hippocampal circuit activity (Tanner et al., 2011). Here we investigate whether elevated KBs paradoxically promote SWDs driven by thalamic circuits. To begin testing this hypothesis, we first determined whether KBs are elevated during heightened SWD activity triggered by acute hypoglycemia. Specifically, we measured blood glucose and KB levels immediately prior to measuring SWD occurrence in non-fasted mice and after a 16 hour fast. Consistent with Reid et al. (2011), we found that a 16 hour fast increases the number of SWDs (mean % increase: 252.7 ± 2.45%; n=10, p=0.01). Furthermore, we found that fasted mice had significantly elevated levels of ketone bodies immediately prior to EEG recording (mean % increase: 227.3 ± 0.048%; n=10, p<0.0001). As K_{ATP} channels can link metabolism to neural activity, we next sought evidence for K_{ATP} channel expression in the thalamus. Both mRNA analysis and antibody staining revealed that K_{ATP} channels (Kir6.2) are expressed by RT and TC neurons. Moreover, *in vitro* electrophysiological methods revealed a significant K_{ATP} channel conductance in thalamic neurons. Specifically, application of the sulfonylurea receptor agonist, diazoxide, increased the holding current in voltage-clamped neurons in a dose-dependent manner, an effect that was blocked by the K_{ATP} channel antagonist, glibenclamide. Additionally, direct KB application to thalamic neurons also activated a glibenclamide-dependent conductance. In sum, we found that acute hypoglycemia elevates KBs, and that KBs recruit K_{ATP} channel activity in the thalamic brain slices. These findings provide initial support for the hypothesis that KBs elevate SWD activity observed during fasting.

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Poster

220. Epilepsy: Circuits

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Title: Exploration of functional connectivity among spiketrains of thalamic and cortical neurons in absence epilepsy

Authors: *M. V. JONES
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Abstract: The γ2(R43Q) mutation of the GABA-A receptor causes absence epilepsy and febrile seizures in humans and in knock-in mice (RQ). GABAergic tonic inhibition is abolished in these mice, whereas synaptic inhibition is only subtly altered. Furthermore, blocking tonic inhibition in wild type mice (RR), via i.p. injection of L-655,708, induces absence-like EEG spike-wave discharges. Therefore, reduction of tonic inhibition is sufficient to cause absence-like activity. In multielectrode single-unit recordings from thalamocortical slices of RR and RQ mice, spontaneous and repeating patterns of spiking occur that appear to reverberate between thalamus and cortex, and these patterns differ between RR and RQ mice. We therefore wish to determine whether these spike patterns might convey information about the functional connectivity between thalamus and cortex, and the role of tonic inhibition in thalamocortical communication, in health and epilepsy. We used Granger Causality as a measure of functional connectivity (J Neurosci Meth 223:50). Before analyzing experimental data, we tested whether Granger Causality is even suitable to detect known causal relationships in synthetic binary spiketrain data where the causal structure is known. We simulated 100 trials of Izhikevich Networks (IEEE Trans Neural Netw 14:1569) and compared the known causal connectivity with that estimated from Granger Causality analysis of the unfiltered binary spiketrains. For a small network of 10 randomly and sparsely (~7%) interconnected neurons (1/5 inhibitory), Granger Causality identified 100% of the existing connections, and predicted 0.08% false positives and 0% false negatives. For a larger network of 40 neurons (~4% connectivity), Granger Causality identified 90% of the existing connections, with 4% false positives and 0.9% false negatives. Overall, Granger Causality was relatively accurate, more likely to predict false connections than to miss true connections, and had a bias toward missing inhibitory connections. Using Granger Causality as a measure of functional connectivity on spike timing data is therefore tenable. Thus we are

proceeding with applying this method to comparing the functional connectivity of healthy and and epileptic tissue from γ2(R43Q) mice.

Disclosures: M.V. Jones: None.

Poster

220. Epilepsy: Circuits

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FRM, Equipe FRM

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CNRS

Title: How inhibition shapes interictal dynamics in awake epileptic mice

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Abstract: In the normal brain, most cortical network dynamics are associated with sparse neuronal recruitment, shaped in space and time by the action of inhibition provided by GABA (γ-aminobutyric acid)-ergic microcircuit . A dysfunction of GABAergic microcircuits is generally linked to brain disorders, the most common of which is epilepsy. In fact, ictal and interictal discharges, the cardinal electrophysiological symptoms of epilepsy, are classically thought to reflect the breakdown of sparse activity into hypersynchronous population bursts resulting from collapsed inhibition. Although, this traditional view has recently been, the exact contribution of distinctive neurons to epileptiform dynamics *in vivo* remains unknown, largely due to the difficulty in monitoring spontaneous single-cell activity in the absence of anesthetics. Here, we use a chronic model of Temporal Lobe Epilepsy to show that GABAergic neurons are the main contributors to spontaneous interictal activity in the awake epileptic mouse hippocampus. For the first time, we apply two photon calcium imaging to map cellular recruitment within large populations of CA1 neurons in awake epileptic mice. We find that specific cellular dynamics of interictal spikes are highly variable, but always associated with the strong bursting of GABAergic neurons resulting in a perisomatic inhibitory restraint that reduces glutamatergic cell activity. This finding is in striking contrast with the canonical view that epilepsy results from an imbalance of inhibitory and excitatory action in favor of runaway excitation and instead supports earlier work indicating that the GABAergic microcircuits that are spared in the course of

epileptogenesis are hyperactive, hyperconnected, and could contribute in shaping epileptiform. Given the emergence of novel closed-loop therapeutic approaches that target abnormal brain patterns, our results provide valuable insight into microscale epileptiform dynamics which will be essential in developing efficient therapies.

Disclosures: R.A. Cossart: None. **V. Villette:** None. **S. Feldt-Muldoon:** None. **T. Tressard:** None.

Poster

220. Epilepsy: Circuits

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 220.10/D54

Topic: C.07. Epilepsy

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FP7 DESIRE project

Title: Optogenetic activation of parvalbumin interneurons synchronizes afterdischarges of seizures in a mouse model of temporal lobe epilepsy

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Abstract: The epileptic seizure reflects an episode of abnormal activity that involves neuronal ensembles from large portions of the brain. In experimental models, seizure onset is characterized by an intense activity in GABAergic interneurons followed by a rapid recruitment

of principal neurons that fire intensively, but with a low synchrony, i.e., the tonic phase, and by the late phase, i.e., the clonic phase, composed of highly synchronous firing discharges in both principal neurons and interneurons. To gain insights into the role of a GABAergic interneuron subclass, i.e., the Pv interneurons, in epileptiform activities, we expressed the light-gated cation channel Channelrhodopsin-2 (ChR2) in Pv interneurons. We used optogenetic techniques in a model of focal seizures in temporal slice preparations that allows us to follow both the initiation and the progression of seizure-like discharges. We applied a light pulse stimulation (473 nm, 150 ms duration at 0.5 Hz) that induced a sustained firing in ChR2-expressing Pv interneurons from both the epileptogenic focus and the surrounding penumbra region while performing local field potential and patch-clamp recordings from layer V pyramidal neurons. We found that optogenetic activation of Pv interneurons during the clonic phase of evoked seizures, unexpectedly prolonged ictal duration (seizure duration in absence and presence of light stimulation respectively: 144 ± 17 s vs 194 ± 18 s; n = 16). We also found that afterdischarges (ADs) could initiate few milliseconds after the light pulse onset (362 out of 675 ADs from 9 ictals) suggesting a correlation between Pv interneuron activation and AD onset. Consistently, the synchronization of AD with light pulses progressively increased along with the ictal development and almost all events could become synchronized towards the end of the clonic phase (n = 9). The distribution analysis of the time intervals between two successive ADs revealed a large peak at 2 s corresponding to the 0.5 Hz light pulse stimulation frequency (number of peaks at 2 s in absence and presence of light stimulation respectively: 6.7 ± 1.3; n = 10 vs 28.5 ± 8.8; n = 9). Spectrograms from individual ictal events during light pulse stimulation also showed the emergence of a large signal power density at 0.5 Hz that could not be detected from ictal events in the absence of light stimulation (n = 7). In conclusion, our results indicate that the prolonged ictal duration that was observed upon optogenetic stimulation is possibly linked to a synchronization of the afterdischarges by activated Pv interneurons and suggest that synchronous activity in the Pv interneuron network may contribute to afterdischarge generation.

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Poster

220. Epilepsy: Circuits

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Program#/Poster#: 220.11/D55

Topic: C.07. Epilepsy

Title: Hyperexcitability of presubicular neurons in a rat model of Temporal Lobe Epilepsy

Authors: *S. ABBASI, S. KUMAR
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Abstract: Temporal Lobe Epilepsy (TLE) is the most common form of epilepsy in adults and is characterized by recurrent seizures originating in the temporal lobes. Evidence from patient and animal model studies suggests that several temporal lobe structures are affected by TLE, and contribute to seizure generation and propagation. This study looks at the impact TLE has on the presubiculum (PrS), a unique parahippocampal structure which both receives inputs from and sends projections to brain regions affected by TLE. We assessed the state of PrS in TLE using whole-cell electrophysiological recordings to determine which of the previously identified cell types become hyperexcitable in epileptic animals, and whether intrinsic and/or synaptic properties are altered in these neurons. We characterized cells based upon their action potential discharge profiles followed by unsupervised hierarchical clustering. PrS neurons from epileptic animals were divided into three groups consisting of Regular Spiking (RS), Irregular Spiking (IR) and Fast Adapting (FA). RS cells, the predominant cell-type encountered in PrS in both control and epileptic groups, and previously identified as sending long-range axonal projections to neighboring structures including medial entorhinal area, were the only group that was hyperexcitable. Alterations in intrinsic properties of RS cells supported an increased propensity for sustained firing of brief action potentials in rapid succession. Surprisingly, frequency of both spontaneous excitatory and inhibitory synaptic events in RS cells was reduced (49 and 38%, respectively), accompanied by amplitude reductions (23 and 40%, respectively). Further analysis of non-action potential dependent miniature currents (in TTX) led us to conclude that the reduced excitatory drive is likely due to a decrease in excitability of neurons which synapse directly onto RS cells and onto GABAergic interneurons that inhibit them under normal conditions. Alterations in physiology of individual PrS neurons from epileptic animals have not been examined in detail and our findings suggest that parahippocampal circuits, specifically in PrS, are altered, contributing to epileptogenesis of downstream structures including the medial entorhinal area.

Disclosures: S. Abbasi: None. **S. Kumar:** None.

Poster

220. Epilepsy: Circuits

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

Support: FAONS-2010 Overseas Travel Fellowship

Title: The effect of oral administration of thymoquinone on an experimental model of temporal lobe epilepsy in rat: Electrophysiological, behavioral and histochemichal study

Authors: *S. .-. DARIANI SAEED[1,2], T. BALUCHNEJADMOJARAD[3,4], M. ROGHANI[5]
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Abstract: Epilepsy is a chronic neurological condition that characterized by unpredictable and recurrent involuntary seizures. Temporal lobe epilepsy (TLE) is common and can be lead to hippocampal sclerosis and cell dead in different area of hippocampus. Between involved mechanisms, oxidative stress plays an essential role in the pathogenesis of epilepsy and it seems that antioxidants can prevent epilepsy-related disorders. Therefore, we evaluated the effect of Thymoquinone (TQ), as an antioxidant, on kainic acid-induced temporal lobe epilepsy in male rat. Male Wistar rats were randomly divided into four groups: sham, sham+TQ (10 mg/kg), kainic acid (4 μg intra-hippocampal), kainic acid+TQ (5 and 10 mg/kg). We evaluated the antiepileptic effect of TQ using behavioral and histological evidence and electrophysiological (iEEG) recording. Our results showed that induced and spontaneously seizures and the amplitude of iEEG were increased in kainic acid group ($p=0.0001$ and $p=0.03$, respectively). Also, kainic acid administration led to increase mossy fiber sprouting in dentate gyrus and decrease neurons in hilar, CA1 and CA3 regions of hippocampus ($p<0.0001$). Immunohistological results showed that kainic acid administration caused to increase GFAP positive cells in astrocytes and the number of hippocampal glutamate receptors compared to sham group ($p<0.01$ and $p<0.0001$, respectively), while it has no significant effect on number of iNOS positive cells. Oxidative stress assessment showed that kainic acid administration led to decrease SOD enzyme activity ($p<0.0002$) and increase lipid peroxidation ($p<0.0001$) and nitrite levels ($p<0.0001$) in hippocampus. TQ pretreatment in epileptic rats showed that TQ prevented spontaneously seizures incidence. Meanwhile, TQ pretreatment decreased neuron loss in hilar, CA1 and CA3 regions, mossy fiber sprouting in dentate gyrus, hippocampal glutamate receptors, and GFAP positive cells in stratum lucidum. On the other hand, thymoquinone administration caused to intensify SOD enzyme activity and decrease lipid peroxidation and nitrite levels in hippocampus ($P<0.01$-$P<0.0001$). Collectively, the results of this study revealed that TQ pretreatment could attenuate some of behavioral and histological disorders via prevention of astrogliosis, oxidative stress and glutamate toxicity related to TLE.

Disclosures: S.-. Dariani Saeed: None. **T. Baluchnejadmojarad:** None. **M. Roghani:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Topic: C.08. Ischemia

Support: NINDS Grant NS085402

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Title: Early capillary constriction impedes collateral blood flow to the acute stroke penumbra

Authors: *Z. J. TAYLOR, A. N. WATSON, A. Y. SHIH
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Abstract: All blood that flows through the cerebral arteries must pass through dense and narrow capillaries. Mechanisms that resist capillary flow will reduce blood supply to the brain and thus exacerbate stroke outcome (del Zoppo and Machubi, J Cereb. Blood Flow Metab. 2003, 23(8):879-94). Numerous mechanisms of capillary resistance have been described, including intravascular obstruction by leukocytes or thrombi, and extrinsic compression by contractile pericytes and swelling of astrocytic endfeet. However, the prevalence and impact of these events remain poorly understood *in vivo*. We used two-photon microscopy to study the degradation of capillary function during ischemia in the mouse cortex. We generated columnar strokes, spanning >1 mm in diameter, by photo-thrombotic occlusion of single, high flux penetrating arterioles (Shih et al. Nature Neuroscience, 2013, 16(1):55-63). These strokes grew radially outward over the course of 24 hours, providing an ideal means to visualize the evolving core-penumbra interface. Unexpectedly, rather than provide collateral blood flow to limit damage, penetrating arterioles that neighbored the occluded vessel slowly stagnated. To understand the source of this flow resistance, we tracked ~200 capillaries underlying these arterioles (7 mice) and measured their lumen diameter, the presence of intraluminal obstruction, and blood-brain barrier breakdown over 8 hours. Capillary constriction, defined as a decrease to less that 80% of pre-stroke diameter, was the earliest pathological event detected, affecting capillaries within 15 minutes of stroke onset, and progressively worsening until 4 hours post-occlusion. An increase in lumen obstruction lagged closely behind constriction. Indeed, individual capillaries that constricted at an early stage during stroke (0-4 hours post-occlusion) were twice as likely to lose

flow at a later stage (4-8 hours), suggesting that constriction was a key initiator of blood flow impedance in the penumbra. Blood-brain barrier leakage occurred only after capillary flow completely ceased, and was a relatively late event. Astrocyte endfoot swelling was not involved in this phenomenon, because constriction persisted in syntrophin knockout mice, which exhibit reduced edema due to mis-localization of the water channel aquaporin-4. In summary, early capillary constriction initiates a feed-forward spread of ischemia by impeding flow through the domains of neighboring penetrating arterioles during expansion of the acute stroke core.

Disclosures: Z.J. Taylor: None. **A.N. Watson:** None. **A.Y. Shih:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Topic: C.08. Ischemia

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Title: Cofilin: A new target for the treatment of ischemic stroke

Authors: *Q. M. ALHADIDI, Z. A. SHAH
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Abstract: Cofilin is a cytoskeleton-associated protein that plays an important role in controlling actin dynamics and turnover. In the central nervous system, cofilin regulates neurite outgrowth and development and Schwann cell myelination. However, studies have shown that cofilin activation and cofilin-actin rod formation is involved in many neurodegenerative diseases such as Alzheimer Disease and Huntington Disease. In the present study, we conducted *in vitro* experiments to evaluate the role of cofilin in ischemia reperfusion using primary cortical neurons and the oxygen and glucose deprivation (OGD) model of ischemia. Primary cortical neurons were subjected to ischemia (1h OGD and 24 h reperfusion) and compared with sham treatment (no OGD). As compared to sham neurons, we observed elevated levels of cofilin in neurons subjected to OGD. Next, we used the siRNA technique to knock down cofilin and delineate the

role of elevated cofilin in ischemic paradigms. The outcomes were measured by neuronal viability (MTT assay) and caspase 3 activity, a component of the apoptotic pathway. Cofilin knockdown neurons subjected to OGD showed significantly increased neuronal viability compared to control neurons (treated with scrambled siRNA and subjected to OGD). To determine the plausible signaling mechanism and cofilin's interaction with the apoptotic pathway, we evaluated the expression levels of caspase 3 in neurons co-treated with cofilin siRNA and OGD. Compared to control neurons, expression levels of caspase 3 were significantly lower in cofilin knockdown neurons after OGD. Collectively, these data highlight a major role of cofilin in mediating apoptotic neuronal death in ischemia, and targeting this protein could open the doors toward a new group of neuroprotective agents that can aid in the cure of ischemic stroke. The study was partly funded by a grant from NIH (R00AT004197), Higher Committee for Education Development in Iraq (HCED) and start-up funds from The University of Toledo to ZAS.

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Poster

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Topic: C.08. Ischemia

Support: NIH Grant NS074895

Title: The effect of age on the immune cohort in the ischemic cortex of female Sprague-Dawley rats at 48 hours post-stroke

Authors: A. O'KOREEH[1], S. BAKE[3], R. C. ALANIZ[3], *F. SOHRABJI[2]
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Abstract: Our previous research has shown that adult female rats sustain smaller infarcts as compared to middle-aged female rats (Selvamani and Sohrabji, 2010). This is accompanied by increased permeability of the blood brain barrier in the middle-aged females as compared to adult females. Blood brain barrier permeability can result in infiltration of peripheral immune cells, such as T and B cells, and cytotoxic products, that can accelerate neuronal damage. In the

present study we tested the hypothesis that the proportion of T and B cells would be elevated in the ischemic cortex of middle-aged females as compared to adult females. Adult (6 month old) and middle aged (10-12 month old) Sprague Dawley rats were subject to ischemic stroke via stereotaxic injections of endothelin-1 (ET-1) to the middle cerebral artery. Forty-eight hours later, the ischemic tissue was homogenized in X-VIVO media and resuspended in 30% Percoll (Sigma-Aldrich). The suspension was layered on a 70% percoll solution with HBSS and centrifuged at 700g for 30 minutes. The supernatant containing myelin and other debris was removed via pipette. Cells in the interface layer were collected and washed in X-VIVO (Lonza, Basel, Switzerland). Spleens from each animal were pulverized and treated with red blood cell (RBC) lysis buffer, washed in X-VIVO, filtered with a pre-separation MACS 70 μm filter. (Miltenyi Biotec, San Diego, CA). Cells were stained for CD4 (general marker of T-cells), CD5+CD1d (general surface markers for B regulatory cells), CD3 and CD161 (general marker for NK cells). No age differences were seen in CD4+ and CD5+/CD1d+ cells in the spleen, however, middle aged females, as compared to adult females, had significantly higher percentage of CD4+ cells (18.6%+5.16 vs 8.63%+2.94)and CD5+ CD1d+ (11.21%+1.2 vs 3.00%+2.3) The older females thus had a 2-fold increase in CD4+ cells and a 3.5-fold increase in CD5+/CD1d+ double-positive cells, suggesting greater infiltration of immune cells in middle aged females, consistent with greater ischemic damage seen in this group. In addition, small cohorts of CD161+CD3- cells were also observed. Adult females had a 7-fold greater proportion of CD161+CD3- as compared to older females (0.54%+0.1) vs 0.077%+067). Although NK cells increase infarct volume (Gan, et. al. 2014), they also mediate infection and have anti-viral functions. Post stroke infections are well known, especially in the elderly and reduced expression of this cell type in the middle aged female population may contribute to the worse outcome seen in this group.

Disclosures: **A. O'Koreeh:** None. **F. Sohrabji:** None. **S. Bake:** None. **R.C. Alaniz:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Program#/Poster#: 221.04/D60

Topic: C.08. Ischemia

Title: An experimental model of ischemic stroke in transgenic mouse: Assessment of histological and functional impairments

Authors: *S. MARASINI[1,2], S.-Y. PARK[1,2], G.-H. KIM[1,2], T.-Y. KU[3], Y.-D. LEE[1,2], H. SUH-KIM*[1,2], S.-S. KIM[1]
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Abstract: Ischemic stroke remains one of the major causes of death and disability worldwide. Treatment modalities for improving the functional recovery remain limited in clinical practice despite extensive efforts in stroke research. Transgenic mouse stroke models add in better understanding the injury mechanisms in stroke and identifying potential therapeutic targets. Here, we generated a mouse transient ischemic stroke model using an intraluminal suture to block the middle cerebral artery and verified the blockage of blood flow using indocyanine green coupled with near infra-red radiation. The survival rate was as low as 10% in the groups of animals not treated with antibiotics while administration of prophylactic antibiotics significantly increased the survival rate upto 60%. Ischemic injury resulted in severe histological as well as functional impairments in animals but recovered spontaneously starting from the second week. Among various behavior tests, pole tests, neurological severity score tests, and body weight changes remained reliable up to 4 weeks, whereas rotarod and corner tests became less distinctive to assess the severity of ischemic injury. In conclusion, we have developed an improved approach which allows us to investigate the role of the cell death-related genes in the disease progression using genetically modified mice and to evaluate the modes of action of candidate drugs.

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Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Topic: C.08. Ischemia

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Title: Role of autophagy in cerebral ischemia-reperfusion injury in adult rats

Authors: *P. S. BHARTI, T. K. DAS
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Abstract: Cerebral ischemia is a major causative factor of stroke, which is one of the leading causes of death and adult disability worldwide. Ischemic injury is initiated immediately after interruption of cerebral blood flow and neuronal death is the ultimate result of this condition. The ischemic insult has many pathophysiological consequences in which necrotic and apoptotic cell deaths are final outcomes. In the recent years, many studies have shown the importance of other types of cell deaths in ischemic-reperfusion (I/R) injuries, like autophagy and necroptosis. It has been reported that autophagy has great significance in pathological outcome, ischemic preconditioning and development of other therapeutics. In this study, we evaluated expression patterns of autophagic proteins LC3, Beclin-1 and Rubicon (KIAA0226) and ultrastructural morphology of cerebral I/R injury in rat middle cerebral artery (MCA) occlusion model. Adult Wistar male rats (200-250 gms) were used in two groups with sham-controls. Right MCA occlusion was performed and the occlusion period was 2 hours. Chloroquine (CQ) (25mg/Kg i.p.) was administered in one group 1 hour prior to surgery. Rats were sacrificed after 24 hours of surgery and brains were procured and processed for histological and western blotting experiments. Brain infarct was measured by 2,3,5-Triphenyltetrazolium chloride staining. For immunohistochemical and western blotting experiments LC3, Beclin-1 and Rubicon (KIAA0226) primary antibodies were used. We also have performed transmission electron microscopy to observe ultrastructural morphology of autophagy and cerebral I/R injury. In our results, LC3 and Beclin-1 expressions were increased significantly (LC3 is an autophagosomal marker protein and Beclin-1 is an autophagy initiator), whereas, expressions of Rubicon is decreased as it plays a role in inhibition of autophagy initiation in MCAo group. After CQ administration, it was observed that expression of LC3 was significantly more increased compared to MCAo group whereas expressions of Beclin-1 and Rubicon have not been changed significantly compared to LC3. The increase in expression of LC3 after CQ administration is due to accumulation of autophagosomes which could not fuse with lysosomes as in normal autophagic pathway. The accumulation and ultrastructural morphology of autophagosomes and autolysosomes in cytosol were further confirmed with transmission electron-microscopy. The present study concludes that there is an increase in autophagic proteins (LC3 and Beclin-1) and thus overall autophagy after cerebral I/R injury which can play an important role in developing therapeutics.

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Poster

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Topic: C.08. Ischemia

Support: NIH grant 1R01 HD049792

Title: The beneficial effect of cooling on male and female rats with experimentally induced hypoxia ischemia

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Abstract: Common events related to premature birth or term birth complications include reduced oxygen and/or blood flow to the brain (collectively termed hypoxia-ischemia; HI). HI can lead to varying degrees of tissue loss, as well as subsequent cognitive/behavioral deficits. For example, early HI insult can lead to later motor, learning/memory, or auditory processing impairments. Interestingly, clinical data suggest sex differences in outcomes following early HI, with males exhibiting more detrimental effects compared to females with similar damage (Smith et al., 2014). Clinically, hypothermia (i.e., head/whole body cooling) has been used as a neuroprotectant following an HI injury. Cooling may block aspects of apoptosis, thus impeding the downstream effects leading to brain injury. However, little is known about the relative ameliorative effects of cooling as a function of sex. To assess whether the neuroprotective effects of cooling are more beneficial to one sex, the current study used a postnatal day (P) 7 rodent model of HI injury (roughly equating to injury in a term infant). Based on previous data indicating better outcomes in female rats on a rapid auditory processing (RAP) task and a spatial learning task following early HI injury (suggesting an intrinsic female protection), we hypothesized that female P7 HI rats would benefit more from cooling compared to males. Alternatively, we hypothesized that rats kept at hyperthermic temperatures would show enhanced HI effects that might over-ride intrinsic female protection and mask sex differences. We sought to test these hypotheses using rota-rod, auditory discrimination, and spatial and non-spatial learning tasks. Preliminary results revealed a significant benefit of cooling in female HI rats on the rota-rod task, with male HI rats only marginally protected by cooling. However, other results from auditory and learning/memory tasks suggest that cooling was equally beneficial to both male and female rats with HI injury. Taken together, our data suggest that cooling in general appears to benefit both sexes from the deleterious effects of HI injury, but task and sex specific patterns will be presented in more detail. Current findings are consistent with previous studies in our lab, specifically indicating a female advantage following early injury on some but not all behavioral tasks (Smith et al., 2014).

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Poster

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Topic: C.08. Ischemia

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Title: Time-course response of miR-34a, miR-132 and miR-146 to ischemic stroke

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Abstract: Stroke is one of the leading causes of death and disability worldwide. The initial triggers in stroke are deceptively simple: loss of blood flow and energy supply which leads to rapid neuronal cell death. However the subsequent pathophysiology is highly complex by multifactorial cascade of molecular mechanisms. Various mouse and human stroke studies have shown that expression of specific microRNAs (miRNAs) is either increased or decreased in post-stroke brain and serum. miRNAs are especially important candidates for stroke therapeutics because of their ability to simultaneously regulate many target genes. The goal of this study is to analyze expression of three miRNA (miR-34a, miR-132 and miR 146a) profiles in serum samples of ischemic stroke patients over the course of 90 days following recovery, as well as in serum and brain of transient Middle Cerebral Arterial Occlusion (tMCAO) mice by qRT-PCR. All three miRNAs were significantly increased 30 days after stroke in a patient's serum who survived, but no change of the miRNAs was observed in those who did not survive (after 30 days). Additionally, in mouse serum following tMCAO, miR-34a levels were increased by 6 hrs post-stroke, while miR-132 levels were increased by 24 hrs. Interestingly all of the miRNAs levels were increased in the brain of tMCAO mice by 24 hrs but the differences disappeared by 48 hrs. These results suggest that increased expression of these miRNAs may inhibit some of their target genes involved in post stroke neuronal death.

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Poster

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Title: Diet-induced obesity induces angiogenesis and worsens post-ischemic recovery following permanent ischemia

Authors: ***J. TULSULKAR**, S. NADA, B. SLOTTERBECK, M. MCINERNEY, Z. SHAH
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Abstract: Obesity-induced diabetes has substantially increased over the years and has become one of the risk factors for stroke. It is well-documented that obesity accelerates neuronal damage and induces post-ischemic seizures. An obesity-related increase in blood glucose levels leads to type-2 diabetes and hypertension, the number one risk factor for stroke. Diabetes also leads to excessive angiogenesis, causing diabetic retinopathy and impaired neovascularization which further result in coronary and peripheral complications. In this study, we investigated the influence of diet-induced obesity and diabetes on permanent ischemic stroke. Age-matched adult male C57/Bl6 mice were treated with a high fat, high carbohydrate diet (HFCD) or a normal diet (ND) for six months, and fasting blood glucose levels were monitored; these were observed to be higher in HFCD as compared to ND mice at the time of experimental stroke. ND and HFCD-treated mice were subjected to permanent distal middle cerebral artery occlusion (pMCAO) and sacrificed after 7 days. Infarct volume analysis showed no differences in HFCD compared to ND group, possibly due to higher neovascularization and collateral flow in HFCD mice. However, neurological deficits (NDS) were significantly higher in the HFCD group compared to the ND group. Cortical protein expression levels revealed that HFCD mice had significantly lower expression of GSK-3β, a key component in the Wnt signaling pathway. Blood vessel growth is

mainly due to the expression of a large amount of Sirt1 in the vasculature, which was observed to be significantly overexpressed in the HFCD group compared to the ND group. To investigate the cell death mechanisms in the HFCD group, we investigated axonal damage marker, amyloid precursor protein (APP) and apoptosis inducing factor (AIF) and observed a higher expression of APP in the HFCD group compared to the ND group. Translocation of AIF to the nucleus was observed in both the HFCD and ND groups, but HFCD mice showed a higher expression of AIF in the nucleus, suggesting that apoptotic processes are active even after 7 days of permanent ischemia. Together, our result suggests that overexpression of Sirt1 in the HFCD group results in neovascularization, and reduced post-stroke recovery is possibly due to impaired Wnt signaling and active apoptosis.

Disclosures: J. Tulsulkar: None. **S. Nada:** None. **B. Slotterbeck:** None. **M. Mcinerney:** None. **Z. Shah:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 221.09/D65

Topic: C.08. Ischemia

Title: Pin1-mediated Notch activation worsens brain damage and functional outcome in ischemic stroke

Authors: *S.-H. BAIK, Y. CHOI, U. YUN, Y. JANG, J. JEONG, D.-G. JO
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Abstract: Pin1, a peptidyl-prolyl cis/trans isomerase, is the enzyme known to catalyze a number of substrates containing phosphorylated Ser/Thr motif. Pin1 induces conformational change of target proteins and regulates cellular responses. Recently findings suggest that Pin1 has been related to certain disease. Here we found that role of Pin1 in ischemic stroke using mice affected by focal ischemia-reperfusion. Pin1 stimulates Notch1 activation and its pro-apoptotic function following ischemic stroke. Pin1 heterozygote (+/-) and knockout (-/-) mice, each showed reduced brain damage and improved functional outcomes in a model of focal ischemic stroke. These results suggest that Pin1 contributes to the pathogenesis of ischemic stroke and that inhibition of Pin1 is a novel approach for treating ischemic stroke.

Disclosures: **S. Baik:** None. **Y. Choi:** None. **U. Yun:** None. **Y. Jang:** None. **J. Jeong:** None. **D. Jo:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: Halls A-C

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Program#/Poster#: 221.10/D66

Topic: C.08. Ischemia

Title: Effects of anoxia or post mortem changes?

Authors: ***K. SKOLD**[1,2], H. ALM[3], M. BORÉN[1], B. SCHOLZ[4]
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Abstract: Aim A combined literature search and experimental approach has been performed to investigate the likeness of neural protein patterns between ischemia and those induced by the post mortem interval. Reverse phase protein arrays (RPPA) were used to confirm the consequences of different lengths of bioreaction termination intervals (BTI, also known as post-mortem intervals) on the phosphorylation levels of eleven known signal transduction proteins. Introduction There are similarities between the state of ischemia and the acute biological conditions introduced in animals and humans after death. The study of ischemia is often performed by cutting of the blood supply to the area that to be investigated that leads to subsequent hypoxia and anoxia. To ensure cell survival, a constant supply of oxygen and nutrients is needed. Without oxygen, oxidative phosphorylation and subsequently adenosine trisphosphate (ATP) production is halted, causing deficiencies in cell functions. This can in some extent be compared to regular sample handling. The study of tissues samples from patients or model organisms usually exposes samples to a certain time of oxygen and nutrient depletion before homogenization and enzyme inactivation occurs. The advancement of protein analysis techniques provides new ways of studying the protein content in various tissues and cell cultures. In the current study RPPA was used to study the influence from post-sampling BTI on the phosphotylation levels of several signal transduction proteins associated with ischemia in the brain. Results Eleven different well studied protein phosphorylations were analyzed in mouse striatum. The tissue were stored at the bench for 0, 10, 30 minutes before frozen to simulate different post-sampling BTIs. All phosphorylations rapidly decreased to the first time point

whereas there were almost no differences between 10 and 30 minutes. CamKII-Thr286p, ERK1/2-Thr202/185,Tyr204/187 and ATF2_Thr71p were found to be particular sensitive to the initial 10 min of post-sampling BTI. The findings indicate that the animal and sample handling in ischemia research is of great importance for mechanistic data interpretation. It further seems to imply that even small variation in experimental sample handling may lead to increased sample variation.

Disclosures: **K. Skold:** A. Employment/Salary (full or part-time):; Denator AB. **H. Alm:** None. **M. Borén:** A. Employment/Salary (full or part-time):; Denator AB. **B. Scholz:** None.

Poster

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Program#/Poster#: 221.11/D67

Topic: C.08. Ischemia

Support: NIH U54#NS083932 (MSM NI)

MSM Endow (AZ and RPS)

Title: Proteomics of hypothalamus and pituitary in brain ischemia

Authors: J. RICE[1,2], L. CAO[1], T. YANG[1], R. SIMON[1], *A. ZHOU[1]
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Abstract: Molecular mechanisms that underlie systemic responses to focal brain ischemia are poorly understood. In parallel with the neuroanatomic pathways of the CNS and PNS, the hypothalamus-pituitary axis plays a pivotal role in neuroendocrine regulation of systemic responses to stroke. Accordingly, here, we characterized proteomic changes in the hypothalamus (HT) and pituitary (PI) of mice subjected to different degrees of focal brain ischemia. Transient focal cerebral ischemia was induced in adult, male mice by middle cerebral artery occlusion (MCAO) with the suture method followed by reperfusion. Three groups of animals were subjected to sham operation, 30 min MCAO (inducing limited cortical injury) and 60 min MCAO (resulting in hemispheric infarct), respectively. Proteins were extracted from HT and PI tissues and analyzed by a quantitative mass spectrometry-based proteomic approach. Proteins that showed a change in quantities after 30 min or 60 min MCAO, when compared with that in sham controls, were analyzed for their bioinformatics characteristics with the assistance of the

MetaCore program, and compared between the 30 min and 60 min MCAO groups. A total of 952 and 785 proteins were identified and quantified in the HT and PI, respectively. We found that, in both the HT and PI, both anatomically distant from the MCAO territory, the proteomic changes in response to 30 min or 60 min MCAO were largely different, as demonstrated by the association of regulated proteins with different biological processes. Among regulated biological processes, only about half were common between 30 min and 60 min MCAO groups. Similar results have been seen in proteomic changes in several peripheral organs (Zhou et al., unpublished results). Interestingly, following a 30 min but not 60 min MCAO, a significant decrease in neuropeptide biosynthesis-related biological processes was seen in the PI. These results suggest that, in the HT-PI axis, neuroendocrine changes in response to different degrees of focal brain ischemia may involve different cellular regulatory mechanisms, which in turn may determine different systemic changes after brain ischemia.

Disclosures: **J. Rice:** None. **L. Cao:** None. **T. Yang:** None. **R. Simon:** None. **A. Zhou:** None.

Poster

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Topic: C.08. Ischemia

Support: NIH grant NS081179

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Title: Splenic monocytes contribute to cerebral ischemic tolerance induced by LPS

Authors: *C. BENAKIS, L. GARCIA-BONILLA, D. BREA, J. MOORE, C. IADECOLA, J. ANRATHER
The Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: Administration of a sub-lethal dose of the endotoxin lipopolysaccharide (LPS) renders the brain tolerant to a subsequent ischemic insult (preconditioning) [Nat Neurosci 14, 1363, 2011]. Although it is well established that reprogramming of the immune system plays a role, the immune cells involved in the induction of the tolerance remain to be defined. Given the prominent role of monocytes/macrophages as endotoxin sensors and the contribution of splenic

monocytes to post-ischemic inflammation [Science 325, 612, 2009], we tested the hypothesis that LPS preconditioning involves activation and mobilization of splenic monocytes. C57Bl/6 male mice (age 7 weeks) were treated with either LPS (dose ≤ 0.5mg/kg; i.p.) or saline (vehicle; Veh) and flow cytometry was performed on brain, spleen and blood cells after 24, 48 and 72 hrs (n=8/group). LPS increased the proportion of monocytes in the blood at all time points, e.g. at 24 hrs: LPS, 23±3%; Veh, 7±4% of total leukocytes ($p<0.05$; mean±SD), while in the spleen the proportion of monocytes was elevated only at 48 and 72 hrs (48 hrs: LPS, 11±6%; Veh, 3±1%; $p<0.05$). Consistent with a mobilization of splenic inflammatory monocytes, LPS increased $Ly6C^{high}$ monocytes in the blood at 24 hrs (LPS: 63±5%; Veh: 51±5% of total monocytes), and reduced $Ly6C^{high}$ monocytes in the spleen (48 hrs: LPS, 21±3%; Veh, 30±7%; $p<0.05$). In brain, LPS increased the total number of monocytes by 3.9±0.7 folds at 24 hrs, most of which were inflammatory (LPS: 66±18%; Veh: 40±4% of brain monocytes; $p<0.05$). To examine the contribution of splenic monocytes in LPS preconditioning, splenectomy or sham surgery was performed (n=5-10/group). Two weeks later, mice were treated with LPS or Veh and were subjected to transient middle cerebral artery occlusion 24 hrs later. Infarct volume was measured 72 hrs after ischemia. After Veh-treatment, infarct size was comparable in splenectomized (57±19 mm^3) or sham-operated mice (52±15 mm^3; $p>0.05$). However, after LPS, the neuroprotective effect was more marked in sham-operated (21±9 mm^3) than in splenectomized mice (39±23 mm^3; $p=0.066$). Splenectomy had no effect on LPS-induced brain monocyte recruitment (984±443 vs.1324±745 cells in controls, $p>0.05$) but reduced the percentage of $Ly6C^{high}$ cells (splenectomy: 45±6; control: 66±18%; $p<0.05$). The data suggest that splenic inflammatory monocytes are critical for the full expression of the preconditioning effects of LPS. Furthermore, the findings unveil a previously unrecognized role of splenic monocytes in the outcome of cerebral ischemia and suggest new therapeutic approaches based on modulation of peripheral monocyte populations.

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Poster

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Program#/Poster#: 221.13/D69

Topic: C.08. Ischemia

Support: NIH NCCAM R15 (1R15AT006593)

Dupont Nutrition and Health

Title: Diet and stroke: Expression of genes that modulate inflammation and neuronal remodeling in the adult and aged male rat cerebral cortex

Authors: *E. D. GRISLEY[1,2], T. REHNBERG[1,2], J. A. MACLEAN, II[1], W. J. BANZ[3], D. N. BUTTEIGER[4], J. L. CHEATWOOD[2]
[1]Physiol., [2]Anat., SIU Sch. of Med., Carbondale, IL; [3]Animal Science, Food, and Nutr., Southern Illinois Univ., Carbondale, IL; [4]Dupont Nutr. and Hlth., St. Louis, MO

Abstract: Nearly 800,000 Americans are stricken by ischemic stroke each year. Other than care with post stroke rehabilitation there are no specific treatments for improving functional recovery. To improve the recovery of stroke patients we are investigating anti-inflammatory, anti-apoptotic, and neuronal remodeling pathways. Estrogen receptor activators are known to be neuroprotective by initiating pathways through ERβ and ERα. The bioactive soy isoflavones, daidzein and genistein, do bind to these estrogen receptors. However, this binding alone is not sufficient to explain the ability of soy-based diets and purified isoflavones to reduce inflammation and improve neuroprotection and recovery after stroke. Herein, we focused on the PPARγ, ARG1, 14-3-3ε, SIRT1, GAP43, and Synaptophysin pathways to test the hypothesis that diets containing soy isoflavones and/or soy protein isolate will reduce inflammation and promote the expression of neuronal plasticity markers following stroke in adult and aged rats via these mechanisms. Adult and aged male Hooded Long Evans rats were fed a semi-purified diet of either 1) Sodium Caseinate (CAS), 2) Sodium Caseinate plus the isoflavones daidzein and genistein (CAS+ISO), or 3) Soy Protein Isolate (SPI) for two weeks prior to middle cerebral artery occlusion (MCAO). Permanent unilateral MCAO was performed and tissue was collected from both hemispheres at Day +3 (N=6/group) and Day +7 (N=6/group). Control (Day 0) animals (N=4/group) received no MCAO. Rats were maintained on their assigned diet throughout the experiment. RNA was extracted and cDNA synthesized for qPCR reaction. All data were normalized to GAPDH via the ΔΔCt method. qPCR analyses of the contralateral and ipsilateral brain tissue did not show a statistical change in relative mRNA level for any of the genes examined to this point between any of the diet groups at any post-stroke time point observed so far ($p<0.05$). Results are pending for analyses of additional genes of interest.

Disclosures: E.D. Grisley: None. **T. Rehnberg:** None. **J.A. Maclean:** None. **W.J. Banz:** None. **D.N. Butteiger:** A. Employment/Salary (full or part-time):; Dupont Nutrition and Health. **J.L. Cheatwood:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Dupont Nutrition and Health.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 221.14/D70

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

Title: Ethyl pyruvate ameliorates 3-nitropropionic acid-induced striatal toxicity through anti-neuronal cell death and anti-inflammatory mechanisms

Authors: ***M. JANG**[1,2], M. LEE[1], I.-H. CHO[2]
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Abstract: The potential neuroprotective value of ethyl pyruvate (EP) for the treatment of the striatal toxicity is largely unknown. We investigated whether EP promotes the survival of striatal neurons in a 3-nitropropionic acid (3-NP)-induced mouse model of Huntington's disease (HD). EP (5, 10, 20, and 40mg/kg/day, i.p.) was daily injected from 30min before 3-NP intoxication (pretreatment) and from onset/progression/peak point of neurological impairment by 3-NP intoxication. EP produced a neuroprotective effect in dose- and time-dependant manners. EP pretreatment of 40mg/kg/day produced the best neuroprotective effect among other conditions. Pretreatment of EP significantly attenuated neurological impairment and lethality and prevented formation of lesion area and neuronal loss in the striatum after 3-NP intoxication. This neuroprotection afforded by EP was associated with the suppression of succinate dehydrogenase activity, apoptosis, and microglial activation. The suppressive effect of EP corresponded to the down-regulation of mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF-κB) signal pathways, and mRNA expression of inflammatory mediators including tumor necrosis factor-alpha, interleukin (IL)-1β, IL-6, inducible nitric oxide synthase, and cyclooxygenase-2 in the striatum after 3-NP intoxication. Interestingly, the intrathecal introduction of inhibitors MAPKs and NF-κB into control mice decreased the lethality after 3-NP intoxication. Our findings indicate that EP may effectively alleviate 3-NP-induced striatal toxicity by inhibition of the MAPKs and NF-κB pathways in the striatum, and that EP has a wide therapeutic window, suggesting that EP may have therapeutic value in the treatment of aspects of HD's disease related to inflammation.

Disclosures: **M. Jang:** None. **M. Lee:** None. **I. Cho:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Topic: C.08. Ischemia

Support: UH2-TR000918

NS-19108

HD-09402

Title: Exosomes from IFNγ-stimulated dendritic cells mitigate hypomyelination from hypoxic-ischemic injury modeled in brain slice cultures

Authors: *R. P. KRAIG, A. D. PUSIC, K. M. PUSIC
Neurol., Univ. of Chicago, CHICAGO, IL

Abstract: Perinatal hypoxic-ischemic brain injury (HI) is a major cause of mortality and persistent morbidity (1) that includes cerebral palsy, cognitive dysfunction, and epilepsy (2). Though no effective pharmacological agents to reduce these birth-related injuries are in use, non-pharmacological stimuli from environmental enrichment (EE) after HI improves brain function (3). However, EE requires sufficient infant maturation for participation. Our goal is to identify/define the mechanisms by which EE improves brain function, to jump-start this effect. Work from our lab demonstrates that exposure to EE promotes the release of exosomes that promote myelination. These exosomes also improve remyelination following acute demyelination of slice cultures by promoting oligodendrocyte precursor differentiation into myelin producing cells (4). This work was extended to show that IFNγ-stimulated dendritic cells (IFNγ-DC-Exos) can be used to generate exosomes that are similarly therapeutic *ex vivo*. (5). Here, we extend use of these exosomes to HI, another disease involving loss of myelin (6). Hippocampal brain slices were cultured from P10 Wistar rats, and transferred to a serum-free media at P17 to eliminate protective effects of horse serum. Ischemic injury was modeled using a 90 minute epoch of oxygen-glucose deprivation (OGD). OGD-exposed slices were then treated with IFNγ-DC-Exos or left untreated and allowed to recover. Three days post-treatment, slices (n=6/group) exposed to OGD alone showed a significant (p=0.002) reduction in myelin basic protein (MBP; used here as a proxy for myelin). In contrast, levels of MBP in slices treated with 100 μg of IFNγ-DC-Exos did not significantly differ from that of pre-OGD controls. . Electron micrographs taken at 7 days after OGD exposure confirmed this protective effect. Exosomes have the additional benefit of low immunogenicity, and do not require an additional vehicle for delivery. There is evidence that nasal administration allows passage of agents across the blood brain barrier to the CNS (7). Indeed, our lab has already demonstrated that nasal administration of IFNγ-DC-Exos can significantly increase CNS myelination (5). Thus, this proof-of-principle study suggests that IFNγ-DC-Exos may be a promising therapeutic for treatment of perinatal HI.

(1) Kurinczuk JJ et al, 2010, Early Hum Dev; (2) Pisani F et al., Brain Dev, 2009; (3) Rojas JJ et al., 2009, Exp Neurol; (4) Pusic A, et al., 2014, Glia; (5) Pusic A et al., 2014, J Neuroimmunol; (6) Pusic A et al., Exp Rev NeuroTher; (7) Thorne RG and Fry, Clin Pharmacokiet, 2001).

Disclosures: R.P. Kraig: None. **A.D. Pusic:** None. **K.M. Pusic:** None.

Poster

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Topic: C.08. Ischemia

Support: NIH (R00AT004197)

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Title: Neurorestorative effects of flavonoids isolated from Rumex aquaticus L in an oxygen glucose deprivation model of ischemic stroke

Authors: *A. RAGHAVAN[1], O. ORBAN-GYAPAI[2], J. HOHMANN[2], Z. SHAH[1]
[1]Medicinal and Biol. chemistry, college of pharmacy and pharmaceutical sci, Univ. of Toledo, Toledo, OH; [2]Univ. of Szeged, Szeged, Hungary

Abstract: There is heightened interest in the field of stroke recovery as there is need for agents that would prevent the debilitating effects of the disorder, thereby tremendously reducing the societal and economic costs associated with it. We report the isolation of two flavonoids_RUA1 & RUA2_from *Rumex aquaticus* and their neuroprotective effects in the oxygen-glucose deprivation (OGD) model of *in vitro* cerebral ischemia in rat pheochromocytoma (PC12) cells. Bioassay-guided fractionation of the ethyl-acetate extract of *Rumex aquaticus* L. afforded the isolation of quercetin-3-*O*-galactoside and quercetin-3-*O*-arabinoside. The structures of compounds were established on the basis of spectroscopic analyses (UV, MS and NMR). Both compounds were isolated for the first time from this species. We found that both RUA1 and RUA2 (10 μM) significantly improved cell survival in OGD conditions. Moreover, these compounds also increased neurite outgrowth in differentiated PC12 cells subjected to ischemic insult. Investigations on the cellular mechanism for the observed effect revealed that RUA1(10 μM) enhances the expression of synaptophysin_a marker of synapses, and an indicator of synaptic plasticity. Rapid restoration of neurological function following injury is paramount to

the prevention of debilitating consequences of ischemic stroke. This combination of neuroprotection and neuritogenic potential could be particularly useful in the recovery phase of stroke. It would be interesting to study the molecular mechanisms for the observed effects, and also if they would be effective in animal models of stroke.

Disclosures: A. Raghavan: None. **O. Orban-Gyapai:** None. **J. Hohmann:** None. **Z. Shah:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Program#/Poster#: 221.17/E1

Topic: C.08. Ischemia

Title: Pro-apoptotic function of Pin1-mediated Notch1 activation in cell death

Authors: *Y. CHOI, U. YUN, J. JEONG, Y. JANG
Sch. of Pharm., Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: The peptidyl-prolyl cis/trans isomerase (PPIase) Pin1 regulates factors involved in control of cell growth and apoptosis. Notch1 is a transmembrane receptor that plays a crucial role in neurogenesis and neuronal death. The proteolytic cleavage of Notch1 by γ-secretase results in the liberation of the intracellular domain of Notch (NICD), which translocates into the nucleus and regulates gene expression and neuronal death in ischemic stroke. However, the mechanisms underlying Notch activation in ischemic stroke remain unclear. Our findings indicate that Pin1 stimulates Notch1 activation and its pro-apoptotic function following ischemic stroke. Overexpression of Pin1 increased γ-secretase activity and NICD levels, and potentiated neuronal cell death in ischemia-like condition. By contrast, knockdown using siRNA or knockout of Pin1 reduced the NICD level and ischemic neuronal cell death. These results suggest that Pin1 contributes to the pathogenesis of ischemic stroke by promoting Notch signaling.

Disclosures: Y. Choi: None. **U. Yun:** None. **J. Jeong:** None. **Y. Jang:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Topic: C.08. Ischemia

Support: VA Merit BX001696-01

Title: Ischemia-induced cell-type specific alterations in brain mitochondrial homeostasis

Authors: K. OWENS[1], J. H. LEE[1], S. GOURLEY[1], *T. KRISTIAN[2,1]
[1]Veterans Affairs Maryland Hlth. Ctr. Syst., Baltimore, MD; [2]Dept Anesthesiol, Univ. Maryland Sch. Med., BALTIMORE, MD

Abstract: Mitochondrial homeostasis via mitochondrial dynamics and quality control is crucial to normal cellular functions. To study the cell-type specific changes in mitochondrial homeostasis following ischemic insult, we used our transgenic mouse model that expresses mitochondrially targeted yellow fluorescence protein (mito-eYFP) either in astrocytes or neurons. Both neuron-, and astrocyte-specific mito-eYFP expressing mice were subjected to transient 10 min global cerebral ischemia. Following 2, 4, 24 hours and 3 days of recovery, the mice were perfusion-fixed or hippocampal samples were collected for further analysis by western blots. By utilizing serial z-sectioning with a Zeiss confocal microscope, we examined the morphological alterations of the entire mitochondrial network within the whole cell. Images were taken from each sub-region of the hippocampus. At the designated recovery time points, we examined the localization and distribution of proteins that regulated fission, fusion and proteins that are involved in mitophagy mechanisms. Interestingly, we found that only neuronal mitochondria became heavily fragmented following ischemia. This intense fragmentation of neuronal mitochondria was observed at 2 hours of reperfusion and persisted during the whole 3 day recovery period in all hippocampal sub-regions. In both neurons and astrocytes, the total mitochondrial mass was decreased during reperfusion suggesting an activation of mitophagy. This notion was supported by dramatic increase in hippocampal PINK1 levels and co-localization of PARKIN, LC3 and lysosomal protein LAMP2 with mitochondria. At 2 hours of recovery, the PARKIN colocalization was observed only in the perinuclear region in both cell types. At 3 days of recovery, we also observed PARKIN co-localization with mitochondria located in astrocytic processes. Remarkably, in neurons, intracellular localization pattern of PARKIN did not change at later reperfusion times. Thus, only perinuclear mitochondria recruited PARKIN in hippocampal neurons. This data suggests that ischemic insult causes pathologic disturbances in mitochondrial homeostasis as reflected in massive fission preferentially of neuronal mitochondria. This is followed by activation of mitophagy in both neurons and astrocytes. In neurons, the mitophagy recruits mainly mitochondria localized in the perinuclear region. In astrocytes, the mitophagy also target peripheral mitochondria localized in processes at

3 days of recovery when the neuronal cell death is apparent in the CA1 sector of the hippocampus.

Disclosures: **K. Owens:** None. **T. Kristian:** None. **J.H. Lee:** None. **S. Gourley:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Program#/Poster#: 221.19/E3

Topic: C.08. Ischemia

Title: The interaction between Notch-1 and HIF-1α enhances ischemic neuronal death

Authors: Y. CHOI, *D.-G. JO, U. YUN, Y. JANG, J. JEONG
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Abstract: Previous studies have shown that γ-secretase mediated Notch-1 signaling induces ischemic neuronal cell death, but the underlying mechanisms are poorly understood. Hypoxia inducible factor-1α (HIF-1α), a transcription factor of cellular adaption to hypoxia, can interact with Notch and modulate its signaling during hypoxic stress. We found that γ-secretase inhibitor and HIF-1α inhibitor protect primary cortical neurons under ischemia-like conditions, and combined inhibition of Notch-1 and HIF-1α further reduced neuronal cell death. HIF-1α and Notch-1 intracellular domain (NICD) are co-localized in the neuronal nucleus, and co-immunoprecipitated in cultured neurons and in brain tissue from mice subjected to focal ischemic stroke. Overexpression of NICD and HIF-1α in human neuroblastoma cells potentiated cell death under ischemic conditions, and a HIF-1α inhibitor rescued the cells. Knockdown of endogenous Notch-1 and HIF-1α using siRNA also decreased cell death under ischemia-like conditions. Finally, mice treated with the γ-secretase inhibitor and the HIF-1α inhibitor exhibited improved outcome and reduced brain injury after stroke. In conclusion, Our findings suggest that agents that target the Notch-1 and HIF-1α pathway may prove effective in reducing neuronal cell death and brain injury after ischemic stroke.

Disclosures: **Y. Choi:** None. **D. Jo:** None. **U. Yun:** None. **Y. Jang:** None. **J. Jeong:** None.

Poster

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Topic: C.08. Ischemia

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National Natural Science Foundations of China 30871264

Title: Maged1 deficiency improves ischemic cell death

Authors: ***J. GAO**, L.-S. JU
Dept. of Neurobio., Nanjing Med. Univ., Jiangsu, China

Abstract: Maged1 is a member of the type II MAGE (melanoma antigen) family of proteins, which is well known to contribute to the p75NTR-dependent cell death. Recently, Maged1 has been reported to be involved in depression, impaired sexual behavior and memory in mice. However, the role of Maged1 in the ischemic cell death remains unknown. The aim of the present study was therefore to investigate whether Maged1 deficiency can protect neuron from ischemia-induced death. Focal cerebral ischemia was induced by middle cerebral artery occlusion (MACO) for 90 min. Infarct volumes and neurological scores were evaluated at 24 h after MACO. Neuronal apoptosis and the Bax/Bcl-2 ratio were also evaluated 24 h after reperfusion. Our results showed that MACO significantly enhanced neuronal expression of Maged1 in the ischemic penumbra 6 h after reperfusion. However, Maged1 deficiency increased infarct volume, decreased neurological outcome, and improved neuronal apoptosis following reperfusion. Moreover,in Maged1 knockout mic, we found the decreased levels of miR-200, which is showed to improve neural cell survival via prolyl-hydroxylase mRNA silencing and subsequent HIF-1α stabilization. In conclusion, Maged1 deficiency decreased miR-200 to improve cerebral ischemia.

Disclosures: J. Gao: None. **L. Ju:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Topic: C.08. Ischemia

Support: NSC-102-2314-B-038-025-MY3

Title: Neuroprotective effects of emodin against ischemia/reperfusion injury through inducing parkin expression *in vitro* and *in vivo*

Authors: *J.-H. LAI, ESQ
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Abstract: **Introduction/Background** It is well recognized that ischemic stroke is a major cause of adult disability and death worldwide. Ischemia/reperfusion-induced injury can lead to irreversible cell damage and death. In recent studies, emodin has been reported to possess the antioxidative capability and protective effects in against myocardial ischemia/reperfusion injury. However, the underlying mechanism and neuroprotective function on rat middle cerebral artery occlusion model (MCAO) of ischemic stroke is still unknown. This study aimed to investigate the neuroprotective effects of emodin against oxygen-glucose deprivation/reperfusion (OGD/RP) damage on PC12 cells. **Materials and Methods** PC12 Cells were subjected to oxygen-glucose deprivation and treated with emodin. We further investigated neuroprotective effects of emodin in rat middle cerebral artery occlusion model. **Results** Results demonstrated that emodin reduced the infract volume and cells death in focal cerebral ischemia injury. The viability and ROS production of PC12 cells and glutamate release were restored by emodin treatment under an ischemiahypoxia environment. Emodin enhanced the Bcl-2, GLT-1 and Parkin expression, but suppressed Bax and Caspase 3 level through AKT/ERK-1/2 signaling pathway. **Discussion/Conclusions** The phosphorylation level of AKT and ERK-1/2 and the parkin expression were enhanced by emodin in a dose- and time- dependent manner. In summary, experimental findings indicated that emodin could induce the parkin for inhibiting neuronal apoptosis, ROS generation and depressing the release of glutamate toxicity via the AKT/ERK-1/2 signaling pathway. Furthermore, emodin could alleviate the injury of nerve cells to against ischemia/reperfusion-induced brain injury in rat models of middle cerebral artery (MCA) ligation. Parkin induced by Emodin has neuroprotective effects against ischemia/reperfusion injury both *in vitro* and *in vivo* through AKT and ERK-1/2 signaling pathway. Key words: Ischemia/reperfusion, MCAO, Emodin, Parkin, GLT-1

Disclosures: **J. Lai:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 221.22/E6

Topic: C.08. Ischemia

Support: CIHR MOP-106651

Title: Cerebral metabolic changes in chronic stroke, assessed by Magnetic Resonance Spectroscopy

Authors: *J. K. FERRIS[1,2], K. E. BROWN[3], C. S. MANG[3], K. P. WADDEN[3], M. R. BORICH[4], S. K. MEEHAN[5], L. A. BOYD[3]
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Abstract: Background: Previous research has shown alterations in motor cortex excitability following chronic ischemic stroke, yet the neurochemical mechanisms underlying these changes remain poorly understood. MRS allows for *in vivo* examination of metabolite concentrations in target tissues. MRS may provide valuable information about metabolic changes occurring between the affected and unaffected hemisphere of the brain following an ischemic stroke. The primary objective of this study was to examine alterations to cerebral neurochemistry between cerebral hemispheres following chronic stroke, using magnetic resonance spectroscopy (MRS). **Methods**: 31 individuals with chronic ischemic stroke were recruited to participate (6 females 25 males, average age, average post-stroke duration: 62 months). All participants were experiencing some degree of hemiparesis. We assessed NAA, glutamate, myo-inositol and creatine concentrations with MRS in a 3T MR scanner, using LC model to extract metabolite concentrations. The voxel of interest was placed over the hand area of primary motor cortex. Upper extremity motor function was assessed with the Fugl-Meyer scale. **Results:** NAA, glutamate, and creatine concentrations were all significantly lower in ipsilesional primary motor cortex (NAA: $t = -3.549$, $p= 0.001$; Glu: $t= -4.416$, $p = 0.0001$; Cre: $t = -2.417$, $p = 0.022$). There was no difference in myo-inositol concentrations between hemipheres. There was a significant correlation of NAA in the affected hemisphere with Fugl-Meyer score ($r = 0.472$, $p = 0.007$), with no other correlations between metabolite concentrations and Fugl-Meyer score **Conclusions:** There appears to be several alterations to neurochemistry in the lesioned cerebral

hemisphere in chronic stroke. First, NAA, a marker of neuronal integrity, is reduced in M1 of the affected hemisphere, and this reduction in NAA is correlated with the degree of functional impairment of the upper extremity, as indexed by the Fugl-Meyer scale. Next, glutamate is reduced in the affected motor cortex, which may relate to previous reports of increased inhibition of the affected hemisphere in chronic stroke. Finally, creatine is reduced in the affected hemisphere, which is perhaps indicative of reduced energy availability in the affected hemisphere. Taken together these data indicate alterations in cerebral metabolites in the primary motor cortex in chronic ischemic stroke. Improved understanding of the neurochemical changes occurring in chronic stroke may provide valuable insights into future rehabilitative avenues for individuals suffering motor impairment following ischemic insult. Funding provided by CIHR: MOP-106651

Disclosures: J.K. Ferris: None. **K.E. Brown:** None. **C.S. Mang:** None. **K.P. Wadden:** None. **M.R. Borich:** None. **S.K. Meehan:** None. **L.A. Boyd:** None.

Poster

221. Ischemia: Cellular Mechanisms and Neuroprotection II

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 221.23/E7

Topic: C.08. Ischemia

Support: Swedish Research Council

Swedish Heart Lung Association

Title: MEK1/2 inhibition diminishes ETB receptor-mediated vasoconstriction and improves neurological function after cerebral ischemia in female rats

Authors: *H. AHNSTEDT[1], M. MOSTAJERAN[1], F. W. BLIXT[1], K. WARFVINGE[1], S. ANSAR[1], D. N. KRAUSE[2], L. EDVINSSON[1]
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Abstract: Sex differences are well-known in cerebral ischemia as demonstrated by a higher incidence of stroke in men. Male-female differences may also impact the effects of stroke treatments. In male rats, the MEK1/2 inhibitor U0126 reduces brain infarct size and improves neurological function after experimental stroke; however, responses to this treatment in females

are not known. In males, U0126 also attenuates ET_B receptor upregulation that occurs in arterial smooth muscle of cerebral arteries subjected to experimental stroke *in vivo* or organ culture *in vitro*. This effect would thereby mitigate reduction in blood flow and increased tissue damage after ischemia in males, but these effects have also not been examined in females. Therefore, the present study investigated whether ET_B receptor upregulation occurs in cerebral arteries of female rats and, if so, whether the effect is mediated by activation of the MEK/ERK1/2 pathway. Furthermore U0126 was tested for potential benefit in female rats after cerebral ischemia. Transient middle cerebral artery occlusion (tMCAO, 120 min) was performed in female rats with and without U0126 treatment (30 mg/kg i.p.) administered at 0 and 24 h of reperfusion. Prior to the surgeries the estrous cycle of the animals were characterized by daily collection of vaginal smears. At the day of surgery only female rats that were under low influence of estrogen, i.e. not in proestrus, were selected. Additionally, *in vitro* organ culture of isolated cerebral arteries from female rats was studied as a model for ET_B receptor upregulation. Stroke infarct volumes were assessed with neuron-specific nuclear protein NeuN staining, and neurological examination was performed using a 6-point and 28-point neuroscore. ET_B receptor-mediated contraction was studied with wire myographs, and protein expression with immunofluorescence and western blot. Cerebral ischemia in female rats resulted in increased arterial ET_B receptor expression and vasoconstriction. U0126 treatment attenuated ET_B receptor-mediated vasoconstriction and inhibited ischemia-induced ET_B receptor expression and ERK1/2 activation in female cerebral arteries. Importantly, U0126 treatment of female rats after experimental stroke improved the neurological function. MEK1/2 inhibition is therefore a promising therapeutic strategy for stroke in both males and females.

Disclosures: **H. Ahnstedt:** None. **M. Mostajeran:** None. **F.W. Blixt:** None. **K. Warfvinge:** None. **S. Ansar:** None. **D.N. Krause:** None. **L. Edvinsson:** None.

Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: This research was supported by the Intramural Research Program of the NIH, NINDS.

Title: Lentiviral-mediated administration of interleukin-4 promotes alternative activation of microglia and reduces endothelin-1-induced cerebral ischemia

Authors: *S.-H. CHOI, A. C. SILVA
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Abstract: The precise role of microglia in cerebral ischemia has been contentious. Microglia are capable of synthesizing numerous soluble and membrane-bound molecules, some of which are known to be neurotoxic, whereas others have neuroprotective bioactivities. The molecular mechanisms through which microglia activate these molecules with apparently opposing physiological roles have thus become an important area of research in cerebral ischemia. To determine the impact of interleukin-4 (IL-4) in post-ischemic inflammation and to elucidate the mechanisms of IL-4-induced polarization of activated microglia, IL-4 was locally expressed into the mouse brain via recombinant lentivirus driven by the mouse phosphoglycerate kinase promoter. Focal cerebral ischemia was induced by intracortical injection of the vasoconstrictor peptide endothelin-1. We found that CD11b-positive cells in the ischemic core increased over time with a concurrent decrease in neurons. CD11b-positive cell population was initially formed of inducible nitric oxide synthase and CD16/32-positive M1 phenotype, which later shifted towards CD206 and Ym1-positive M2 phenotype. Lentiviral-mediated administration of IL-4 was able to modulate microglia towards an anti-inflammatory, neuroprotective M2 phenotype and ischemic brain injury was partly reduced compared to controls. This study may provide a promising strategy to investigate the potential role of protective microglia in neurological disorders.

Disclosures: S. Choi: None. **A.C. Silva:** None.

Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: Brain and Spinal Cord Injury Research Trust Fund, McKnight Brain Institute, UF, Project 000107575

Title: Neurovascular protection by post-ischemic injections of a lipoxin A4 receptor agonist, BML-111, in a rat model of ischemic stroke

Authors: *K. E. HAWKINS[1], K. M. DEMARS[1], J. SINGH[1], J. C. FRANKOWSKI[1], S. DORÉ[2], E. CANDELARIO-JALIL*[1]

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Abstract: Background: Resolution of inflammation is an emerging new strategy to reduce damage following ischemic stroke. Lipoxin A4 (LXA4) is an anti-inflammatory, pro-resolution lipid mediator with high affinity binding to its receptor ALX. Since LXA4 is rapidly inactivated, potent analogs have been created, including the ALX agonist BML-111. We hypothesized that post-ischemic intravenous administration of BML-111 would provide protection to the neurovascular unit and reduce neuroinflammation in a rat stroke model. Method: Wistar rats were subjected to 90 min of middle cerebral artery occlusion (MCAO) and BML-111 (1 mg/kg) was administered intravenously 100 min and 24 h after stroke onset and sacrificed 48 h after reperfusion. Infarct size and vasogenic edema were detected by TTC staining. Blood brain barrier (BBB) disruption and hemorrhagic transformation were calculated by measuring IgG and Hb extravasation, respectively. Matrix metalloproteinase (MMP)-9 was measured with gelatin substrate zymography and MMP-3 levels were determined with an immunocapture FRET peptide assay. Levels of CD68, myeloperoxidase (MPO), intracellular adhesion molecule (ICAM)-1, and zona occludens-1 (ZO-1) were detected by western blotting. Results: Post-ischemic treatment with BML-111 reduced infarct size, decreased vasogenic edema, protected against BBB disruption, and reduced hemorrhagic transformation. MMP-9 and MMP-3 were significantly reduced following BML-111 treatment. Administration of BML-111 dramatically decreased microglial activation, as seen with CD68, as well as neutrophil infiltration and recruitment, as assessed by levels of MPO and ICAM-1. The tight junction protein ZO-1 was protected from degradation by activation of ALX with BML-111. Conclusion: These results indicate that post-ischemic activation of ALX has resolution effects that limit the inflammatory damage in the penumbra and helps maintain BBB integrity after ischemic stroke.

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Poster

222. Ischemia: Neuroprotection

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 222.03/E10

Topic: C.08. Ischemia

Title: Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a novel therapeutic target in neonatal hypoxic-ischemic encephalopathy (HIE)

Authors: *T. AKAMATSU[1,2], T. TANAKA[3], M. MIZUGUCHI[3], Y.-I. GOTO[2], M. ITOH[2]
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Abstract: Background: Neonatal care has remarkably progressed for the last decades. However the incidence of nHIE has not been decreased, and severe neurological sequelas in nHIE. Major pathologies of nHIE are neuronal apoptosis and breakdown of neurovascular unit. Although hypothermia is the typical and efficient treatment against nHIE and improves neuronal apoptosis and neurovascular unit, more convenient and spreadable treatment is needed. We have reported that the suppression of the expression of oxidized low density lipoprotein receptor 1 (Olr1) was associated with the neuroprotection of hypothermia and Olr1 was expressed in neurons and endothelial cells in nHIE brains. Objectives: We assessed LOX-1 as a novel therapeutic target in nHIE, using rodent nHIE model. Materials & methods: We made nHIE rats, using postnatal-day 7 rats, and performed hypothermia at 28℃ for 3 hours. We tried the administration of anti-LOX-1 neutralizing antibody as a novel treatment against nHIE. We assessed infarct size by Nissl staining, apoptosis by TUNEL staining. We assessed the level of malondialdehyde (MDA) by ELISA and the level of cleaved caspase 3 (cCASP 3) by western blot, with proteins from infarct-side hemispheres. We assessed brain edema by water content and the levels of tight junction proteins (TJPs) by western blot. Results: The administration of anti-LOX-1 antibody significantly decreased infarct size and TUNEL-positive cells in nHIE rats. Moreover the antibody significantly decreased the levels of MDA and cCASP 3 which were increased in nHIE rats. The antibody suppressed brain edema and suppressed the degradation of TJPs in nHIE rats. These effects of antibody were equal to those of hypothermia. Conclusions: We revealed that anti-LOX-1 neutralizing antibody had the neuroprotective effects against nHIE to the same degree with hypothermia. The antibody improved the major pathologies of nHIE, neuronal apoptosis and breakdown of neurovascular unit. The antibody might directly suppress neuronal death induced by reactive oxygen species and improved neurovascular unit by acting on endothelial cells. Father research is necessary for clinical administration.

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Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: ANPCyT

CONICET

UBA

Title: Enhanced survival of melanopsin-expressing ganglion cells and the non-image-forming visual system after acute retinal ischemia

Authors: *D. DORFMAN, M. F. GONZALEZ FLEITAS, M. L. ARANDA, P. H. SANDE, R. E. ROSENSTEIN
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Abstract: Retinal ischemia/reperfusion injury is an important cause of visual impairment. The loss of retinal ganglion cells (RGCs) is a key sign of retinal ischemia. A subset of RGCs expressing the photopigment melanopsin (mRGCs) regulates non-image-forming visual functions such as the pupillary light reflex (PLR) and circadian rhythms. We studied the effect of retinal ischemia on melanopsin expressing RGCs and the non-image-forming visual system. For this purpose, ischemia was induced in male *Wistar* rats by increasing intraocular pressure (120 mm Hg for 40 min). Retinal function (electroretinogram (ERG)), the number of Brn3a(+) and melanopsin(+) RGC (immunohistochemistry), Brn3a and melanopsin levels (Western Blot), and the consensual pupil light reflex (PRL) (after 10-s light flash) were examined. Anterograde transport was assessed after an intravitreal injection of cholera toxin β-subunit, and circadian rhythms of general locomotor activity were registered in cages equipped with infrared detectors of motion. After 4 weeks of ischemia, clear alterations in the visual function (ERG)and retinal histology were observed. Concomitantly with a significant decrease in the number of Brn3a(+) RGC and in Brn3a levels, no differences in the number of melanopsin(+) cells, and melanopsin levels were observed between non-ischemic and ischemic retinas.Ischemia decreased anterograde transport to the superior colliculus and lateral geniculate nucleus, whereas the anterograde transport to the suprachiasmatic nucleus and the olivary pretectal nucleus remained unaffected. At high light intensity, consensual PLR was conserved in ischemic eyes, whereas at low light intensity, a decrease in pupil constriction was observed in intact eyes contralateral to ischemic eyes. Animals with ischemia in both eyes showed a conserved locomotor activity pattern and a photoentrainment rate which did not differ from control animals. The present results indicate a marked preservation of a unique subtype of RGCs, mRGCs, which was functionally and structurally protected even after an extensive retinal damage such as that observed at 4 weeks after ischemia.

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Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: JSPS KAKENHI Grant 23111004

JSPS KAKENHI Grant 25670099

Title: Hippocampal pyramidal neurons lacking LC3A and LC3B are resistant to hypoxic-ischemic brain injury in neonatal mice

Authors: J. YAMAGUCHI[1], T. SUNABORI[2], M. KOIKE[2], T. NANAO[1], M. SHIBATA[3], *A. FURUTA[1], Y. UCHIYAMA[1]
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Abstract: Although LC3, a mouse homologue of yeast Atg8, has mainly been used for a marker protein of autophagy, little is known about roles of subtypes of LC3, LC3A and LC3B, or whether these proteins compensate their roles for each other during autophagic processes. For this, we produced LC3A and LC3B (LC3A/B)-double deficient (KO) mice, which were born normally and were grown-up to be capable of reproduction. Using these KO mice, both mRNA and protein levels of LC3 homologous proteins, a GABARAP family of proteins, were examined in brain and other tissues, and found that they did not significantly alter between wild-type and LC3A/B-double deficient mice, except for that the expression level of GABARAP was significantly higher in liver tissue than in brain tissue. These results indicate that basic autophagy may work normally in neurons of LC3A/B-deficient brains. Since we have previously shown that Atg7-deficiency rescues mouse neonatal hippocampal pyramidal neurons from hypoxic-ischemic injury (H/I), LC3A/B-double deficient mice were applied to this H/I model. As previously reported, hippocampal pyramidal neurons in wild-type mice (wt) were vulnerable to H/I injury, while the protein amount of LC3-II was significantly more elevated in the ipsilateral hippocampus than in the contralateral hippocampus from 9 to 24 hours after H/I injury. In contrast, hippocampal pyramidal neurons deficient in LC3A/B were resistant to the H/I injury,

while the protein amount of LC3 II in the double KO mouse hippocampus was not increased after H/I injury, compared to that in the wt hippocampus. These results suggest that LC3A/B, but not GABARAP family of proteins, are involved in H/I injury-mediated pyramidal neuron death that occurs in the hippocampal pyramidal layers of neonatal mouse brains. As shown in our previous study, excess autophagic stress may induce neuron death. Our present data using LC3A/B-deficient mice strongly support the presence of H/I injury-mediated autophagic neuron death in neonatal brains. Of course, further study is required to elucidate the molecular mechanism of why H/I injury-mediated neuron death is suppressed by deficiency in Atg7 or LC3A/B.

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Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: SNF grant n° 31003A-140957 to LP

Title: Changes in monocarboxylate transporter (MCT) expression following ischemia : an *in vitro* and *in vivo* study

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Abstract: Accumulating evidence indicate that lactate represents an important neuronal energy substrate. Moreover, it has been suggested that lactate could be neuroprotective under various pathological conditions, including stroke. Indeed, 1Berthet et al. have demonstrated a beneficial effect of lactate injections following middle cerebral artery occlusion (MCAO) in both rat and mouse models. However, the precise mechanism by which lactate exerts this neuroprotective effect remains unknown. In parallel, we have recently demonstrated that a reduction in oxygen tension promotes glycolytic activity in cultured astrocytes and induces the expression of the monocarboxylate transporter MCT4. Thus, it is purported that alterations in lactate metabolism,

including changes in expression of the different MCTs found in the brain might participate to the recovery from an ischemic insult and could be necessary to explain the beneficial effects of lactate in this context. To obtain further insight about this question, we determined the level of expression of MCT1, MCT2 and MCT4 in the brain of mice following MCAO. The expression of all three MCTs was decreased at the protein level in the ischemic striatum compared to the contralateral hemisphere 1 hour after the ischemic event, while their expression was restored 24 hours after ischemia. When we looked at the expression of MCT4 by immunohistochemistry, the appearance of cells strongly labeled for MCT4 was observed in the striatum, hippocampus and cortex of the ischemic hemisphere, although these cells were not GFAP positive when examined by double labelling and confocal microscopy. In order to complement our *in vivo* data, we undertook a study of MCT expression on rat hippocampal organotypic culture submitted to oxygen-glucose deprivation (OGD). Preliminary data show that MCT2 expression is decreased following OGD, an observation that might be indicative of neuronal cell death. MCT4 expression also decreased while MCT1 expression was unaffected. Thus, our data indicate that MCT expression is modified under hypoxic/ischemic conditions. It remains to be determined how important are these changes for the functional recovery of the animal and whether they are necessary for the beneficial effects of lactate in these conditions 1Berthet et al (2009) J Cereb Blood Flow Metab 29 :1780-9 ; Berthet et al. (2012) Cerebrovasc Dis 34 :329-35.

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Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: NIH Grant NS050730

Title: GPR30 mediates estrogen rapid signaling and neuroprotection

Authors: R. WANG[1,3,4], H. TANG[4], Q. ZHANG[1,3], L. YANG[4], Y. DONG[1], M. KHAN[1], F. YANG[4], *D. W. BRANN[1,3,2]
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Abstract: G-protein-coupled estrogen receptor-30 (GPR30), also known as G-protein estrogen receptor-1 (GPER1), is a putative extranuclear estrogen receptor whose precise functions in the brain are poorly understood. Studies using exogenous administration of the GPR30 agonist, G1 suggests that GPR30 may have a neuroprotective role in cerebral ischemia. However, the physiological role of GPR30 in mediating estrogen (E2)-induced neuroprotection in cerebral ischemia remains unclear. Also unclear is whether GPR30 has a role in mediating rapid signaling by E2 after cerebral ischemia, which is thought to underlie its neuroprotective actions. To address these deficits in our knowledge, the current study examined the effect of antisense oligonucleotide (AS) knockdown of GPR30 in the hippocampal CA1 region upon E2-BSA-induced neuroprotection and rapid kinase signaling in a rat model of global cerebral ischemia (GCI). Immunohistochemistry demonstrated that GPR30 is strongly expressed in the hippocampal CA1 region and dentate gyrus, with less expression in the CA3 region. Furthermore EM and confocal analysis showed that GPR30 localized at extranuclear sites, including the plasma membrane, endoplasmic reticulum, and dendritic spines in the brain. E2-BSA exerted robust neuroprotection of hippocampal CA1 neurons against GCI, an effect abrogated by AS knockdown of GPR30. Missense control oligonucleotides had no effect upon E2-BSA-induced neuroprotection, indicating specificity of the effect. The GPR30 agonist, G1 also exerted significant neuroprotection against GCI. E2-BSA and G1 also rapidly enhanced activation of the prosurvival kinases, Akt and ERK, while decreasing proapototic JNK activation. Importantly, AS knockdown of GPR30 markedly attenuated these rapid kinase signaling effects of E2-BSA. As a whole, the studies provide evidence of an important role of GPR30 in mediating the rapid signaling and neuroprotective actions of E2 in the hippocampus.

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Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

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Title: Subacute administration of zinc caused an increase of CXCL1 and CXCR2 during a cerebral hypoxia-ischemia process in rat

Authors: *V. M. BLANCO ALVAREZ[1], G. SOTO-RODRIGUEZ[2], J. A. GONZALEZ-BARRIOS[5], C. PIÑA-LEYVA[6], C. TOMAS-SANCHEZ[3], D. MARTINEZ-FONG[7], E. BRAMBILA[3], M. TORRES-SOTO[4], A. RUIZ-TAGLE[4], B. A. LEON-CHAVEZ[3]
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Abstract: Cerebral ischemia causes an increase of the immune response by releasing mediators involved in leukocyte chemoattraction (chemokines) in cell damage. The subacute administration of ZnCl2 (2.5 mg/kg/day for 4 days) has shown to reduce the lipoperoxidation and cell death. The aim of this work is know if zinc subacute administration causes the expression of CXCL1 and CXCR2 during cerebral hypoxia-ischemia. Male Wistar rats (220 ± 190 g) with subacute administration of zinc was performed the common carotid artery occlusion (CCAO) for 10 min., other group was only administered with zinc and a positive control group with only CCAO. The cerebral cortex samples were obtained at different times (24h, 48h, 72h and 96h during the administration, and 4h, 8h, 12h, 36h, 96h and 168h post-reperfusion). Protein levels of CXCL1 and CXCR2 were measured by ELISA and mRNA by RT -PCR. The results show that CCAO caused upregulation of CXCL1 mRNA levels by 101.6% ± 18.7%, protein levels were increased by 70.0% ± 23% at 4 h, 28% ± 2.7% at 12 h, and 59 % ± 10.3% since 96h postreperfusion as compared with the sham group. The subacute administration of Zinc caused downregulation by 56.7% ± 4.1% in the CXCL1 mRNA levels since the first to fourth administration, the protein levels was increased by 56.7% ± 4.1% after of the first administration, by 79.5%± 4.7% at the 4 h after last administration. The subacute administration of zinc caused an upregulation in mRNA levels CXCR2 at 8 h post-reperfusion. These results suggest that zinc subacute administration causes a preconditioning effect through of CXCL1 and CXCR2 during brain hypoxia-ischemia process.

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Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: The Research Fund of the IRP, NIMH, NIH

Title: Pharmacological inhibition of HDAC6 alleviates brain infarction and functional deficits after experimental stroke: Potential roles of α-tubulin acetylation, FGF-21 up-regulation and excitotoxicity mitigation

Authors: Z. WANG[1], J. WANG[1], Y. LENG[1], J. BERGMAN[2], P. LEEDS[1], A. KOZIKOWSKI[2], *D.-M. CHUANG[1]
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Abstract: Histone deacetylase (HDAC) 6 exclusively exists in the cytoplasm and mainly uses cytoplasmic proteins such as α-tubulin as its substrate. Dysfunction of HDAC6 is known to be associated with CNS pathological conditions. Therefore, HDAC6 has recently emerged as a potential target for treatment of neurodegenerative diseases. The role of HDAC6 in the pathophysiology of ischemic stroke remains to be elucidated. The present study investigated the beneficial effects of tubastatin A (TubA), a novel specific HDAC6 inhibitor, in a rat model of ischemic stroke and an *in vitro* model of excitotoxicity. Male Sprague Dawley rats underwent middle cerebral artery occlusion (MCAO) for 60 minutes followed by reperfusion. TubA (i.p.) was administrated immediately after ischemic onset, and once daily for up to three days. Post-ischemic TubA treatment at both 25 and 40 mg/kg robustly reduced brain infarction on day three after MCAO. TubA treatment at both doses also facilitated functional recovery in MCAO rats at least three days after ischemia. Specifically, MCAO rats receiving TubA had much longer retention time on an accelerating rotarod compared with the untreated MCAO group. Concurrently, the increased neurological deficit score and body tilting percentage in MCAO rats were markedly attenuated by TubA treatment. Of interest, when given at 24 hours after MCAO, 25 mg/kg TubA still exhibited significant effects in reducing brain infarction and improving functional outcomes for at least three days after ischemia. Levels of acetylated α-tubulin were decreased in the ischemic cortex on days one and three after MCAO, and this was significantly restored by TubA (25 mg/kg). We recently reported that fibroblast growth factor-21 (FGF-21), a novel metabolic regulator, is inducible in brain neurons and has a robust neuroprotective role. Notably, FGF-21 was markedly down-regulated in the ischemic cortex after MCAO, and this down-regulation was significantly alleviated by TubA treatment. In addition, TubA conferred neuroprotection in primary cortical neuronal cultures against glutamate-induced excitotoxicity. Live cell imaging showed that TubA appeared to improve the impaired transport of mitochondria induced by glutamate. Together, our results suggest that TubA reduces brain infarction and promotes functional recovery in MCAO rats, and protects against glutamate-induced excitotoxicity. The neuroprotective effects of TubA likely involve HDAC6 inhibition and the

subsequent up-regulation of acetylated α-tubulin and FGF-21. These findings suggest HDAC6 as a promising target for the treatment of ischemic stroke and the clinical utility of TubA for this brain disorder.

Disclosures: **Z. Wang:** None. **J. Wang:** None. **Y. Leng:** None. **J. Bergman:** None. **P. Leeds:** None. **A. Kozikowski:** None. **D. Chuang:** None.

Poster

222. Ischemia: Neuroprotection

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 222.10/F2

Topic: C.08. Ischemia

Support: VIEP grant SAIC121

Title: Chronic administration of zinc decreased nitrosative stress in cerebral cortex and heart in rat

Authors: ***C. T. SANCHEZ**[1], C. PIÑA-LEYVA[2], V. M. BLANCO-ALVAREZ[3], G. SOTO-RODRIGUEZ[3], J. A. GONZALEZ-BARRIOS[6], D. MARTINEZ-FONG[2], E. BRAMBILA[3], M. TORRES-SOTO[4], A. UGARTE[5], B. A. LEON-CHAVEZ[3]
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Abstract: In previous studies have been demonstrated that subacute administration of zinc decreased the lipoperoxidation and caspase-3 during hypoxia-ischemia and Wilson's disease. The zinc treatment has caused an increase of presynaptic/extracellular zinc concentration in brain and distribution in liver and kidney. The aim of this work is investigate if chronic administration of zinc decreases the nitrosative stress in different tissues (cerebral cortex, liver, heart, kidney and serum). Male Wistar rats were treated with different concentrations of ZnCl2 (0, 0.1, 0.5, 1 and 2.5 mg/kg each 24 h during 14 days, i.p.) and sacrificed a later day. Lipoperoxidation was measured in the supernant by using Gerard-Monnier method; the pro-caspase-3 and nitrotyrosine levels of each organ were measured in the homogenized by ELISA. The results showed that chronic treatment of zinc decreased the lipoperoxidation by 35.1% ± 2.8% since the

administration of 0.5 mg/kg in the cerebral cortex, by 53.8% ± 2.5% with 0.1 mg/kg in the heart, and by 62.5% ± 3.2% only to 0.5 mg/kg in the serum. In addition there was an increase by 73.2% ± 22.6% of lipoperoxidation to the zinc administration of 2.5 mg/kg in kidney. The levels of procaspase-3 decreased by 35.1% ± 4.5% with zinc treatment since 0.5 mg/kg only in cerebral cortex. The optimal concentration of chronic administration of zinc below of 1.0 mg/kg decreased the lipoperoxidation and procaspase-3 levels. These findings provide crucial information about use a zinc optimal concentration to decrease nitrosative stress in these organs.

Disclosures: C.T. Sanchez: None. **C. Piña-Leyva:** None. **V.M. Blanco-Alvarez:** None. **G. Soto-Rodriguez:** None. **J.A. Gonzalez-Barrios:** None. **D. Martinez-Fong:** None. **E. Brambila:** None. **M. Torres-Soto:** None. **A. Ugarte:** None. **B.A. Leon-Chavez:** None.

Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: Grants-in-Aid for Young Scientists (B) (25750366) from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: The role of vagus nerve in the recovery of post-ischemic glucose intolerance and neuroprotective effect by hypothalamic orexin-A

Authors: *S. HARADA, S. TOKUYAMA
Dept. of Clin. Pharmacy, Sch. of Pharmaceut. Sci., Kobe Gakuin Univ., Kobe, Japan

Abstract: Orexin-A (a neuropeptide in the hypothalamus) plays an important role in many physiological functions, including the regulation of glucose metabolism. We have previously found that the development of post-ischemic glucose intolerance is one of the triggers of ischemic neuronal damage, which is suppressed by hypothalamic orexin-A (Harada et al., J. Pharmacol. Exp. Ther., 2013). Other reports have shown that the communication system between brain and peripheral tissues through the autonomic nervous system (sympathetic, parasympathetic and vagus nerve) is important for maintaining glucose and energy metabolism. The aim of this study was to determine the involvement of the hepatic vagus nerve on hypothalamic orexin-A-mediated suppression of post-ischemic glucose intolerance development and ischemic neuronal damage. Male ddY mice were subjected to middle cerebral artery

occlusion (MCAO) for 2 h. The hepatic vagotomy mice created to selectively transect at the hepatic branch vagus nerve. Neuronal damage was estimated by histological and behavioral analyses. Expression of each protein levels was analyzed by western blot and immunofluorescence staining. Intrahypothalamic orexin-A (5 pmol/mouse) administration significantly suppressed the development of post-ischemic glucose intolerance and neuronal damage on day 1 and 3, respectively after MCAO. MCAO-induced decrease of hepatic insulin receptors and increase of hepatic gluconeogenic enzymes on day 1 after was reversed to control levels by orexin-A. This effect was reversed by intramedullary administration of the orexin-1 receptor antagonist, SB334867, or hepatic vagotomy. In the medulla oblongata, orexin-A induced the co-localization of cholin acetyltransferase (cholinergic neuronal marker used for the vagus nerve) with orexin-1 receptor and c-Fos (activated neural cells marker). These results suggest that the hepatic branch vagus nerve projecting from the medulla oblongata plays an important role in the recovery of post-ischemic glucose intolerance and mediates a neuroprotective effect by hypothalamic orexin-A (Harada et al., PLos One, 2014).

Disclosures: S. Harada: None. **S. Tokuyama:** None.

Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: Academia grants

National Science Council grants

Title: Activating transcription factor 3 by inhibiting carboxyl-terminal modulator protein transcription alleviates ischemia-reperfusion induced brain injury

Authors: H.-D. TSAI[1], C.-Y. HUANG[1], J.-S. WU[1], W.-M. CHEUNG[1], *T.-N. LIN[2]
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Abstract: Activating transcription factor 3 (ATF3) is a stress-induced transcription factor with diverse functions in multiple cell types and disease states. ATF3 has been shown to have neuroprotective action against cerebral ischemia which may involve caspase 3. However, the molecular mechanisms underlying ATF3 regulation of apoptosis is largely unknown. Recently,

we identified an endogenous neuroprotective ATF3→CTMP (carboxyl-terminal modulator protein) signaling that binding of ATF3 to the ATF/CREB site blocked NF-κB binding to the CTMP promoter, which repressed CTMP expression, and the subsequent Akt inactivation and caspases activation in primary cortical neurons subjected to oxygen-glucose deprivation and reoxygenation. However, whether this signal cascade also plays an important role in the ischemic brain remains to be studied. In the present *in vivo* studies, with gain- and loss-of-function and rescue approached we showed that ATF3 overexpression or knockout led to respective down- and up-regulation of CTMP expression in the ischemic brain. Moreover, post-ischemic CTMP siRNA treatment not only reduced infarct volumes but also improved functional outcome. In summary, we report an endogenous neuroprotective signaling cascade under cerebral ischemic stress entailing ATF3 regulation of CTMP. The ATF3→CTMP signaling cascade is a promising therapeutic target for reducing ischemic brain injury.

Disclosures: **H. Tsai:** None. **C. Huang:** None. **J. Wu:** None. **W. Cheung:** None. **T. Lin:** None.

Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: NSFC 81071061

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Title: Effect of limb remote ischemic preconditioning on angiogenesis

Authors: L. YU, S.-S. DUAN, W.-S. DONG, J. CHEN, W. JIN, Z. JIN, *C. REN
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Abstract: Abstract Objectives Remote ischemic preconditioning(RIPC) has emerged as a feasible and attractive procedure for cerebroprotection. However, its molecular mechaniams remain poorly understood. Angiogenesis, the growth of blood vessels from the existing vasculature, is an important natural process in the body used for healing and reproduction. This study's aim was to test whether RIPC has an effect on angiogenesis and the expression pattern of angiogenic molecules, through which RIPC exert its protective function. Methods Stroke was

generated by a permanent occlusion of the left distal middle cerebral artery (dMCAO) combined with a 30min occlusion of the bilateral common carotid arteries (CCA) in male rats. Limb preconditioning was generated by 15min occlusion followed with the same period of reperfusion of the left hind femoral artery, and repeated for hree cycles. Microvessel density (using anti-CD31) of both ischemic penumbra and ingarction core was counted, expression of VEGF/VEGFR2 system and Ang/Tie-2 system was measured semi-quantitatively by means of immunohistochemistry and western blotting. Results Microvessel density and the expression of VEGFR2 in ischemic penumbra was significantly increased in RIPC group than in ischemic control group from 3 hours to 2 weeks after dMCAO, both groups peaking at 7 days. Level of Tie-2 was upregulated more remarkably in RIPC group than in ischemic control group (compared with baseline level) from 2 days to 14 days after stioke, the former peaked at 7 days and the latter 14 days. For secretory factors, expression of VEGF and Ang-2 was more abundant in RIPC group than in control group at 2 days and 7 days after operation. Just the opposite, level of Ang-1 was lower in RIPC group. Conclusions RIPC enhanced process of angiogenesis and changed the expression pattern of angiogenic molecules after stroke in rats. This may be an important molecular mechanism of neuroprotection exerted by RIPC.<!--EndFragment-->

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Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: National Natural Science Foundation of China 81361128010

National Natural Science Foundation of China 81301011

Title: Increased oligodendrogenesis by humanin promotes axonal remyelination and neurological recovery in hypoxic/ischemic brains

Authors: ***X. XU**[1], J. CHEN[2], H. ZHAO[2], C.-F. LIU[2]
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Abstract: Oligodendrocytes are the predominant cell type in white matter and are highly vulnerable to ischemic injury. The role of oligodendrocyte dysfunction in ischemic brain injury is unknown. In this study, we used a 24-amino acid peptide S14G-Humanin (HNG) to examine oligodendrogenesis and neurological function recovery in a hypoxic/ischemic (H/I) neonatal model. Intraperitoneal HNG pre-treatment decreased infarct volume following H/I injury. Delayed HNG treatment 24 h after H/I injury did not reduce infarct volume but did decrease neurological deficits and brain atrophy. Delayed HNG treatment did not attenuate axonal demyelination at 48 h after H/I injury. However, at 14 d after H/I injury, delayed HNG treatment increased axonal remyelination, the thickness of corpus callosum at the midline, the number of Olig2+/BrdU+ cells, and levels of brain-derived neurotrophic factor (BDNF). Our results suggest that targeting oligodendrogenesis via delayed HNG treatment may represent a promising approach for the treatment of stroke.

Disclosures: **X. Xu:** None. **J. Chen:** None. **H. Zhao:** None. **C. Liu:** None.

Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: Junta de Castilla y Leon. Reference LE 184A12-2

Diego Perez Rodriguez is a fellowship of the Junta de Castilla y León co-financed by Fondo Social Europeo.

Title: Lipid therapy significantly reduces the infarct volume in a rat model of transient focal cerebral ischemia

Authors: *A. FERNANDEZ-LOPEZ[1], M. SANTOS-GALDIANO[1], B. ANUNCIBAY-SOTO[1], D. PEREZ-RODRIGUEZ[1], A. I. CORTINA-RIVERO[2], F. SANROMAN-LLORENS[2], M. GARCIA-GOMEZ[2], P. V. ESCRIBA[3]
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Abstract: LP204A1 is a synthetic polyunsaturated fatty acid, with the properties of a non-steroidal anti-inflammatory drug (NSAID), that modifies the membrane lipid composition (lipid therapy). In this study we compare the effect of LP204A1 and celecoxib, an anti-inflammatory agent considered the most specific COX-2 inhibitor and a neuroprotective agent against cerebral ischemia. We used a transient focal cerebral ischemia model in rats where the middle cerebral artery was occluded for 60 minutes. One hour and 24 hours after the reperfusion onset (filament withdrawal), animals were treated with LP204A1 (1 g/kg), celecoxib (20 mg/kg) or vehicle. Forty eight hours after the ischemia the infarct volume and the unfolded protein response (UPR) were measured. The infarct volume with respect to the animals with vehicle was about 40% lower in celecoxib treated animals and 70% lower in LP204A1 treated animals. UPR markers were also strongly modified by these agents. We concluded that lipid therapy with LP204A1 constitutes a promising therapy to treat patients that suffered a stroke.

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Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: Biaggi Foundation

CONACYT Grant 215456

Title: Lactate neuroprotection in cerebral ischemia: What is the mechanism?

Authors: *X. CASTILLO[1], K. ROSAFIO[2], L. PELLERIN[2], L. HIRT[1]
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Abstract: Stroke is a highly disabling disease that accounts for one death every four minutes in the United States (Go AS et al., 2014). Despite enormous efforts worldwide for new treatments, rTPA given within 4.5 h of symptom onset remains the only approved treatment for ischemic stroke, the major stroke sub-type. We have previously shown that L-lactate administration during

reperfusion exerts long lasting protection in mice against ischemic damage after transient middle cerebral artery occlusion (tMCAO) (Berthet et al., 2009; 2012). New evidence suggests the possible involvement of the Hydroxy-Carboxylic Acid Receptor-1 (HCA1), a lactate receptor, in nervous system effects of lactate (Bergersen et al., 2013; Bozzo et al., 2013). The objective of the present work is to elucidate if the neuroprotective effects of lactate are exerted by lactate acting as a metabolic substrate or lactate acting on the HCA1 receptor. *In vitro* experiments subjecting organotypic hippocampal slice cultures (OHC) to 1h oxygen and glucose deprivation (OGD) were used to model ischemia. D-lactate, pyruvate, acetate, glucose or 3-5 DHBA, a specific agonist of the HCA1 receptor, were added to the culture medium after OGD. *In vivo* experiments used transient MCAO in adult CD1 mice with administration of Lactate (L or D isoform) or pyruvate at reperfusion. The endpoints were cell survival (*in vitro*), lesion size and neurological performance 48 hours after the insult *in vivo*. For protein expression analysis, mice were sacrificed either 1 or 24h after transient MCAO. Administration of D-lactate, pyruvate and the HCA-1 receptor agonist 3-5 DHBA after OGD improved cell survival, while the administration of acetate and glucose did not. *In vivo*, D- Lactate administration during reperfusion significantly reduced lesion size and improved the neurological performance. HCA1 expression is increased 24h after transient MCAO around the lesion site. As both lactate and it's metabolites, as well as the receptor agonist lead to neuroprotection, we suggest a dual mechanism of it's mode of action. Experiments using HCA1 -/- mice are underway to confirm the physiological relevance of these findings.

Disclosures: X. Castillo: None. **K. Rosafio:** None. **L. Pellerin:** None. **L. Hirt:** None.

Poster

222. Ischemia: Neuroprotection

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Topic: C.08. Ischemia

Support: NIH Grant UL1TR000117

Title: Selective intra-arterial administration of neuroprotective agents directly to the site of ischemic injury after experimental stroke improves neuron survival and functional outcome

Authors: *M. E. MANISKAS[1], J. F. FRASER[2], G. J. BIX[3]
[1]Anat. & Neurobio., [2]Dept. of Neurosurg., [3]Sanders Brown Ctr. on Aging, Univ. of Kentucky, Lexington, KY

Abstract: There has long been a recognized disconnect between laboratory stroke therapy models and the clinical human condition. This has contributed to the translational failure of many promising preclinical neuroprotective stroke treatments. Thus, there is an urgent need to develop a reliable and reproducible animal stroke model that mirrors contemporary acute management for large vessel stroke which includes endovascular blood clot removal (thrombectomy) for larger clots that cannot be adequately dissolved with tissue plasminogen activator. We hypothesize that experimental neuroprotective stroke therapies may fail, in part, due to their failure to reach stroke-affected brain tissue if the blood clot has not been removed prior to treatment. To that end, we propose to use the transient ipsilateral common carotid artery (CCA)/middle cerebral artery (MCA) occlusion (MCAo) mouse model in combination with post-reperfusion selective (i.e. directly into the recanalized CCA) intra-arterial (IA) neuroprotectant administration to accurately mimic large vessel occlusion and the IA techniques used during surgical thrombectomy in patients, respectively. This stroke-targeted administration model also decreases potential systemic side effects. We have chosen to administer two distinct neuroprotective agents, a calcium channel blocker (CCB) that is currently FDA approved for IA cerebrovascular administration and is thus "shovel-ready" for stroke clinical trials, and an experimental N-methyl-D-aspartate receptor (NMDA) modulator. Selective administration of either of these agents significantly reduced mean brain infarct volumes and improved functional outcomes without any systemic side-effects. We conclude that the selective IA administration of potential neuroprotective agents can be successfully modeled in the laboratory to mimic contemporary human large vessel acute stroke management and may result in the successful translation of experimental stroke treatments.

Disclosures: **M.E. Maniskas:** None. **J.F. Fraser:** None. **G.J. Bix:** None.

Poster

222. Ischemia: Neuroprotection

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 222.18/F10

Topic: C.08. Ischemia

Title: Ischemia postconditioning relieves cerebral ischemia injury

Authors: *Y. LUO[1], H. ZHAO[1], Z. TAO[1], R. WANG[1], F. YAN[2], X. JI[3]
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Abstract: Ischemic postconditioning (IPostC) protects against ischemic brain injury. To date, no study has examined the role of (T-LAK-cell-originated protein kinase) TOPK in IPostC-afforded neuroprotection. We explored the molecular mechanism related with TOPK in antioxidant effect of IPostC against ischemia-reperfusion. Focal ischemia was induced in male Sprague-Dawley rats by transient middle cerebral artery occlusion (tMCAO). Reactive oxygen species (ROS) production in the peri-infarct area was detected using dihydroethidium (DHE), MDA level, as a marker of lipid peroxidation, and 3-Nitrotyrosine (3-NT) level, as a marker of protein oxidation, were detected by ELISA. The expression or location of antioxidant proteins and signal molecules TOPK, Phosphatase and tensin homolog (PTEN) and Akt was analyzed by immunofluorescence and western blotting. Our results revealed that IPostC relieved tMCAO-induced oxidative damage by reducing ROS, MDA and 3-NT accumulation in the peri-infarct area, and raised levels of antioxidants peroxiredoxin 1 (Prx-1), Prx-2, and thioredoxin1 (Trx1). In addition, IPostC increased p-AKT and p-TOPK levels, which colocalized in neural cells. *In vitro*, TOPK knockdown, by siRNA, decreased the levels of antioxidants Prx-1, Trx, and MnSOD, and MnSOD activity in PC12 cells. *In vivo*, intracerebroventricular injection of TOPK siRNA reversed IPostC-induced neuroprotection by increasing infarct volume and nitric oxide content, and reducing MnSOD activity. Moreover, IPostC-evoked Akt activation was blocked by TOPK siRNA *in vivo*, but the decreased p-PTEN level in ischemia-reperfusion was not influenced by IPostC or by TOPK siRNA treatment. Our results suggest that the antioxidative effects of TOPK/Akt might contribute to the neuroprotection of IPostC treatment against tMCAO.

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Poster

222. Ischemia: Neuroprotection

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Support: PAPIIT Grant IN201713

DGAPA-UNAM fellowship 070/2012

Title: Tilia americana reduces the infarct area in a model of middle cerebral artery occlusion (MCAO) in rats

Authors: *R. VENTURA-MARTINEZ[1], G. E. ANGELES-LOPEZ[1], M. E. GONZALEZ-TRUJANO[2]
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Abstract: Tilia americana is a plant widely used in mexican traditional medicine for their effects on the central nervous system. The aim of this study was to determinate the infarct area in animals treated with hexane and aqueous extracts of T. americana in the middle cerebral artery occlusion (MCAO) model. Adult male Wistar rats were subjected to right carotid occlusion by intraluminal filament introduction. Two hours after the induction of ischemia, the filament was removed to allow reperfusion. Just before reperfusion, vehicle or extracts of T. americana (AQUOS 300 mg/kg or HEX 300 mg/kg) were administered intraperitoneally. After 2 h of reperfusion, animals were sacrificed and their brains were removed to determinate the infarct area using the TTC staining. Results showed that treatment with both extracts of T. americana, hexane or aqueous, significantly reduced the infarct area in the MCAO procedure, suggesting that this medicinal plant has neuroprotective property in brain ischemia.

Disclosures: R. Ventura-Martinez: None. **G.E. Angeles-Lopez:** None. **M.E. Gonzalez-Trujano:** None.

Poster

222. Ischemia: Neuroprotection

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Program#/Poster#: 222.20/F12

Topic: C.08. Ischemia

Title: The role of neuronal expression of P2X7 receptors in the processes of brain injury in mice

Authors: *A. B. CAGLAYAN[1], M. C. BEKER[1], U. KILIC[2], B. CAGLAYAN[1], T. KELESTEMUR[1], G. OZTURK[1], E. KILIC[1]
[1]Physiol., Istanbul Medipol Univ., Istanbul, Turkey; [2]Bezmialem Vakif Univ., Istanbul, Turkey

Abstract: P2X7 receptors (P2X7R) are members of the family of cationic-selective ion channels gated by extracellular ATP. They are involved in regulation of receptor trafficking, inflammation and ATP-mediated cell death. There is an ongoing debate in the literature about the expression pattern of P2X7R in the brain. Studies submitted unclear data whether it is expressed in neurons or glial cells. To examine these conflicting results, we designed a series of experiments to

demonstrate the cellular expression pattern of these receptors and their roles and effect mechanisms in the development of neuronal injury after optic nerve transection and focal cerebral ischemia in mice. Here, we analyzed cellular expression profiles of P2X7R in retinal layers and brain cells using double immunohistochemistry. Furthermore, we investigated the role of these receptors in the processes of cellular injury by evaluating DNA fragmentation tests, neuronal survival, anti-apoptotic Bcl-XL, pro-apoptotic Bax, survival- and stress kinases by Western blot analysis. The cellular expression profiles of P2X7 receptors are only observed on neuronal cells in the brain and retinal ganglion cells and bipolar cells in the retina. In contrast to previous studies, double IHC studies with glial and microglial markers revealed that P2X7R expression was only observed in the neuronal cells. In addition, we observed that the activation of P2X7 receptor with varying concentrations of BzATP has no significant effect on neuronal survival. However, modulation of P2X7 receptors by Brilliant Blue G (BBG) significantly improved neuronal survival and infarct volume after ON transection and cerebral ischemia. Number of Fluoro-Gold positive RGCs was significantly higher in BBG treated animals than in vehicle treated animals. Moreover, inhibition of P2X7 receptors decreased infarct volume, brain swelling, blood-brain barrier permeability and neurological scores on animals following a 90 min focal cerebral ischemia and 24 hours reperfusion. In addition, inhibition of P2X7 receptors decreased DNA fragmentation and increased neuronal survival after 30 min of focal cerebral ischemia which was assocciated with increased phosphorylation of survival kinases AKT and ERK-1/2. With this study, we provide evidence that the cellular expression of P2X7 receptors is mainly observed on the neuronal cell and P2X7 receptor modulation can be important in neuronal cell survival. We predict that the clinical implementation of P2X7 receptor antagonists can be beneficial not only in patients with acute ischemic stroke, but also with more delayed degenerative neurological diseases.

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Poster

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Support: AHA Grant-In-Aid 63517

Title: Thrombopoietin reduces injury and cognitive impairment in cerebrovascular disease models

Authors: D. M. ROSENBAUM, J. ZHOU, J. ZHUANG, C. POON, J. LI, *F. C. BARONE
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Abstract: Objectives: Thrombopoietin (TPO) reduces brain injury and sensory-motor deficits following stroke in the rat. TPO brain protection is mediated by vascular protection. TPO reduces stroke-induced inflammatory cytokines, matrix metalloproteinase's and blood brain barrier injury. Here we demonstrate that TPO protects the brain and reduces vascular cognitive impairment in: [1] rat embolic stroke (+/- tissue plasminogen activator; tPA), [2] mouse suture-focal stroke, and [3] mouse chronic carotid stenosis-induced forebrain hypoperfusion. Methods: Rats (Wistar) underwent embolic middle cerebral artery occlusion (MCAO). Vehicle, tPA (10 mg/kg, iv), TPO (0.1 μg/kg, iv) or TPO plus tPA were administered 2 hours post-stroke. Mice (C57Bl/6) underwent suture-MCAO or carotid artery stenosis-induced forebrain hypoperfusion and then received Vehicle or TPO (0.3 or 0.1 μg/kg, iv) at 1 hr or 1 day after surgery. Neurological deficits, complex learning and hemispheric infarct size were measured for 1-21 days post-surgery. Results: In rat embolic stroke, tPA or TPO plus tPA improved stroke-induced neurological deficits significantly. Significant post-stroke-induced deficits in APA cognitive performance were improved 87.2±16.4% by TPO or 69.4±9.7% by TPO plus tPA, but not by tPA alone. In mouse suture-focal stroke, brain infarcts were reduced by 64.5±7.7% and neurological deficits were reduced by 90.3±6.4%. In mouse carotid artery stenosis-induced forebrain hypoperfusion a single administration of TPO 1 day after surgery improved APA performance 84.8+3.1% 3 weeks later (all $p<0.01$). Conclusions: We have demonstrated TPO long-term protection and safety with and without tPA. TPO exhibits protection in mouse suture-focal and in mouse forebrain hypoperfusion-induced complex learning deficits. These data present multiple model and species work that supports the potential "multiple use" of TPO in the future.

Disclosures: D.M. Rosenbaum: None. **J. Zhou:** None. **F.C. Barone:** None. **J. Zhuang:** None. **C. Poon:** None. **J. Li:** None.

Poster

223. Demyelinating Disorders

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 223.01/G2

Topic: C.09. Demyelinating Disorders

Title: The role of low density lipoprotein receptor-related protein 1 (lrp1) in cns myelin development, stability and repair

Authors: *J.-P. LIN[1,2], R. GIGER[3]
[1]Univ. of Michigan, Ann Arbor, ; [2]Cell and developmental biology, [3]Cell and developmental biology, Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: The Role of Low Density Lipoprotein Receptor-related Protein 1 (LRP1) in CNS Myelin Development, Stability and Repair. Jing-Ping Lin[1], Roman J Giger[1,2] University of Michigan, Department of Cell and Developmental Biology[1], Department of Neurolgy[2] Ann Arbor, MI Low density lipoprotein receptor-related protein 1 (LRP1) is a large cell surface receptor abundantly expressed in the developing and adult mammalian CNS, where it is thought to function as an endocytic receptor. LRP1 is required for proper myelination of the peripheral nervous system (PNS).Schwann cell conditional *LRP1* knockout mice present with thinner myelin sheaths during development, and faster axon degeneration after sciatic nerve crush injury. However, it is not clear whether LRP1 is important for proper myelination of the central nervous system (CNS) during development or during white matter repair. *In vitro* studies suggest that LRP1 is required for oligodendrocyte progenitor cell (OPC) differentiation, which is a critical step during myelin formation. LRP1 binds and internalizes myelin debris by direct binding to myelin components such as myelin basic protein (MBP) and myelin-associated glycoprotein (MAG). Moreover, LPR1 regulates inflammation and subsequent endocytosis of dead cells after injury, which is an important step for generating an environment permissive to myelin repair. These observations suggest that LRP1 functions in OPCs and/or oligodendrocytes (OLs) and potentially other cell types to regulate CNS myelination and/or remyelination. Germline deletion of *LRP1* in mice is embryonic lethal, therefore we generated *LRP1* OL lineage conditional knockout mice (*LRP1$^{flox/flox}$; Olig2-Cre*) to address its role in CNS myelination. Our preliminary data show that knocking out *LRP1* in oligodendrocyte linage cells results in reduced expression of proteolipid protein (PLP), a myelin-associated protein and a positive regulator of axonal integrity. To study the function of LRP1 in adulthood, we generated inducible *LRP1* knockout mice (*LRP1$^{flox/flox}$; CMV-ER Cre*). Currently, studies are investigating if *LRP1* is required for myelin repair. Injection of lysophosphatidylcholine (LPC) into the corpus callosum will be used to compare the extent of remyelination of the white matter lesions between control and *LRP1* mutant animals. A more detailed understanding of the role of LRP1 in the CNS may provide new insights into the molecular mechanisms of remyelination, and may further our understanding of the pathophysiology of demyelinating disorders.

Disclosures: J. Lin: None. **R. Giger:** None.

Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: NMSS RG 4224

Title: Leukemia-lymphoma related factor (LRF) is a transcriptional repressor of Hes5 and MBP that exhibits stage-specific expression during remyelination

Authors: *N. DAVIDSON[1], N. KIJPAISALRATANA[2], T. Q. LE[1], R. C. ARMSTRONG[1]
[1]USUHS, Bethesda, MD; [2]Chulalongkorn Univ., Bangkok, Thailand

Abstract: Leukemia/lymphoma-related factor (LRF) is a zinc-finger transcription factor that regulates differentiation, proliferation, and maintenance of multiple cell types. This study is the first to demonstrate the potential regulatory effect of LRF on oligodendrocyte progenitor (OP) cell differentiation in the adult mouse, and to provide evidence for the different mechanistic pathways affected by LRF in OP cells and the mature oligodendrocyte. Adult male C57BL/6 mice were treated with murine hepatitis virus strain A59 (MHV-A59) by intracranial injection to induce a demyelinating injury and then compared to vehicle-injected controls. We show that LRF is expressed in a small subset of OP cells and in nearly all mature oligodendrocytes. This expression pattern is tightly correlated with cell stage and remains consistent between MHV-A59 and vehicle treated mice despite significant changes in total OP and oligodendrocyte populations throughout the progression of demyelination and remyelination. LRF may therefore play a stage-dependent role with critical activity just prior to OP differentiation and with continuing activity in the mature oligodendrocyte. With this in mind, we examined the role of LRF in transcriptional regulation of the Notch effector gene hairy and enhancer of split 5 (Hes5), an OP differentiation inhibitor. In P2 rat brain cultures enriched in OP cells, LRF transfection significantly reduced luciferase activity driven by the Hes5 promoter. This supports our theory that LRF overcomes inhibition of OP differentiation by opposing Hes5 in a derepression model. When examining the effect of LRF expression on myelin-specific proteins, we find that LRF reduces the activity of promoters for both 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) and myelin basic protein (MBP). Using a truncated MBP-promoter, we show that LRF exerts its regulatory effect on MBP primarily over a 135 base pair region. We further demonstrate that LRF-specific inhibition of the MBP promoter is lost with a 3 base pair mutation of the truncated MBP promoter at a well-

defined site necessary for specificity protein 1 (Sp1) binding and activity. Consequently, LRF may be necessary to maintain homeostasis in the mature oligodendrocyte by regulating the production of myelin-specific proteins. Taken together, this data supports a clear mechanistic pathway whereby LRF enhances OP differentiation by opposing the Notch effector Hes5, and then modulates myelin protein transcription in the mature oligodendrocyte; in at least the case of MBP, this LRF effect requires the presence of a specific 3 base pair subset of an established Sp1 binding site.

Disclosures: N. Davidson: None. **N. Kijpaisalratana:** None. **T.Q. Le:** None. **R.C. Armstrong:** None.

Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: Training Program in Organogenesis T32HD007505

NIH R01-NS081281-01

Title: Cell-autonomous and non-cell-autonomous functions of lipid phosphatase FIG4/SAC3 in oligodendrocyte development, axon myelination, and white matter stability

Authors: *Y. MIRONOVA[1], G. M. LENK[2], J.-P. LIN[1], T. L. DICKENDESHER[3], M. H. MEISLER[2], P. SHRAGER[4], R. J. GIGER[1]
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Abstract: Mutations of human *FIG4(SAC3)* are responsible for Charcot-Marie-Tooth 4J disease, a severe form of peripheral neuropathy (Chow et al, Nature 2007), Yunis-Varon Syndrome (Campeau et al, AJHG 2013), and polymicrogyria with epilepsy (Baulac et al, Neurology 2014). *FIG4* encodes an evolutionarily conserved lipid phosphatase that regulates intracellular vesicle trafficking in the endo-lysosomal compartment. Mice null for *Fig4* have severe myelination defects in the PNS and CNS. We are studying the cell-autonomy of FIG4 function in CNS myelination using mouse genetics. Remarkably, on a *Fig4* null background, neuron-specific

overexpression of transgenic *Fig4* is sufficient to rescue myelination defects in the optic nerve and corpus callosum, reduce tremor, and prevent premature lethality (Winters et al., J. Neurosci. 2011; Ferguson et al., HMG 2012). To examine *Fig4* loss-of-function in specific neural cell types, we generated *Fig4* conditional knockout ($Fig4^{flox/flox}$) mice (Ferguson et al., HMG 2012). Neuron-specific ablation of *Fig4* in $Fig4^{-/flox}$*,SynCre* mice results in severe hypomyelination of the optic nerve, demonstrating that neuronal *Fig4* is necessary for proper CNS myelination. Biochemical studies of $Fig4^{-/flox}$*,SynCre* brain tissue revealed reduced abundance of MBP (myelin basic protein) and MAG (myelin-associated glycoprotein). Recordings of compound action potentials on acutely isolated optic nerves revealed a delay in nerve conduction. Interestingly, selective loss of CNS *Fig4* in the oligodendrocyte (OL) lineage ($Fig4^{-/flox}$*, Olig2Cre*) also results in CNS hypomyelination. The data indicate that correct CNS myelination requires expression of *Fig4* in both neurons and OLs. To test whether FIG4 is required for myelin stability and repair, we generated mice that permit inducible gene ablation ($Fig4^{-/flox}$*,CMV-ER-Cre*). Preliminary data indicate that juvenile and adult inducible *Fig4* ablation does not result in reduced levels of myelin-associated markers, suggesting that Fig4 may be dispensable for CNS myelin maintenance and stability. Intriguingly, electron microscopy imaging of sciatic nerve in $Fig4^{-/flox}$*,CMV-ER-Cre* reveals axonal and myelin pathology, indicating that induction of *Fig4* deletion is deleterious in the adult PNS. Ongoing experiments include assessing whether inducible *Fig4* deletion thwarts axonal remyelination in the lysolecithin (LPC) white matter lesion model.

Disclosures: Y. Mironova: None. **G.M. Lenk:** None. **J. Lin:** None. **T.L. Dickendesher:** None. **M.H. Meisler:** None. **P. Shrager:** None. **R.J. Giger:** None.

Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: Consejo de Investigaciones Científicas y Técnicas (CONICET)

Agencia Nacional de Promoción Científica y Tecnológica (BID 1201/OCAR, PICT 2011-0799)

Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (SeCyT-UNC)

Title: Alterations in presynaptic release machinery and synaptic vesicle mobility in the frontal cortex of rats with experimental autoimmune encephalomyelitis

Authors: *N. L. CHANADAY[1], A. A. VILCAES[1], A. L. DE PAUL[2], A. I. TORRES[2], A. L. DEGANO[1], G. A. ROTH[1]
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Abstract: Experimental autoimmune encephalomyelitis (EAE) is an animal model that mimics many of the clinical and pathological features of multiple sclerosis (MS). Both are inflammatory demyelinating and neurodegenerative pathologies of the central nervous system associated with motor, sensory, and cognitive deficits. In MS gray matter atrophy is related to the emergence of cognitive deficits and contributes to clinical progression. However the molecular bases of these changes are still unknown. Taking advantage of EAE similitudes, we herein analyze functional and morphological changes in isolated cortical presynaptic terminals (synaptosomes) from an acute rat model. We show that cortical functional changes during EAE are concentrated at the frontal region and appear concomitantly with the onset of EAE clinical manifestations, disappearing when animals begin to recover. In this region, nerve terminals exhibit reduced Ca2+-dependent glutamate release, with no changes in total synaptosomal glutamate content. We propose that this is due to a dysfunction in the presynaptic release machinery, mainly the synapsin I (SynI) pathway. In this regard, we found reduced basal levels of CaMKIIα associated to synaptic vesicles (SVs) and impaired SynI phosphorylation at site 3 (CaMKIIα substrate) after depolarization. In consequence, SynI fails to detach from SVs. Moreover, our study shows an increase in total and phosphorylated Erk1/2 accompanied by higher basal and stimulated levels of SynI phosphorylated at sites 4/5 (Erk1/2 substrates). This might imply that resting SVs-actin network is more disorganized. Overall, this changes lead to reduced SVs mobility as evidenced by electron microscopy, with no apparent morphological or SVs content differences. These are the first evidences unraveling the molecular pathways involved in neuronal dysfunction in the frontal cortex during EAE. The same mechanisms might be responsible for functional abnormalities in MS and could contribute to the development and progression of cognitive impairments and fatigue.

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Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: TEVA Pharmaceuticals

Title: Glatiramer acetate confers neuroprotection in experimental autoimmune encephalomyelitis through activation of SIRT1

Authors: *V. K. NIMMAGADDA[1], K. LAM[1], C. T. BEVER, Jr[1,2], D. TRISLER[1,2], S. I. V. JUDGE[1,2], T. K. MAKAR[1,2]
[1]Neurol., Univ. of Maryland Baltimore, Baltimore, MD; [2]MS Ctr. of Excellence-EAST, VA Maryland Hlth. Care Syst., Baltimore, MD

Abstract: Background: Recent evidence suggests a potential neuroprotective role for sirtuin1 (SIRT1), a NAD+-dependent deacetylase, in multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). Also, SIRT1 was reported to increase mitochondrial biogenesis and rescue mitochondrial density and fission-fusion balance in neurodegeneration models. Our recent studies indicate a relationship between the SIRT1 pathway and the brain derived neurotrophic factor (BDNF)-TrkB signaling pathway in EAE mice. Glatiramer acetate (GA) is one of the disease modifying therapies (DMTs) approved for MS. Previous studies showed that GA treatment increased BDNF secretion in both MS and EAE. Objective: Determine whether neuroprotective effect of GA is mediated via SIRT1 pathway in EAE. Methods: EAE was induced in C57Bl/6 female mice by immunization with myelin oligodendroglial glycoprotein peptide 35-55. GA (150 μg/mouse/day) was administered intraperitoneally starting at disease onset. Mice were euthanized on day 20 of GA treatment and lumbar spinal cords were examined histologically. Results: GA treated mice have less inflammation and demyelination as compared to untreated mice. Increased secretion of BDNF was observed in the spinal cords of GA treated mice. GA treatment increased the expression of SIRT1, SIRT3 and Nampt (a rate limiting enzyme of NAD synthesis) in the CNS of these mice. We also found an increased expression of Mito fusion-2 and OPA-1(regulators of fusion) and decreased expression of mitochondrial Fis1 and DNM1-L (regulators of mitochondrial fission) GA treated EAE mice. These mice also showed increased levels of peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1α) and nuclear factor (erythroid-derived 2)-like 2 (NRF-2), indicators of mitochondrial biogenesis. Conclusions: Taken together, these findings indicate that GA confers neuroprotection in EAE by activating the SIRT1 pathway and thereby controlling mitochondrial biogenesis and dynamics. Studies are ongoing to further evaluate if GA acts directly on SIRT1 targets or indirectly through the BDNF-TrkB pathway. However, both the TrkB and SIRT1 pathways may contribute to novel neuroprotective targets in EAE and MS.

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Poster

223. Demyelinating Disorders

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Title: Lineage tracing reveals dynamic changes in PDGF alpha receptor-derived cells following cuprizone-induced demyelination

Authors: ***J. DEBRUIN**[1], E. BAXI[2], D. E. BERGLES[2], P. A. CALABRESI[2]
[2]Neurol., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Oligodendrogenesis is essential for successful remyelination and repair in demyelinating diseases such as multiple sclerosis (MS). Understanding the time course of the oligodendrocyte progenitor cell (OPC) response to demyelination in the cuprizone model may help determine the kinetics of the remyelinating response and could allow further modeling of why the process does not efficiently occur in MS patients. The aim of this study was to observe the accumulation and maturation of OPCs during cuprizone induced demyelination and subsequent remyelination after return to normal diet. Fate mapping of OPCs was performed using PDGFαR-CreER;Rosa26-eYFP mice that conditionally express YFP in OPCs after induction of recombination with 4-hydroxytamoxifen (4-HT). Mice were fed a diet of 0.2% cuprizone, a demyelinating neurotoxin, or regular chow for 6 weeks. Both groups then received regular chow for an additional 4 weeks. Recombination was induced with 4-HT at week 2. Mice were sacrificed weekly, beginning 2 weeks after recombination. The fate of the OPCs was determined imunohistochemically via colocalization of eYFP with multiple cellular makers including PDGFαR (OPCs), CC1 (oligodendrocytes), GFAP (astrocytes), and NeuN (neurons). At 4 weeks the density of YFP+ PDGFαR+ cells (OPCs) was 3 fold higher in the corpus

callosum of cuprizone treated animals as opposed to controls, while the density of YFP+CC1+ cells (mature oligodendrocytes) was low in both groups. This was followed by a 6 fold increase in the density of YFP+CC1+ cells at the 6 week time point and a return of YFP+ PDGFαR+ cell density to control levels. Interestingly, the kinetics of OPC differentiation and maturation in gray matter areas appeared to be quite different with much less expression of recombined cells at these time points. Additionally, we observed no deviation of OPCs into astrocytes (YFP+GFAP+) or neurons (YFP+NeuN+) in any brain regions. Analysis at other time points is ongoing. These results, in combination with knowledge of key demyelination/remyelination time points in white matter vs gray matter may provide unique insight into the capacity and mechanisms of remyelination in different parts of the brain. The weekly time course of these events allows insight into OPC proliferation, accumulation, maturation, and remyelination, and could provide insight into how this may occur in MS.

Disclosures: **J. Debruin:** A. Employment/Salary (full or part-time):; Johns Hopkins University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH and Silverman foundation. **E. Baxi:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **D.E. Bergles:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **P.A. Calabresi:** A. Employment/Salary (full or part-time):; Johns Hopkins University.

Poster

223. Demyelinating Disorders

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Title: Prostaglandin $F_{2\alpha}$ FP receptor inhibitor suppress demyelination and motor dysfunction in a cuprizone-induced multiple sclerosis mouse model

Authors: *K. YOSHIKAWA, K. IWASA, S. YAMAMOTO, K. MARUYAMA
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Abstract: Previously, we have demonstrated that prostamide/PGF synthase, catalyzes the reduction of prostaglandin (PG) H_2 to $PGF_{2\alpha}$, is constitutively expressed in myelin sheaths and cultured oligodendrocytes, suggesting that $PGF_{2\alpha}$ has functional significance in myelin-forming oligodendrocytes. To investigate the effects of $PGF_{2\alpha}$/FP receptor signaling on demyelination, we administrated FP receptor agonist and antagonist to cuprizone-exposed mice, a model of multiple sclerosis. Mice were fed a diet containing 0.2% cuprizone for 5 weeks, which induces severe demyelination, glial activation, proinflammatory cytokine expression, and motor dysfunction. Administration of the FP receptor antagonist AL-8810 attenuated cuprizone-induced demyelination, glial activation, and TNFα expression in the corpus callosum, and also improved the motor function. These data suggest that during cuprizone-induced demyelination, $PGF_{2\alpha}$/FP receptor signaling contributes to glial activation, neuroinflammation, and demyelination, resulting in motor dysfunction. Thus, FP receptor inhibition is a potential therapy for reducing the extent of demyelination and the severity of motor dysfunction in multiple sclerosis.

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Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: Else Kröner-Fresenius Foundation (EKFS), Research Training Group Translational Research Innovation – Pharma (TRIP)

Title: Lack of CerS2 suppresses the development of experimental autoimmune encephalomyelitis

Authors: J. MÄNNICH[1], K. SCHMITZ[1], Y. PEWEZNER-JUNG[2], A. FUTERMAN[2], *A. SCHMIDTKO[3], G. GEISSLINGER[1], S. GRÖSCH[1], S. SCHIFFMANN[1]
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Abstract: Ceramide synthases (CerS) synthesize chain length specific ceramides (Cer) which are known to mediate cellular processes in a chain length dependent manner. In an animal model of multiple sclerosis (MS), the experimental autoimmune encephalomyelitis (EAE) model, we observed a significant elevation of CerS2 in neutrophils of EAE mice at disease onset. Mice with a total lack of CerS2 or a specific lack in blood cells develop clinical symptoms later than wild type mice. This is accompanied by a reduced amount of immune cells in the lumbar spinal cord of CerS2KO EAE mice as compared to CerS2WT EAE mice. Chemokine receptors (CCR) expressed on neutrophils promote the migration of neutrophils into the CNS which is a prerequisite for the recruitment of further immune cells and the inflammatory process leading to the development of MS. Interestingly, white blood cells isolated from CerS2KO EAE mice show lower CCR1, CXCR1 and CXCR2 mRNA expression levels as compared to CerS2WT EAE mice. Most importantly, the lack of CerS2 reduces the migratory potential of neutrophils isolated from EAE mice. In conclusion, our data indicate strongly that CerS2 is involved in the signaling process of the synthesis of chemokine receptors and promote thereby the migration of neutrophils and possibly contributes by these pro-inflammatory effects to the development of EAE and MS.

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Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Title: Semaphorin4A causes an apoptotic response in oligodendrocytes

Authors: *D. F. LEITNER[1], B. TODORICH[3], X. ZHANG[2], J. R. CONNOR[1]
[1]Neurosurg., [2]Penn State Univ. Col. of Med., Hershey, PA; [3]Duke Univ. Eye Ctr., Durham, NC

Abstract: We have previously established that there is an H-ferritin (Hft) receptor on oligodendrocytes. This receptor was subsequently identified as Tim2 (T cell immunoglobulin and mucin domain containing 2). In addition to Hft, Tim2 is known to bind Semaphorin 4A (Sema4A), a class IV semaphorin expressed by macrophages and lymphocytes. We report herein that recombinant Sema4A binds to rat primary oligodendrocyte progenitor cells (OPCs) but not astrocytes and induces process collapse and apoptosis in OPCs. Sema4A also induces lactate

dehydrogenase (LDH) release in the human oligodendrocyte cell line MO3.13. Because oligodendrocytes are enriched for iron *in vivo*, we determined the influence of cellular iron status on the Sema4A effect. In rat primary oligodendrocytes and the MO3.13 cell line, the Sema4A effect on LDH release was increased following iron chelation in mature cells but iron loading did not alter the Sema4A effect. This finding indicates that both rat and human oligodendrocytes are vulnerable to Sema4A induced apoptosis and the vulnerability is influenced by iron status. Because peripheral macrophages express Sema4A, a potential source of Sema4A in the brain may come from microglia. In a cell culture model we found that rat primary microglia express Sema4A, and the level of expression was increased after activation with lipopolysaccharide (LPS). Because microglia accumulate iron when activated, we determined whether iron status influenced Sema4A expression by microglia. We observed that *in vitro* iron chelation decreased expression of Sema4A when microglia were activated with LPS. Iron loading in the presence of LPS increased Sema4A expression. These data suggest that Sema4A could be a link between the immune system and oligodendrocytes and that the response of these cells is iron sensitive. To determine the clinical relevance of our finding, we examined cerebrospinal fluid (CSF) and tissue samples from patients who had multiple sclerosis (MS). Sema4A depleted CSF from a primary-progressive MS patient improved the viability of OPCs, but Sema4A depleted CSF from a relapsing-remitting patient did not alter OPC viability when compared to control. We also found that 10% of MS patients had elevated Sema4A in chronic active lesions. This study provides a novel link between the immune system and oligodendrocytes and identifies the role of Sema4A in destruction of oligodendrocytes.

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Poster

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The Miami Project To Cure Paralysis, University of Miami

Title: Oligodendroglial TNFR2 mediates the protective effects of transmembrane tumor necrosis factor (tmTNF) by promoting remyelination in experimental autoimmune encephalomyelitis

Authors: *P. MADSEN[1,2], S. K. PATEL[1], H. GAO[1], D. E. SZYMKOWSKI[3], J. R. BETHEA[4], R. BRAMBILLA[1]

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Abstract: Tumor necrosis factor (TNF) has been associated with the pathophysiology of multiple sclerosis (MS), as MS patients have elevated concentrations of TNF in cerebrospinal fluid and active lesions. TNF exists in two forms, transmembrane (tmTNF) and soluble (solTNF), whose functions are mediated by TNFR1 and TNFR2. Due to their different binding affinities, solTNF primarily signals through TNFR1 and tmTNF through TNFR2. The cellular processes activated by the two receptors are often opposite: TNFR1 mediates apoptosis and inflammation whereas TNFR2 is associated with cell survival, immunity and myelination. Numerous studies have linked MS to the detrimental effects of solTNF and TNFR1. In our own work we demonstrated not only that solTNF is detrimental, but that tmTNF is protective and important for repair and remyelination. We showed that mice treated with XPro1595, a selective solTNF inhibitor, have improved clinical outcome, preserved axon integrity and increased remyelination following MOG-induced EAE. Since TNFR2 is expressed throughout the oligodendrocyte lineage, we hypothesized that activation of TNFR2-dependent cascades in oligodendrocytes is associated with the protective functions of tmTNF in EAE. To test this hypothesis we used a genetic/pharmacological approach with oligodendrocyte-specific TNFR2 conditional knockout (CNPcreTNFR2fl/fl mice) in combination with XPro1595 treatment. Naïve CNPcreTNFR2fl/fl mice do not display any abnormal locomotor phenotype or differences in oligodendrocyte precursor and mature oligodendrocyte numbers compared to control mice. This suggests that TNFR2 does not play a major role in oligodendrocyte function during development. However, following EAE CNPcreTNFR2fl/fl mice show a significantly worse clinical outcome compared to TNFR2fl/fl littermates, and this behavior is not improved by XPro1595 treatment, which suppresses EAE in TNFR2fl/fl mice. Finally, we find that CNPcreTNFR2fl/fl mice have decreased axon and myelin preservation, as well as remyelination compared to TNFR2fl/fl mice. Collectively, our data demonstrate that TNFR2 activation in oligodendrocytes is key to the protective effect of tmTNF in EAE. A better understanding of how oligodendroglial TNFR2 signaling regulates remyelination may lead to new therapeutic strategies for MS, especially chronic progressive MS. Indeed, this form of MS is associated with failure of remyelination and irreversible axonal damage, and no therapies are currently available. This work is supported by: FISM (Italian Multiple Sclerosis Foundation) grant 2012/R/2 (RB); NINDS grant NS084303-01A1 (RB); The Miami Project To Cure Paralysis (RB, JRB).

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Poster

223. Demyelinating Disorders

Location: Halls A-C

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Topic: C.09. Demyelinating Disorders

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The National Multiple Sclerosis Society

The Civitan International Research Foundation

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Title: System xc- transporter modulates CNS infiltration of immune cells in autoimmune inflammatory disease

Authors: *K. S. EVONUK[1], B. J. BAKER[1], R. E. DOYLE[1], C. M. SESTERO[1], B. P. JOHNSTON[1], P. DE SARNO[1], A. TANG[1], I. GEMBITSKY[1], S. J. HEWETT[2], H. SONTHEIMER[1], C. RAMAN[1], T. M. DESILVA[1]
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Abstract: T cell infiltration into the central nervous system (CNS) is a significant underlying pathogenesis in autoimmune inflammatory demyelinating diseases. Mechanisms that regulate immune cell infiltration into the CNS are not well understood. The neurotransmitter glutamate has been implicated in modulating blood-brain barrier permeability and may contribute to the vulnerability of the CNS to immune cell infiltration during pathological conditions. The system xc- transporter is a source of glutamate release during oxidative stress and is upregulated in leukocytes from human blood samples in patients with multiples sclerosis as compared to control samples. Therefore, we tested the role of system xc- in regulating immune cell infiltration in experimental autoimmune encephalomyelitis (EAE) using pharmacological and genetic approaches. Pharmacological blockade of system xc- in the C57Bl/6 animal model of EAE attenuated T cell, macrophage, and neutrophil infiltration into the CNS. These data were consistent with reduction in both clinical disease and myelin loss. Precursor frequency of peripheral MOGp-reactive T cells (CFSE assay) and their ability to enter into cell cycle was not affected by inhibitors of system xc-. These data suggest that low numbers of infiltrating

inflammatory cells in the CNS of mice treated with inhibitors of system xc- was not due to compromised activation of MOGp T cells in the periphery. Furthermore, mice harboring an Slc7a11 (xCT) mutation that inactivated system xc- were resistant to EAE corroborating a central role for system xc- in the pathogenesis of EAE. Taken together our data support a novel role for the system xc- transporter in mediating T cell infiltration into the CNS in autoimmune inflammatory diseases.

Disclosures: **K.S. Evonuk:** None. **B.J. Baker:** None. **R.E. Doyle:** None. **C.M. Sestero:** None. **B.P. Johnston:** None. **P. De Sarno:** None. **A. Tang:** None. **I. Gembitsky:** None. **S.J. Hewett:** None. **H. Sontheimer:** None. **C. Raman:** None. **T.M. DeSilva:** None.

Poster

223. Demyelinating Disorders

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Program#/Poster#: 223.12/H1

Topic: C.09. Demyelinating Disorders

Support: KAKEN 25430056

Title: *In vitro* models for the study of amiodarone-induced peripheral neuropathy

Authors: ***N. NIIMI**[1], M. TSUKAMOTO[2], H. YANAGISAWA[1], K. WATABE[1], K. SANGO[1]
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Abstract: Amiodarone (AMD), a class III anti-arrhythmic agent prescribed for patients with atrial fibrillation and ventricular arrhythmias, has a number of adverse effects, including peripheral neuropathy. AMD and its metabolites (desethylamiodarone and bis-desethylamiodarone) are likely to inhibit lysosomal phospholipases and induce accumulation of phospholipids and other substances in the lysosomes of Schwann cells, thereby being a cause of Schwannopathy or myelinopathy. However, the molecular mechanisms of AMD toxicity in Schwann cells remain unknown. We have established a spontaneously immortalized adult rat Schwann cell line IFRS1, which displays distinct Schwann cell phenotypes and fundamental ability to myelinate neurites in coculture with dorsal root ganglion neurons (Sango et al., 2011) and PC12 cells (Sango et al., 2012). In this study, we investigated the effects of AMD on the viability and biochemical properties of IFRS1 cells and the myelination in PC12-IFRS1 coculture system. IFRS1 cells were maintained in Dulbecco's modified Eagle's medium

(DMEM) with 1% fetal calf serum and N2 supplement. Treatment of IFRS1 cells with AMD (1, 5, and 10 μM) for 24 h dose-dependently reduced the cell viability, increased the intracellular phospholipids, and upregulated the expression of a lysosomal marker LIMPII, an oxidative stress marker 4-hydroxy-2-nonenal, autophagy markers LC3-II and p62, and sterol regulatory element-binding protein (SREBP)-1. The upregulation of p62 suggests impaired autophagy, whereas SREBP-1 plays a role in lipid metabolism and its upregulation may be correlated with the AMD-induced phospholipid accumulation. PC12-IFRS1 cocultures were incubated in myelination medium (DMEM with B27 supplement, 50 μg/ml ascorbic acid, 10 ng/ml nerve growth factor and 25 ng/ml neuregulin-1 type III) for 21 days. Treatment of the cocultures with 20 μM of AMD for 48 h induced detachment of IFRS1 cells from the neurite networks as seen under a phase-contrast microscope and downregulated the expression of myelin protein P0 and neuregulin-1 receptor ErbB3 as revealed by western blot analysis.

Disclosures: N. Niimi: None. **M. Tsukamoto:** None. **H. Yanagisawa:** None. **K. Watabe:** None. **K. Sango:** None.

Poster

223. Demyelinating Disorders

Location: Halls A-C

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Program#/Poster#: 223.13/H2

Topic: C.09. Demyelinating Disorders

Title: Role of N-Acylethanolamine Acid Amidase (NAAA) in the experimental autoimmune encephalomyelitis model of multiple sclerosis

Authors: *S. PONTIS, V. CAPURRO, A. GUIJARRO, M. SUMMA, D. PIOMELLI, A. REGGIANI
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Abstract: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) characterized by autoimmune response and aberrant inflammatory response involving T cells, macrophages and microglia which leads to the gradual loss of myelin sheath that protects nerve fibers. Fatty acid ethanolamides (FAEs) are endogenous bioactive lipids involved in the modulation of inflammatory and immune response. Previous studies demonstrated that palmitoylethanolamide (PEA) exerts anti-inflammatory, analgesic and neuroprotective effects acting mainly through PPAR-α receptors in several models of inflammation. The administration of PPAR-α agonists gemfibrozil and fenofibrate inhibits

clinical signs of experimental autoimmune encephalomyelitis (EAE), an experimental model of MS. Moreover clinical studies showed that both plasma and cerebrospinal fluid levels of NAEs are altered in MS patients. N-Acylethanolamine Acid Amidase (NAAA) has been identified as the enzyme responsible for the degradation of palmitoylethanolamide (PEA). We have previously demonstrated that NAAA inhibition normalizes PEA levels in several inflammatory models. In order to investigate the role of NAAA in MS we produced EAE in mice and examined the expression of NAAA and inflammatory markers. EAE was induced in mice by immunization with myelin oligodendrocyte (MOG) 35-55 peptide, 200 μg/mouse and 200 ng pertussis toxin (PTX) in 200μL PBS after MOG injection and 48 h later. Control-immunized mice in addition to unimmunized mice served as negative controls. Mice were weighed and scored daily for clinical signs, killed at different time points and spinal cord tissue was processed for qPCR and immunohistochemistry. Gene expression of NAAA and markers of inflammation (iNOS , COX2, TNF-alpha) was evaluated. Immunofluorescence studies were conducted to determine the identity of NAAA-expressing cells. Analysis of qPCR data showed that, at day 7 and 10 post immunization, NAAA and iNOS mRNA levels were significantly up-regulated in mice showing symptoms at the moment of sacrifice compared to non-symptomatic mice ($p<0.0001$, Student's t test). Analysis of fluorescence in spinal cord sections showed significant increase of NAAA immunofluorescence in EAE mice vs control immunized mice (integrated density average in EAE mice 136 ±16 vs control immunized 0.29 ± 0.11; $p<0,001$ Student's t test). Overall, these preliminary results suggest that NAAA is altered during the development of MS. Further studies are needed to clarify whether their modulation could be beneficial for treatment.

Disclosures: S. Pontis: None. **V. Capurro:** None. **A. Guijarro:** None. **M. Summa:** None. **D. Piomelli:** None. **A. Reggiani:** None.

Poster

223. Demyelinating Disorders

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Program#/Poster#: 223.14/H3

Topic: C.09. Demyelinating Disorders

Title: Human remyelination promoting IgM antibody rescues myelin-mediated inhibition of oligodendrocyte precursor cell process outgrowth

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Abstract: rHIgM22 is an experimental recombinant antibody in clinical studies in patients with multiple sclerosis (MS). In MS, demyelinated central nervous system (CNS) lesions fail to sufficiently remyelinate despite the presence of oligodendrocyte precursor cells (OPCs) capable of differentiating into mature oligodendrocytes and producing new myelin. rHIgM22 is an IgM isotype antibody previously shown to promote remyelination in Theiler's murine encephalomyelitis virus-mediated model of demyelination. While rHIgM22 improves remyelination in animal models, its mechanism of action has not been fully defined. In mixed glial cultures, rHIgM22 improves OPC survival and increases OPC proliferation via PDGF-mediated paracrine signaling. While purified OPCs can bind rHIgM22, rHIgM22 does not show direct proliferative effects in isolated OPC cultures. MS lesions contain damaged myelin debris, which has been suggested to inhibit OPC process outgrowth. In this study, we examined if myelin inhibits OPC process outgrowth, and if this effect can be directly rescued by treatment with rHIgM22 by interfering with external inhibitory signals from the damaged myelin. OPCs were isolated by shaking postnatal (P1) rat mixed glial cultures, and expanded in proliferation media with growth factors. To determine if OPCs could directly bind rHIgM22, OPCs were differentiated using thyroid hormone (T3) and stained with rHIgM22 antibody along with markers of OPC differentiation. We found that rHIgM22 binds to OPCs over multiple stages of differentiation indicated by co-labeling of cells with rHIgM22 and phenotypic markers A2B5, O4 or MBP. For OPC process outgrowth assays, OPCs were plated on poly-d-lysine or adult rat myelin fraction coated slides, and treated with rHIgM22 in the presence of T3 for 48 hours. OPCs were imaged live using Calcein AM, and process outgrowth was analyzed using ImageJ software. We found that when plated on permissive poly-d-lysine substrate, OPCs adhere to the surface and put out multiple thin processes. However, when plated on myelin-coated substrate, OPCs form loosely adhering cell clusters with very few processes, suggesting that myelin inhibits OPC process outgrowth. Addition of rhIgM22 partially rescues this morphological effect, and allows OPCs to put out more processes on myelin substrate, resembling the more complex morphology observed on poly-d-lysine. Therefore, in addition to paracrine trophic support of OPC population, rhIgM22 may play a direct role in overcoming myelin-mediated inhibition of OPC process outgrowth, thereby providing an additional mechanism for promoting remyelination.

Disclosures: Y. Zorina: A. Employment/Salary (full or part-time):; Acorda Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics. **P. Sarmiere:** A. Employment/Salary (full or part-time):; Acorda Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics. **A.O. Caggiano:** A. Employment/Salary (full or part-time):; Acorda Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual

property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics. **D. Button:** A. Employment/Salary (full or part-time):; Acorda Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics.

Poster

223. Demyelinating Disorders

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FISM (Italian Multiple Sclerosis Foundation) grant 2012/R/2

The Miami Project To Cure Paralysis

Title: Role of Tumor Necrosis Factor Receptor 2 signaling in microglia and macrophages in experimental autoimmune encephalomyelitis

Authors: *H. GAO[1], P. M. MADSEN[1,2], R. BRAMBILLA[1], R. BRAMBILLA[1]
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Abstract: Tumor necrosis factor (TNF) is a pleiotropic cytokine involved in numerous physiological and pathological processes. It exists in two forms, transmembrane (tmTNF) and soluble (solTNF). The biological functions of TNF are mediated by TNFR1 and TNFR2. Due to their different binding affinities, solTNF signals preferentially via TNFR1, which is involved in activation of pro-inflammatory pathways and apoptosis, and tmTNF via TNFR2, which is associated with immunity, anti-inflammatory and pro-myelinating effects. To date, most studies have focused on TNFR1, and the role of TNFR2 in health and disease is poorly understood. TNF is involved in the pathophysiology of multiple sclerosis (MS). Interestingly, a clinical trial with a non-selective TNF inhibitor blocking both solTNF and tmTNF resulted in MS exacerbation, while selective blockade of solTNF resulted in ameliorated experimental autoimmune encephalomyelitis (EAE). Furthermore, TNFR2 knockout mice showed worse EAE outcomes than WT mice. These data suggest that tmTNF and TNFR2 are likely to mediate the beneficial functions of TNF in EAE/MS and modulation of TNFR2 could become a promising therapeutic

target in MS. Since TNFR2 is the only functional TNF receptor in microglia and is highly expressed in macrophages, we sought to elucidate the functions of TNFR2 in microglia and macrophages *in vivo* by conditionally ablating TNFR2 in both populations. To do so, we generated novel LysMcreTNFR2fl/fl mice. LysMcreTNFR2fl/fl mice do not display obvious phenotypical abnormalities. Under naïve conditions, immune cell profiles in the spleen, numbers of microglial cells, activity levels and locomotor behavior are comparable to TNFR2fl/fl littermates, suggesting that TNFR2 does not play a major role in microglia and macrophage function during development. However, following induction of EAE, LysMcreTNFR2fl/fl mice develop significantly delayed and suppressed EAE, contrary to expectations. This result may have multiple implications: 1) TNFR2, in either or both cell types, signals to the production of detrimental factors that sustain the EAE pathology; 2) TNFR2 is key to the immunological functions of macrophages, and in its absence the induction of EAE is prevented. Our data underscore that TNFR2 functions are cell type-dependent, likely not only associated with beneficial effects after EAE, and cell-specific conditional knockout models represent an invaluable tool to better understand TNFR2 signaling and function in health and disease. This work is supported by: NINDS grant NS084303-01A1 (RB); FISM (Italian Multiple Sclerosis Foundation) grant 2012/R/2 (RB); The Miami Project To Cure Paralysis (RB).

Disclosures: H. Gao: None. **P.M. Madsen:** None. **R. Brambilla:** None. **R. Brambilla:** None.

Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: Department of Defense, Congressionally Directed Medical Research Programs
MS120120

Title: Potential neuroprotective effect of PAC1 signaling in experimental autoimmune encephalomyelitis

Authors: *V. MAKHIJANI[1], M. C. CONDRO[1], Y. V. TAN[3], S. K. TIWARI-WOODRUFF[2], J. WASCHEK[1]
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Abstract: Pituitary adenylyl cyclase activating peptide (PACAP) is a neuropeptide that exerts strong anti-inflammatory actions *in vitro* and *in vivo*, through two receptor subtypes, VPAC1 and VPAC2, that also bind the related peptide vasoactive intestinal peptide (VIP), and one PACAP-specific receptor, PAC1, thought to mediate much of its neuroprotective actions. In the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, PACAP knockout mice exhibit more severe disease symptoms than wild type controls and VIP knockout mice, suggesting that PAC1 signaling might play a role in modulating this disease. PAC1 knockout mice were thus immunized with MOG35-55 and monitored for disease symptoms. PAC1 knockout mice exhibit more severe disease than wild type controls, similar to the PACAP knockout mice. Postmortem cortex and hippocampal samples were assayed for levels of total and non-phosphorylated neurofilament, as a measure of axonal degeneration. Protein lysates from the cortex and hippocampus in PAC1 knockout mice have an increased ratio of non-phosphorylated to total neurofilament relative to controls. These data support the hypothesis that PAC1 signaling exerts a neuroprotective effect in the EAE model.

Disclosures: V. Makhijani: None. **M.C. Condro:** None. **Y.V. Tan:** None. **S.K. Tiwari-Woodruff:** None. **J. Waschek:** None.

Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: CONICET PIP830

UBA 20020090100281

Title: The role of the notch signaling pathway in the demyelination-remyelination process

Authors: *A. M. ADAMO, F. ALMEIRA GUBIANI, M. PEREIRA LUPPI, P. MATHIEU
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Abstract: In the CNS, the oligodendroglial cell (OL) is responsible for normal myelination, while demyelination is a pathological process characterized by myelin loss around axons. Demyelination is followed by remyelination, the process by which myelin sheaths are restored

around demyelinated axons resolving the functional deficit. In this work we examined the Notch signaling pathway involvement in the demyelination-remyelination process in a toxic model of demyelination induced by cuprizone (CPZ) ingestion. Twenty-one-day-old Wistar rats were fed a diet containing 0.6% (w/w) CPZ during 2 weeks. Demyelinated animals were sacrificed 7d before CPZ withdrawal (-7d), the day of CPZ withdrawal (0d), and 7, 14 and 21d after (+7d, +14d and +21d). Control animals were sacrificed at the same times. We characterized Notch signaling in the SVZ and CC through Notch intracellular domain (NICD) levels and the non-canonical Notch ligand F3/Contactin (F3) by WB and IHC, concomitantly with oligodendroglial progenitor cell (OPC) marker NG2 and OL marker Olig2. We also evaluated the expression of Notch downstream genes Hes1, Hes5 and MAG in SVZ and CC of control and demyelinated animals by Real Time-PCR. In addition, we characterized cell population in primary cell cultures of the SVZ from CPZ and control animals at the time points described above. Results show that Notch signaling pathway was activated in NG2+ OPCs in the CC and SVZ as a consequence of demyelination induced by CPZ. The level of F3 increased significantly in the SVZ of CPZ-treated animals at -7d, +7 and +21d, while this increase was observed at -7d, 0d and +7d in the CC of demyelinated rats. The number of Olig2+ cells expressing F3 was larger than that of NG2+ cells expressing F3, indicating that non-OPC further differentiated OLs express Notch's non-canonical ligand. There was a concomitant increase in the expression of Hes1 in the SVZ in CPZ demyelinated rats at 0d. This result suggests that Notch signaling activation through the non-canonical ligand F3 may be involved in OL maturation and, therefore, in remyelination.

Disclosures: A.M. Adamo: None. **F. Almeira Gubiani:** None. **M. Pereira Luppi:** None. **P. Mathieu:** None.

Poster

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Topic: C.09. Demyelinating Disorders

Support: NIH

Title: Expression of cxcl12 chemokine receptor cxcr7 in normal and demyelinated mouse brain

Authors: *G. BANISADR[1], J. R. PODOJIL[2], S. D. MILLER[2], R. J. MILLER[3]
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Abstract: The chemokine stromal cell-derived factor-1 (SDF-1)/CXCL12 acting via its G-protein coupled receptor CXCR4 has been implicated in neurogenesis, neuromodulation, brain inflammation, HIV-1 encephalopathy and tumor growth in adult brain. Recently, CXCR7 was identified as a novel, alternate receptor for SDF-1/CXCL12. Like CXCR4, CXCR7 serves as a co-receptor for some HIV-1 strains and has also been shown to be involved in tumor growth. Characterization of CXCR7-deficient mice showed a role for CXCR7 in fetal endothelial biology, cardiac development, and B-cell localization. Despite its ligand binding properties, CXCR7 does not seem to signal like a conventional receptor. It has been suggested that CXCR7 may not function alone but may modulate the function of CXCR4. However, the *in vivo* role of CXCR7 within the CNS remains largely unexamined. Here, we investigated the regional localization of CXCR7 receptors in adult mouse brain using CXCR7-EGFP transgenic mice. We also studied the expression pattern of CXCR7 during Experimental Autoimmune Encephalomyelitis (EAE), an animal model for Multiple Sclerosis (MS). We found that the receptors were expressed in various brain regions including the olfactory bulb, cerebral cortex, hippocampus, subventricular zone, ventricular walls, hypothalamus, cerebellum and spinal cord. Extensive CXCR7 expression was also associated with cerebral blood vessels. In the cortex, CXCR7 expressing cells were predominant in layer IV-V and to a lesser extent in layers II and VI. In the hippocampus, CXCR7 expression was confined to the subgranular layer, molecular layer, pyramidal layer and hilus. Only a few cells expressed CXCR7 in the granular layer. Using cell type specific markers, CXCR7 expression was found in neurons, astrocytes and endothelial cells. GAD-expressing neurons showed CXCR7 expression in the hippocampus. We also compared the distribution of SDF-1/CXCL12 and CXCR7 using bitransgenic mice expressing both CXCR7-EGFP and SDF-1-mRFP. CXCR7 and SDF-1/CXCL12 expression overlapped particularly in blood vessels and neuronal cell populations in both normal conditions and after LPS treatment. In LPS-treated mice, SDF-1/CXCL12 expression was up-regulated in glial cells and blood vessels expressing CXCR7. High expression of SDF-1/CXCL12 was observed in the subventricular zone, white matter and cerebellum. In addition, we found that CXCR7 expression was increased during EAE and is co-localizes with oligodendrocyte progenitors (OPCs). Our findings suggest CXCR4-independent functions for CXCR7 in normal brain and during CNS inflammation.

Disclosures: **G. Banisadr:** None. **J.R. Podojil:** None. **S.D. Miller:** None. **R.J. Miller:** None.

Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: National MS Society RG4019

NINDS R01NS045702

NINDS R01NS056427

Title: Astrocyte ETB-R activation delays OPC differentiation during remyelination

Authors: *T. R. HAMMOND, P. MORTON, M. RAYMOND, V. GALLO
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Abstract: Elevated levels of the signaling peptide Endothelin-1 (ET-1) are found in demyelinated subcortical white matter lesions in adults. ET-1 has been shown to promote reactive astrogliosis and inhibit OPC differentiation and remyelination in these lesions. ET-1 acts through two G-protein coupled receptors, ETA-R and ETB-R, but our understanding of their expression patterns and cell-specific function in demyelinated lesions is still poorly understood. We find that selective inhibition of the ETB-R - but not ETA-R - accelerates OPC differentiation in lysolecithin white matter lesions in mice. Conditional deletion of the ETB-R in astrocytes using ETB-R fl/fl hGFAPcreERT2 mice increased the rate of OPC differentiation, while ETB-R deletion in OPCs using ETB-R fl/fl PDGFrαcreERT2 had no effect on OPC proliferation, migration, or differentiation. Our findings demonstrate that ET-1 acts predominantly through the ETB-R on astrocytes - and not OPCs - to indirectly regulate the rate of OPC differentiation during remyelination. These results shed further light on the specific action of ET-1 in demyelinated lesions, and could lead to the development of cell- ET-R-specific therapeutic options to promote repair of demyelinated tissue.

Disclosures: T.R. Hammond: None. **P. Morton:** None. **M. Raymond:** None. **V. Gallo:** None.

Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: Multiple Sclerosis Society of Canada

Natural Sciences and Engineering Research Council of Canada

Title: The inflammasome pyrin contributes to pertussis toxin-induced IL-1β synthesis, neutrophil intravascular crawling and autoimmune encephalomyelitis

Authors: *L. VALLIERES[1], A. DUMAS[2], N. AMIABLE[2], J. DE RIVERO VACCARI[3], J. CHAE[5], R. KEANE[4], S. LACROIX[1]
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Abstract: Microbial agents can aggravate inflammatory diseases, such as multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). An example is pertussis toxin (PTX), a bacterial virulence factor commonly used as an adjuvant to promote EAE, but whose mechanism of action is unclear. We have reported that PTX triggers an IL-6-mediated signaling cascade that increases the number of leukocytes that patrol the vasculature by crawling on its luminal surface. In the present study, we examined this response in mice lacking either TLR4 or inflammasome components and using enzymatically active and inactive forms of PTX. Our results indicate that PTX, through its ADP-ribosyltransferase activity, induces two series of events upstream of IL-6: 1) the activation of TLR4 signaling in myeloid cells, leading to pro-IL-1β synthesis; and 2) the formation of a pyrin-dependent inflammasome that cleaves pro-IL-1β into its active form. In turn, IL-1β stimulates nearby stromal cells to secrete IL-6, which is known to induce vascular changes required for leukocyte adhesion. Without pyrin, PTX does not induce neutrophil adhesion to cerebral capillaries and is less effective at inducing EAE in transgenic mice with encephalitogenic T lymphocytes. This study identifies the first microbial molecule that activates pyrin, a mechanism by which infections may influence MS and a potential therapeutic target for immune disorders.

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Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Title: Protein glycation affects peripheral nerve myelination mechanism and is involved in the pathogenesis of demyelinating disorders

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Abstract: Advanced glycation endproducts (AGEs), which are cross-linked products that result from a reaction between glucose and protein, accumulate during aging or in diabetes. Several evidences suggest that AGEs accumulation is related to the age-related diseases (e.g. degeneration of intervertebral disk, Cataracta senilis) or the diabetes complications (e.g. diabetic pheripheral neuropathies, blood vessel impairments). Recently, it has been reported that AGEs accumulation is a risk factor of several neurodegenerative diseases, but little is known about the association between AGEs accumulation and development of the nerve disorders. To understand the involvement of AGEs accumulation in the pathogenesis of peripheral nerve disorders, we used *in vitro* myelination model to identify the specific target(s) in protein glycation. We found that pyridoxine, which is a subtype of vitamin B6 in culture media, increases the AGEs accumulation level and inhibits the nerve myelination. We also observed that CRMP2 is specifically glycated in *in vitro* myelination model in the presence of pyridoxine. CRMP2 is known to regulate microtubule formation and stabilization. Taken together, these observations suggest that the inhibition of the myelination in the presence of pyridoxine and the glycation of CRMP2 are involved in the pathogenesis of diabetic peripheral neuropahy. Now, we try to identify the specific glycation site(s) of CRMP2 and their effects on the CRMP2 function.

Disclosures: H. Hagiwara: None. **F. Saito:** None. **S. Wakatsuki:** None. **T. Araki:** None.

Poster

223. Demyelinating Disorders

Location: Halls A-C

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Topic: C.09. Demyelinating Disorders

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The National Multiple Sclerosis Society

The Civitan International Research Foundation

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Title: Inhibition of system Xc- transporter protects the CNS in autoimmune inflammatory demyelination

Authors: *R. DOYLE, K. S. EVONUK, B. J. BAKER, C. M. SESTERO, B. JOHNSTON, P. DE SARNO, A. TANG, I. GEMBITZKY, C. RAMAN, T. M. DESILVA
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Abstract: Mechanisms underlying demyelination in autoimmune neuroinflammatory diseases are not well understood. Several lines of evidence suggest that glutamate dysregulation in the CNS is an important consequence of immune cell infiltration in neuroinflammatory demyelinating diseases. Excitotoxicity as a mechanism in autoimmune encephalomyelitis is supported by animal studies where blocking the AMPA-type glutamate receptor prevents myelin degradation. Evidence in this regard is further substantiated by studies in MS patients where elevated levels of glutamate are found in white matter lesions and cerebral spinal fluid. However, the causal link between inflammation and glutamate dysregulation is not well understood. Here, we examine the role of the system Xc- transporter as a glutamate release mechanism during inflammatory conditions. We report that myelin-specific CD4+ T cells stimulated nitric oxide production from microglia that was blocked by neutralizing antibodies to the cytokines IFN-gamma and TNF-alpha. Microglia stimulated with IFN-gamma and TNF-alpha showed increased expression of the system Xc- transporter, which enhanced glutamate release, causing excitotoxic death to mature oligodendrocytes, the cells that comprise the myelin sheath. We next examined the role of the system Xc- transporter in the CNS after immune cell infiltration. We observed increased expression of system Xc- in reactive glia in spinal cords of C57Bl/6 mice subjected to EAE compared to control. Pharmacological inhibitors of the system Xc- transporter administered after the first relapse in a SJL animal model of relapsing-remitting EAE abrogated clinical disease. This observation was consistent with a reduction in reactive gliosis and myelin loss. Taken together these studies support an important role for the system Xc- transporter in promoting myelin destruction after immune cell infiltration in autoimmune inflammatory demyelination.

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Poster

223. Demyelinating Disorders

Location: Halls A-C

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Program#/Poster#: 223.23/H12

Topic: C.09. Demyelinating Disorders

Title: Chronic oral riluzole or caloric restriction ameliorate symptoms of experimental autoimmune encephalomyelitis

Authors: *R. ROTOLO[1], J. DEMURO[2], G. DRUMMOND[1], J. WOOD[3], E. LAZAROFF[3], S. LUPINSKI[3], C. LITTLE[3], A. WOLF[3], G. VANN[3], L. TELISKA[3], D. RILEY[3], J. BAHGAT[3], J. VIDAL[3], M. ALBALAWI[2], L. JOHNS[1], A. BETZ[3]
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Abstract: Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS). We characterized the impairments that represent EAE in female C57BL/6 mice. Mice were immunized subcutaneously with 100 μg of myelin oligodendrocyte glycoprotein emulsified in incomplete Freund's adjuvant supplemented with 500ug mycobacterium tuberculosis H37RA and 200 ng of intraperitoneal pertussis toxin on days 0 and 2. Tail paralysis was observed daily. In Experiment 1, we found that a battery of behavioral tasks delayed the onset and severity of EAE but did not affect nociception. In Experiment 2, we found that caloric restriction (CR) and chronic oral administration of riluzole, a glutamate antagonist, delayed the onset and severity of EAE. Additionally, CR and riluzole both reduced nociceptive behavior throughout disease progression. Glutamate neurotoxicity has been proposed as major determining factor that accompanies the demyelination and axonal degeneration observed during the course of MS. Further, CR plus riluzole decreased symptomology more so than CR alone. Lastly, we found altered levels in proteins important for normal immune reactions such as TNFα, Treg, IL-6, BDNF, pSTAT3, and leptin. Altered immunological function was also indicated by reduced demyelination in the spinal cords of mice treated with riluzole. These findings indicate a compelling need to delineate the roles of glutamate, the immune response, and CR in EAE.

Disclosures: R. Rotolo: None. **J. DeMuro:** None. **G. Drummond:** None. **J. Wood:** None. **E. Lazaroff:** None. **S. Lupinski:** None. **C. Little:** None. **A. Wolf:** None. **G. Vann:** None. **L. Teliska:** None. **D. Riley:** None. **J. Bahgat:** None. **J. Vidal:** None. **M. Albalawi:** None. **L. Johns:** None. **A. Betz:** None.

Poster

223. Demyelinating Disorders

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Program#/Poster#: 223.24/I1

Topic: C.09. Demyelinating Disorders

Title: Acetylation changes regulate cytokine induced cell death and IRF-1 signalling in the HOG (Human Oligodendroglioma) cell line

Authors: *E. LODA, Y. WANG, J. PATEL, D. LIEBENSON, R. GOSWAMI, D. STEFOSKI, R. BALABANOV
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Abstract: Objective: To investigate epigenetic mechanisms, specifically acetylation and its involvement in IRF-1 signaling, in cytokine induced cell death in the human oligodendroglioma (HOG) cell line. Background: Acetylation is key epigenetic modification that is involved in transcription, protein synthesis and posttranslational modification. Chromatin remodeling involves histone deacetylating enzymes (HDAC) which removes acetyl groups from lysine contributing to chromatin condensing and suppression of transcription. The opposing enzyme, histone acetylating enzyme (HAT) adds acetyl groups contributing to chromatin decondensing and activation of transcription. Our earlier studies with cell free plasma DNA have shown that epigenetic changes take place during the inflammatory stage of MS. We hypothesized that the interferon regulatory factor 1 (IRF-1) transcription factor, which is upregulated around MS lesions in oligodendrocytes and contributes to the inflammatory process, is modified by epigenetic mechanisms involving acetylation. Methods: HOG cells were treated with HDAC or HAT modifiers such as lysophosphatidic acid (LPA) and garcinal in the presence or absence of the cytokines interferon gamma, tumor necrosis factor alpha, and lipopolysaccaride. MTT and LDH assays were used to measure cell viability and cytotoxicity respectively. Immunopercipitation was used to analyze the acetylation status of IRF-1. Western blot was used for protein expression analysis. Results: Cells treated with the HDAC activator LPA, were rescued from cytokine induced cell death. The p300 inhibitor garcinal reduced expression of IRF-1 and acetylated IRF-1 expression. Conclusion: We demonstrate that acetylation epigenetic changes are involved in cytokine induced death in HOG cells. Limiting acetylation tends to rescue cells from cytokine induced death whereas increasing acetylation tends to increase cytokine induced cell death by mechanisms that involve IRF-1. Understanding the significance

of epigenetic modificatins in oligodrocyte cell death may provide a basis for development of novel therapies for cell protection in MS.

Disclosures: **E. Loda:** None. **Y. Wang:** None. **J. Patel:** None. **D. Liebenson:** None. **R. Goswami:** None. **D. Stefoski:** None. **R. Balabanov:** None.

Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

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MS Society of Canada Grant

Canadian Institute of Health Research Grant

Title: Inducible deletion of myelin regulatory factor is a cell selective mechanism to impair oligodendrocyte remyelination

Authors: ***G. J. DUNCAN**[1], J. R. PLEMEL[2], J. LIU[3], R. E. HIRATA[3], Y. CHAEICHI[3], M. BERSON[3], W. TETZLAFF[3]
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Abstract: Evidence from multiple sclerosis lesions suggests chronic demyelination is associated with sustained degeneration of axons. Remyelination, is essential for the restoration of conductance following demyelination and is correlated to improved axon preservation. During remyelination, oligodendrocyte precursor cells (OPCs) are recruited to the lesion epicenter where they differentiate and mature into new myelinating oligodendrocytes, a process requiring intricate transcriptional regulation. Therapies that promote myelin repair hold enormous promise, but require a greater understanding of the key factors regulating transcription during oligodendrocyte maturation and subsequent remyelination. One factor, myelin regulatory factor (Myrf) is essential for the maturation of oligodendrocytes and the formation of central nervous system (CNS) myelin during development. However, its role in remyelination has not been elucidated. We crossed Myrffl/fl with PDGFRα Cre ERT2 mice to remove Myrf from OPCs (referred to as Myrf iKO OPC) while leaving mature oligodendrocytes intact. Mice were then

injected with lysolecithin to induce a focal demyelinating lesion in the corpus callosum, and examined at different time points post lesion. This allows for the direct assessment of the role of Myrf on different stages of OPC differentiation and remyelination. Myrf was not required for recruitment of OPCs following demyelination, but there was a marked reduction in the total number of CC1-Olig2+ mature oligodendrocytes at ten days post lesion (dpl). Additionally, by crossing Myrf iKO OPC mice with an inducible reporter mouse Rosa26-eYFP, reveals that recombined cells fail to robustly express GST-π, a marker of mature oligodendrocytes by 14 dpl. Thus, MYRF is required for the full maturation of OPCs into mature oligodendrocytes following demyelination. New nodes of Ranvier, suggestive of compact myelin formation, can be labelled by punctate caspr and ankG staining within the lesion. The number of nodes of Ranvier were reduced 14dpl in Myrf iKO OPC. Recombined cells visualized with a membrane tethered reporter reveal that when Myrf is deleted in recombined cells, they rarely contributed to forming new myelin internodes. Thus, the inducible deletion of Myrf from OPCs prior to demyelination mimics aspects of chronically demyelinated MS lesions: the inability of OPCs to mature and successfully remyelinate and can be used as new model to determine the long-term effects of impaired remyelination on demyelinated axons and its contribution to functional repair.

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Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Title: A remyelinating antibody rHIgM22 induces proliferation in mixed glial cells

Authors: I. HAKIMI[1], *M. SRINIVAS[2], J. CAO[2], A. M. VECCHIONE[2], P. D. SARMIERE[2], M. RODRIGUEZ[3], A. O. CAGGIANO[2], D. C. BUTTON[2]
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Abstract: The recombinant monoclonal IgM antibody known as rHIgM22 is in Phase 1 clinical trials for treatment of multiple sclerosis (MS). MS is characterized by inflammation, demyelination and axonal degeneration. rHIgM22 has been shown to induce remyelination in the Theiler's Murine Encephalomyelitis Virus and lysolecithin models of demyelination. While the

mechanism of action is not fully understood, there is evidence suggesting that rHIgM22 may influence the local environment and enable proliferation of oligodendrocyte precursors, thus supporting remyelination. To further elucidate the mechanisms of action of rHIgM22 we compared the effects of rHIgM22 with those of a human isotype control IgM (IC) on the proliferation of cells in mixed glial cultures (MGCs). While rHIgM22-induced OPC proliferation in MGCs has been shown previously (Watzlawik et al 2013), our results extend those observations by showing that microglia also proliferate in response to rHIgM22. MGCs were prepared from P2/P3 rat cortex and cultured in serum-containing medium for 5 days. Cultures were immunophenotyped using cell specific markers to ensure that a uniform complement of cells was generated from all dissections. Our MGCs are composed of GFAP-positive astrocytes (~60%), A2B5-positive OPCs (~20%), O4-positive oligodendrocytes (~10%) and IB4-positive microglia (~10%). Parallel cultures were switched to serum free medium in the presence of rHIgM22 or IC at concentrations ranging from 0.05 to 33µg/mL and incubated for 48 hours. Growth factors were included as positive control for proliferation. 5-ethynyl-2´-deoxyuridine (EdU) was added for the last 18 hours of stimulation to label proliferating cells. Fluorescent labeling of EdU was performed followed by DAPI labeling of nuclei. A Zeiss LSM 700 confocal microscope was used for image acquisition and the proliferation index was calculated as a ratio of EdU to DAPI staining. We observe a dose dependent increase in cell proliferation in rHIgM22-treated cultures that is at least two fold greater than IC with little to no proliferation in vehicle controls. To identify what cell types of the MGCs proliferate in response to rHIgM22, we co-labeled treated cultures with EdU and the cell specific markers listed above. Our results suggest that microglia and OPCs proliferate in response to rHIgM22. In addition to previously demonstrated effects on oligodendrocytes, it is also possible that rHIgM22 has an indirect activity through microglia which in turn influences OPC proliferation. Further studies are being pursued to examine actions of rHIgM22 on responsive cells in MGCs treated for either short (2 days or less) or long times (5-7 days).

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Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: PIP 830 CONICET, Argentina

Title: Is PGJ2 involved in the effect of indomethacin on bone marrow mononuclear cell migration to the sciatic nerve of the rat after a lesion?

Authors: V. USACH, C. I. CASALIS, K. WEBER, G. PIÑERO, M. FERNÁNDEZ-TOMÉ, *P. C. SETTON-AVRUJ
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Abstract: Demyelination is one of the hallmarks of the Wallerian degeneration (WD) process and cell therapy is among the strategies under study to induce remyelination. Results from our group obtained in a reversible model of WD induced by the crush of the rat sciatic nerve demonstrated the spontaneous migration of endogenous or transplanted bone marrow mononuclear cells (BMMC) exclusively to the injured nerve. Once in the ipsilateral nerve, some BMMC colocalized with Schwann cell markers and nerve fiber markers, which accelerated the degeneration process and, as a consequence, the regeneration process too. On the basis of these results, the aim of the present work was to evaluate whether prostaglandins (PGs), one of the molecules generated during the inflammatory process associated with the injury, is one of the signals involved in the migration and recruitment of BMMC to the demyelinated nerve. To that end, adult Wistar rats were submitted to sciatic nerve crush and one group of animals was transplanted with BMMC through the sacra artery immediately. The presence of BMMC in the injured nerve was evaluated through confocal microscopy 24 h, 3 and 5 days post injury; the expression of ciclooxigenases (Cox-1 and Cox-2) and the synthesis of PGs were evaluated between 0 and 24 h post crush, through Western blot and PGs radioconversion, respectively. Besides, the effect of a non-steroidal anti-inflammatory drug as indomethacin on the migration of BMMC and PGs biosynthesis was analyzed, treating the animals with a subcutaneous injection of indomethacin 50 mg/kg/day the day of the lesion and the previous day, and 5 mg/kg/day the subsequent days. The results obtained show that, as soon as 24 h post injury, BMMC arrived at the edges of the ipsilateral nerve, and after 3 days they became part of it. As regards PGs biosynthesis, the expression of the inducible form of ciclooxigenase, Cox-2, was observed in the ipsilateral nerve 2 hours after the nerve injury and continued at 24 h. Our results demonstrate the biosynthesis of PGE2, PGD2 and PGJ2 in sciatic nerve homogenates, and that their levels did not change significantly as a consequence of the lesion. Although indomethacin inhibited the migration of transplanted BMMC to the injured sciatic nerve, the biosynthesis of PGE2 and PGD2 was not affected. Surprisingly, indomethacin promoted a significant increase in PGJ2 both in the contralateral and the ipsilateral nerves. Further experiments are necessary to elucidate the specific participation of PGs in BMMC migration and to understand the effect of indomethacin on the degeneration-regeneration process.

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Poster

223. Demyelinating Disorders

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Title: Zinc transporter 3 (ZnT3) gene deletion reduces spinal cord white matter damage and behavioral deficits in a murine MOG-induced multiple sclerosis model

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Abstract: The present study aimed to evaluate the role of zinc transporter 3 (ZnT3) on multiple sclerosis pathogenesis. Experimental autoimmune encephalomyelitis (EAE), a disease model of multiple sclerosis, was induced by immunization with myelin oligodendrocyte glycoprotein (MOG 35-55) in female mice. Three weeks after the initial immunization, demyelination, immune cell infiltration and blood brain barrier disruption in the spinal cord were analyzed with Luxol Fast Blue (LFB) staining, cresyl violet staining and immunohistochemistry. Clinical signs of EAE first appeared on day 11 and reached a peak level on day 18 after the initial immunization. ZnT3 gene deletion profoundly reduced the daily clinical score of EAE mice. The ZnT3 gene deletion-mediated inhibition of the clinical course of EAE was accompanied by suppression of inflammation and demyelination in the spinal cord. Cresyl violet staining of wild-type mice with EAE revealed intensive infiltration of mononuclear cells around microvessels in the spinal cord. LFB staining of the spinal cord also exhibited severe demyelination in wild-type mice with EAE. The behavioral deficits accompanying neuropathological changes associated with EAE were dramatically reduced in ZnT3 knockout mice with EAE. This reduction was accompanied by coincident reductions in demyelination and infiltration of encephalitogenic immune cells including CD4+ T cells, CD8+ T cells, CD20+ B cells and F4/80 cells in the spinal cord. These results demonstrate that ZnT3 gene deletion inhibits the clinical features and neuropathological changes associated with EAE. ZnT3 gene deletion also remarkably inhibited

EAE-associated BBB disruption and MMP-9 activation. The present study found that MOG-induced EAE increased ZnT3 expression in the spinal cord. Therefore, amelioration of EAE-induced clinical and neuropathological changes by ZnT3 gene deletion suggests that vesicular zinc may be involved in several steps of multiple sclerosis pathogenesis.

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Poster

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Topic: C.09. Demyelinating Disorders

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UW Vision Research Core P30EY01665

Title: Histological correlates of oct measures of retinal nerve fiber layer thickness in a large animal model of dysmyelination

Authors: ***I. D. DUNCAN**[1], J. VER HOEVE[3], B. GLATTING[3], L. TEIXEIRA[2], J. MAYER[1], R. DUBIELZIG[2], C. SMITH[1]
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Abstract: Axon loss in multiple sclerosis (MS) is the likely reason that patients with relapsing-remitting disease decline and enter a secondary progressive phase. Determining axon loss in patients has relied primarily on MRI of the brain and spinal cord. Recently, non-invasive optical coherence tomography (OCT) imaging of the thickness of the retinal nerve fiber layer (RNFL).has been promoted as a means to study axon loss in MS patients. However, at present, there is no direct (histological) evidence of loss of retinal ganglion cell axons in MS. We studied the correlation between OCT measurement of RNFL thickness and histology in a large animal model of a genetic dysmyelinating disorder: dogs with a mutation in proteolipid protein gene *plp1*, allelic to the mutation causing Pelizaeus-Merzbacher disease in humans. We hypothesized that chronic dysmyelination may cause a chronic loss of axons in the optic nerve of *plp1* mutant dogs and result in atrophy of the RNFL. Here we present the results from four dogs (2 with *plp1*

mutation and 2 controls) over a period of 2 years, measuring spectral-domain OCTs (sdOCTs) and flash visual evoked potentials (VEPs) at quarterly intervals. Axon numbers in the optic nerve and in the retina were then quantitated histologically. We found sdOCT estimates of RNFL thickness were significantly thinner in the affected dog retina and correlated with histologically-determined measures. Microcystic changes in outer retina, reported in MS, were not observed in these dogs. Functional measures, including behavioral assessments and VEPs from affected dogs were markedly reduced. PLP1 mutant dogs show evidence of RNFL thinning on OCT and corresponding histology and therefore may be a valuable model for validating the use of non-invasive imaging in demyelinating disorders.

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Poster

223. Demyelinating Disorders

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Topic: C.09. Demyelinating Disorders

Support: IMSS/1129

Title: Brain-derived neurotrophic factor and tau as molecular markers of multiple sclerosis

Authors: *A. ISLAS[1], C. F. CUEVAS[2], L. M. ESPAILLAT[2], B. BERTADO-CORTES[2], P. GARCIA-DE LA TORRE[1]
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Abstract: Multiple Sclerosis (MS) is an inflammatory, progressive, and degenerative disorder of the central nervous system (CNS). It affects mainly white matter, causing axonal demyelination and deterioration. Whith a relapsing-remitting course, followed by a secondary progressive disability, which leads inevitably to deficiencies in life's quotidian activities and functional capacity. Severity of cognitive deterioration in this disease is directly related to the location of the demielinizated tissue. MS development can be measured by MIR, taking into account the presence and number of lesions in the brain. Activation of immune system in response to MS can also be a predictor of the disease´s course. Besides, several proteins have been studied as potential predictors of disease´s development, which could provide quantitative, sensitive, and

reliable information about MS. Among these biomarkers, BDNF has been associated to neuroprotection, and Tau has been studied as a marker for neural damage. Both markers are potential predictors of MS progression, however there are contradictory data about them. Here, we measured BDNF and Tau in peripheral blood of 50 MS patients and 50 control subjects. We found that BDNF levels in peripheral blood of MS patients was lower than in controls, which could be related to a diminished neuroprotection for patients with MS. It could also mean that the consumption of BDNF is higher in MS patients as a consequence of the damage the disease itself produces to the tissue. On the other hand, we found no differences between patients and controls when Tau was measured. In conclusion, our results show that BDNF can be used as a biomarker of MS, as it diminishes in peripheral blood in patients with the disease; on the other hand, Tau cannot be used as a marker.

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Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

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the Natural Science Foundation of Guangdong Province (No. S2011040004895)

Title: Electroacupuncture promotes the differentiation of transplanted trkc gene modified bone marrow mesenchymal stem cells into oligodendrocyte-like cells and remyelination in the demyelinated spinal cord of rat

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Abstract: Our previous study indicated that electroacupuncture (EA) treatment could promote NT-3 expression, increase the cell number and differentiation of endogenous oligodendrocyte precursor cells (OPCs), and remyelination in the demyelinated spinal cord. However, the number of oligodendrocytes differentiated from the endogenous OPCs is limited. It is known that NT-3 promotes the survival and differentiation of cells by preferentially binding to its receptor TrkC. In this study, we attempted to transplant TrkC gene modified MSCs (TrkC-MSCs) into the demyelinated spinal cord to investigate whether EA treatment could promote NT-3 secretion in the demyelinated spinal cord, and to determine whether increased NT-3 could further enhance transplanted TrkC-MSCs to differentiate into oligodendrocytes, remyelination and functional recovery in the demyelinated spinal cord. The spinal cord of adult Sprague-Dawley rats was microinjected with ethidium bromide (EB) at T10, to establish a demyelinated model. Six groups of animals were performed for the experiment: Sham, EB+PBS, MSCs, MSCs+EA, TrkC-MSCs and TrkC-MSCs+EA group. Our results showed that TrkC-MSCs transplantation combined with EA (the TrkC-MSCs+EA group) treatment significantly increased the number of NG2-positive OPCs and APC-positive oligodendrocyte-like cells differentiated from transplanted MSCs compared with the other transplantation groups. By toluidine blue-stained semithin and electron microscopy analysis, we found that concomitantly the number of newly formed myelins was increased and oligodendrocyte-like cells differentiated from TrkC-MSCs formed myelin sheaths. Immunofluorescence histochemistry and Western blot analysis showed that TrkC-MSCs transplantation and EA treatment could promote the MBP expression and Kv1.2 arrangement trending to normal level. Furthermore, behavioural test and cortical motor evoked potentials detection demonstrated a significant functional recovery in the TrkC-MSCs+EA group. In conclusion, our results suggested that EA treatment increased NT-3 expression and promoted oligodendrocyte-phenotype differentiation from TrkC-MSCs and remyelination in the demyelinated spinal cord as well as the functional improvement of demyelinated spinal cord.

Disclosures: Y. Ding: None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

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Topic: C.09. Demyelinating Disorders

Support: The Shockey Family Foundation

Title: Intermittent blockade of the OGF-OGFr axis by low dose naltrexone upregulates OGF and inhibits the progression of established relapse-remitting experimental autoimmune encephalomyelitis

Authors: L. A. HAMMER, P. J. MCLAUGHLIN, *I. S. ZAGON
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Abstract: Relapse-remitting multiple sclerosis (RR-MS) is a chronic immune system related disorder. RR-MS affects approximately 350,000 people in the U.S., with progressive physical and emotional deterioration. The Opioid Growth Factor (OGF)-OGF receptor (OGFr) pathway has been reported to inhibit progression of established chronic progressive EAE as well as reduce astrogliosis and neuronal damage in the spinal cord. RR-EAE was established in SJL/J mice with PLP139-151 immunizations, and previous studies have shown that OGF therapy initiated with induction of disease markedly reduce behavior and neuropathology. OGFr blockade was invoked by daily injections (ip) of 0.1 mg/kg naltrexone (low dose naltrexone, LDN) or saline beginning 2 days after initial signs of disease. Within 9 days of LDN treatment, mice responding to LDN treatment had markedly reduced mean behavioral scores compared to controls, with the sum of behavioral scores being 142.9±11.5 for the RR-EAE+Saline group in comparison to 78.8±9.1 for the RR-EAE+LDN group. In the LDN group, 80% of the mice had complete remission (behavioral scores of 0.5 or less) during the 40 day observation period. Exogenous administration of OGF (10 mg/kg) to mice with established RR-EAE also resulted in changes in behavior within 9 days. OGF-responder mice had markedly reduced mean behavior scores relative to controls; the sum of the behavior scores was 140.1±14.4 for the saline-treated group in comparison to 72.2±11.5 for the group of mice responding to OGF. Disease severity scores were 3.6±1.7 for OGF treated mice in comparison to 16.3± 5 for mice receiving saline. No saline-treated RR-EAE mouse had a complete remission, whereas 83% of OGF mice had complete remissions (behavioral score $\leq$ 0.5); the total length of time in complete remission was 9.1±3.4 days for the RR-EAE+OGF animals over the course of the 40-day experimental period. Approximately 40-50% of mice receiving either LDN or OGF did not respond to treatment when initiated 2 days after established disease. In summary, OGF given exogenously or induced by intermittent receptor blockade , inhibited the severity of initial flair and limited the progression of clinical disease in mice with RR-EAE. These observations support LDN or OGF as non-toxic and safe biotherapies for the treatment of RR-MS.

Disclosures: L.A. Hammer: None. **P.J. McLaughlin:** None. **I.S. Zagon:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.03/I10

Topic: C.09. Demyelinating Disorders

Title: Physical exercise alters the composition of the lesion extracellular matrix and promotes white matter regeneration

Authors: *S. K. JENSEN, M. B. KEOUGH, L. CRAIG, J. PLEMEL, C. BRIDEAU, V. YONG
Univ. of Calgary, Calgary, AB, Canada

Abstract: Physical exercise is considered a critical component of a healthy lifestyle and is known to have efficacy in treating/controlling many systemic disorders. In the central nervous system, regular exercise has been associated with a decrease in the risk of developing neurodegenerative disorders. Indeed, a possible role for exercise in the treatment of Parkinson's disease, age-related cognitive decline and neuroinflammation, and mood/anxiety disorders has been described. Studies conducted within these model systems have identified pleiotropic mechanisms activated by exercise with the capability of promoting neuroregeneration. Specifically, described mechanisms include: increased neurogenesis and oligodendrogliogenesis, enhanced neural plasticity, induction of growth factor expression, and modulation of the immune system and the pathological microenvironment. Our previous data has implicated lesion-accumulated chondroitin sulfate proteoglycan (CSPGs) as inhibitors of oligodendrocyte migration, maturation and remyelination (Lau et al., Ann Neurol 72:419, 2012; Nature Rev Neurosci 14:722, 2013). In the current study, we hypothesized that physical exercise is an efficacious intervention for promoting white matter regeneration following a demyelinating insult, and that its mechanisms of benefits involve affecting the microenvironment/extracellular matrix, particularly CSPG deposition. To examine this, we induced a focal demyelinating lesion via injection of lysolecithin into the dorsal funiculus of the mouse spinal cord. Mice were singly housed and given free access to an electronically monitored running wheel, which were locked in control animals, at the day of injury until sacrifice at either 7, 14, or 21 days post lesion (representing peak demyelination, early remyelination, and late remyelination, respectively). We observed reduced lesion volumes in exercising animal, which was associated with an increased presence of lesion-associated myelin as assessed by eriochrome cyanine staining and myelin basic protein immunohistochemistry. Further, phosphorylated neurofilament immunoreactivity revealed a greater number of axons present in the lesion of exercising animals. The frequency of $GFAP^+$ reactive astrocytes, $Iba1^+$ macrophages/microglia, and $Olig2^+$ oligodendrocyte precursor cells appears to be unaltered by exercise. Impressively, a significant reduction in the accumulation of versican, a member of the CSPG family, was observed. These results indicate that physical exercise can improve white matter regeneration and may be an important tool for the treatment of demyelinating disorders, such as multiple sclerosis.

Disclosures: **S.K. Jensen:** None. **M.B. Keough:** None. **L. Craig:** None. **J. Plemel:** None. **C. Brideau:** None. **V. Yong:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.04/I11

Topic: C.09. Demyelinating Disorders

Title: Fingolimod increases hippocampal histone H3 Lys14 acetylation and ameliorates behavioural and cognitive functions in a mouse model of reactive depression

Authors: L. DI NUZZO[1], R. ORLANDO[2], C. TOGNOLI[3], P. DI PIETRO[4], M. MOTOLESE[4], E. MOSCARDO[3], M. PELLITTERI[3], G. BERTINI[3], P. F. FABENE[3], G. BATTAGLIA[4], *V. BRUNO[1,4,5], F. NICOLETTI[1,4,5]
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Abstract: Since epigenetic regulation of gene expression has been implicated in the hippocampal dysfunction that characterizes the pathophysiology of depressive disorders, drugs able to influence chromatin remodeling in this brain area can have antidepressant properties. Fingolimod, a CNS-permeant sphingosine-1-phosphate (S1P) analogue, is the first oral drug to have received the regulatory approval for the treatment of multiple sclerosis. Independently of its immunological mechanism of action, fingolimod can increase hippocampal BDNF expression when injected intraperitoneally in mice. Considering that S1P can inhibit *in vitro* the activity of type-2 hystone deacetylase (HDAC2), fingolimod could influence gene expression by affecting chromatin regulation in the hippocampus. BDNF levels are reduced in the hippocampus of depressed individuals and this reduction can be due to an alteration in the epigenetic regulation of the bdnf gene promoter. Moreover, it is hypothesized that the clinical efficacy of antidepressant drugs is based, at least partially, on their ability to restore normal concentrations of the neurotrophin. We examined the antidepressant-like activity of fingolimod in mice exposed to four weeks of chronic unpredictable stress (CUS). Different groups of mice were treated daily with vehicle or fingolimod (3 mg/kg). Treatments started from the 3rd week of CUS and continued for 4 weeks (i.e. up to 3 weeks after the end of CUS). We examined depressive-like

behavior (forced swim test), anxiety-like behavior (elevated plus maze), spatial memory (Morris water maze), and social memory (social recognition test). In addition, we measured acetylated and non-acetylated H3 (Lys14) levels in the hippocampus, HDAC2 and BDNF mRNA and protein levels in the hippocampus and prefrontal cortex. Moreover, we evaluated hippocampal neurogenesis (BrdU and Dcx immunostaining). We found a significant antidepressant-like activity of fingolimod in the forced swim test, which was associated with an improvement of cognitive functions measured by the Morris water maze test and with an antidepressant-like neurochemical phenotype. Accordingly, CUS-exposed mice treated with fingolimod showed an increased acetylation of H3 at Lys14, a reduced expression of HDAC2 and an increased expression of BDNF in the hippocampus. In addition, fingolimod-treated mice showed an increased neurogenesis in the hippocampal dentate gyrus. These findings are consistent with the recent clinical evidence that multiple sclerosis patients who switched from first-line disease modifying drugs into fingolimod showed a significant reduction of depressive symptoms.

Disclosures: **L. Di Nuzzo:** None. **R. Orlando:** None. **C. Tognoli:** None. **P. Di Pietro:** None. **M. Motolese:** None. **E. Moscardo:** None. **M. Pellitteri:** None. **G. Bertini:** None. **P.F. Fabene:** None. **G. Battaglia:** None. **V. Bruno:** None. **F. Nicoletti:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.05/I12

Topic: C.09. Demyelinating Disorders

Support: Jean Perkins Foundation

Title: A small molecule ligand for TrkB/TrkC neurotrophin receptors improves motor dysfunction and pathology in trembler mice

Authors: ***F. M. LONGO**[1], T. YANG[1], K. C. TRAN[1], S. M. MASSA[2,3]
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Abstract: Charcot-Marie-Tooth disease (CMT) disease is the most common hereditary peripheral neuropathy and CMT1A is a dominantly inherited form, affecting an estimated 50% of CMT patients. It is most frequently caused by duplication of peripheral myelin protein 22

(PMP22), but is also caused by point mutations in the PMP22 gene. Currently, there is no effective drug therapy for CMT1A. Previous studies showed that neurotrophin-3 (NT-3) administered via gene therapy promoted nerve regeneration and increased myelin thickness in the Trembler-J (TrJ) CMT1A mouse model (Sahenk et al., Molecular Therapy, 2014) and in sural nerves from CMT1A patients (Sahenk et al., Neurology, 2005). However, long-term treatment with NT-3 and other neurotrophins such as brain-derived neurotropic factor (BDNF) is limited by short half-lives and poor bioavailability. Treatment of TrJ mice with TrkB and TrkC agonist antibodies individually or in combination over 20 weeks via subcutaneous injection were found to improve myelin and axonal morphological measures, with the combination TrkB/C therapy most effective for some endpoints. Our laboratories have developed a small molecule, non-peptide ligand, BC-2, that binds to and activates both TrkB and TrkC, but not TrkA or p75 neurotrophin receptors. We hypothesized that BC-2 might have effects similar to TrkB/C agonist antibodies and consequently evaluated its effects on morphological and behavioral deficits in TrJ mice. BC-2 was administered via intraperitoneal injection (50 mg/kg) three times per week for 20-21 weeks starting at 10-12 weeks of age in male TrJ mice, which begin to manifest tremor and demyelination at 4-6 weeks of age. BC-2 significantly increased myelin thickness in the sciatic nerve of TrJ but not wild type mice, and resulted in diminished clasping behavior and improved performance on gait and catwalk testing relative to vehicle treatment. Further, BC-2 treatment increased the number and size of myelinated axons and partially reversed the deficit in levels of phosphorylated neurofilament (NF)-H. Overall, these results support the idea that concomitant targeting of TrkB and TrkC might offer a novel, pharmacologically feasible small molecule strategy for treatment of patients with CMT1A and other neuropathies.

Disclosures: **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PharmatrophiX, Patent interest in BC-2. **S.M. Massa:** Other; Patent interest in BC-2. **T. Yang:** None. **K.C. Tran:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.06/J1

Topic: C.09. Demyelinating Disorders

Title: Acute desipramine restores presynaptic cortical defects in murine experimental autoimmune encephalomyelitis by suppressing central CCL5 overproduction

Authors: *A. PITTALUGA, S. DI PRISCO, E. MEREGA, A. UCCELLI, S. CASAZZA
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Abstract: Altered glutamate exocytosis and cyclic adenosine monophosphate (cAMP) production in cortical terminals of Experimental Autoimmune Encephalomyelitis (EAE) mice occur at the early stage of disease (13 days post immunization, d.p.i.). Neuronal defects were paralleled by central chemokine Regulated upon Activation Normal T cell Expressed and Secreted (RANTES or CCL5) overexpression, suggesting its role in presynaptic impairments. We propose that drugs able to restore CCL5 content to physiological levels could also rescue presynaptic defects. Because of its efficacy in controlling CCL5 overexpression, desipramine (DMI) appeared to be a suitable candidate to test our hypothesis. Control and EAE mice at 13 d.p.i. were acutely or chronically DMI administered and monitored for behaviour and clinical score. Noradrenaline and glutamate release, cAMP, CCL5 and TNF-α productions were quantified in cortical synaptosomes and homogenates. Peripheral cytokine production was also detected. Noradrenaline exocytosis and α2-autoreceptor-mediated activity were unmodified in EAE mice at 13 d.p.i. when compared to control. Acute, but not chronic, DMI reduced CCL5 level in cortical homogenates of EAE mice at 13 d.p.i., leaving unaltered peripheral IL-17 and TNF-α contents as well as CCL5 plasma levels. Acute DMI caused a long-lasting restoration of glutamate exocytosis, rescued endogenous cAMP production, and impeded the shift from inhibition to facilitation of the CCL5-mediated control of glutamate exocytosis. Finally, DMI ameliorated anxiety-related behaviour but not motor activity and clinical scores. We propose DMI as an add-on therapy to normalize neuropsychiatric symptoms in MS patients at the early stage of disease.

Disclosures: A. Pittaluga: None. **S. Di Prisco:** None. **E. Merega:** None. **A. Uccelli:** None. **S. Casazza:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

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Program#/Poster#: 224.07/J2

Topic: C.09. Demyelinating Disorders

Support: UH2-TR000918

NS-19108

HD-09402

Title: Pro-myelinating serum-derived exosomes from environmentally enriched rats are secreted by peripheral blood mononuclear cells

Authors: *A. D. PUSIC, K. M. PUSIC, R. P. KRAIG
Univ. of Chicago, Chicago, IL

Abstract: Grey matter demyelination is an important component of multiple sclerosis (MS) pathogenesis, particularly in the secondary progressive disease phase. Extent of damage is strongly correlated to decline in memory and cognitive dysfunction. Aging likewise occurs with cognitive decline from myelin loss, and age-associated failure to remyelinate significantly contributes to MS progression.Evidence shows that parabiotic exposure of aged animals to a youthful systemic milieu improves remyelination. We discovered that this effect involves serum exosomes that increase oligodendrocyte precursor cells and their differentiation into mature myelin-producing cells - both under normal conditions and after acute demyelination (1). Environmental enrichment (EE) of aging animals produced exosomes that mimicked this pro-myelinating effect. We found that both young and EE serum-derived exosomes were enriched in miR-219, which is necessary and sufficient for production of myelinating oligodendrocytes (2). Thus, peripherally produced exosomes found in the serum of young or environmentally enriched animals may be a useful therapy for remyelination. Here, we aimed to better characterize these exosomes. Exosomes found in the blood can originate from a multitude of sources, and it is precisely this attribute that makes them ideal candidates as biomarkers of disease. For example, in a variety of solid organ cancers, tumor-derived exosomes can be isolated from blood (3). However, based on work demonstrating that the properties of serum-derived EE exosomes could be reproduced *ex-vivo* using cultured primary dendritic cells (Pusic et al., 2014, J Neuroimmunol), we focused on immune cells. As a first step in determining the cellular source of pro-myelinating exosomes, we briefly cultured peripheral blood mononuclear cells (PBMCs) isolated from whole blood of EE and control animals and harvested exosomes from the conditioned media. Like EE serum exosomes, PBMC exosomes increased baseline myelin content when applied to slice cultures. This begins to confirm our hypothesis that these exosomes are produced by immune cells and allows us to further focus on specific cell populations within the broad category of 'PBMCs'. Accordingly, we sorted PBMCs into constituent cell populations (T cells, B cells, dendritic cells and macrophages) for collection of exosomes. Functional assays are in progress, and miRNA screens of exosome content for each group will be performed and expression profiles compared to that of exosomes harvested from EE serum. (1) Pusic and Kraig 2014, Glia; (2) Dugas et al., 2010, Neuron; (3) Kosaka et al., 2010, Cancer Sci.

Disclosures: **A.D. Pusic:** None. **K.M. Pusic:** None. **R.P. Kraig:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

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Program#/Poster#: 224.08/J3

Topic: C.09. Demyelinating Disorders

Support: UH2-TR000918

NS-19108

HD-09402

Title: Nasal administration of IFNγ-stimulated dendritic cell exosomes inhibits spreading depression

Authors: *K. M. PUSIC, A. D. PUSIC, R. P. KRAIG
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Abstract: Migraine with aura (MwA) and multiple sclerosis (MS) are clinically correlated, and both display brain imaging white matter abnormalities suggestive of myelin disruption (1). Likewise, MS modeled in rats reduces the threshold to spreading depression (SD), the likely cause of aura and pain in MwA (2). Using brain slice cultures, we discovered that SD transiently disrupts grey matter myelin. Like MS, this SD-induced demyelination involves T cells, their release of IFNγ and increased oxidative stress, which may serve to increase neutral sphingomyelinase-2 (nMase-2) activity by depleting glutathione. (3). These changes are consistent with a shift in microglia to a predominantly M1 (pro-inflammatory) phenotype (3). Importantly, we have shown that exosomes derived from IFNγ-stimulated dendritic cells (IFNγ-DC-Exos) increase brain myelination and microglial glutathione (4), and may do so through modulation of microglial polarization states. Since microglia are essential for SD and their M2a polarization by environmental enrichment prevents SD (5), we probed the impact IFNγ-DC-Exos on SD. We showed that an hour of recurrent (6) neocortical SDs *in vivo* (n=5) also significantly ($p<0.001$) reduced grey and adjacent white matter myelin (as measured via myelin basic protein levels) a day later compared to the contralateral areas. When applied to slice cultures, IFNγ-DC-Exos evoked a 13-fold significant ($p<0.001$) increase in SD threshold compared to controls (n=8/group) three days later. Similarly, nasal administration of IFNγ-DC-Exos (n=6/group) caused a significant ($p<0.001$) 57-fold increase in SD threshold compared to unstimulated exosome treated shams and untreated control animals (n=5,3,4/group). Furthermore, IFNγ-DC-

Exo treatment reduced the M1 polarization shift normally observed with SD. Treatment with unstimulated exosomes did not have this effect. Examination of the contralateral hemisphere, which was not exposed to SD, showed reduced expression of M1 microglia products with IFNγ-DC-Exo relative to unstimulated exosome treatment. (n=5,3/group). Taken together, these results support a pathophysiological link between myelin dysfunction in MwA and MS. Additionally, they suggest that IFNγ-DC-Exos, which have been suggested as a novel treatment to reduce inflammation and promote remyelination in MS (6) may also be beneficial for MwA and perhaps migraine in general. (1) Bashir A et al., 2013, Neurology; (2) Merkler D et al., 2009, Ann Neurol; (3) Pusic A et al., 2011, Soc Neurosci; (4) Pusic A et al., 2014, J Neuroimmunol; (5) Pusic K et al., 2014, Glia; (6) Pusic A et al., Exp Rev Neurother.

Disclosures: K.M. Pusic: None. **A.D. Pusic:** None. **R.P. Kraig:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.09/J4

Topic: C.09. Demyelinating Disorders

Title: Multiple sclerosis therapeutic glatiramer acetate enhances oligodendrocyte precursor cell differentiation and remyelination

Authors: *S. COTTRELL-CUMBER, A. ROSEN, A. FERNANDEZ-CASTANEDA, A. GAULTIER
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Abstract: Multiple Sclerosis (MS) is a progressive neurological illness characterized by destruction of the myelin sheath surrounding neurons in the central nervous system. The destruction of the myelin sheath is mediated by autoreactive T cells. Current pharmacological therapies seek to decrease the severity of the inflammatory response on the damaged axons by suppressing various aspects of the immune system. One such current MS treatment, Glatiramer Acetate (GA), is believed to act as an immunomodulatory peptide that shifts the immune response by simulating myelin basic protein. However, evidence suggests that GA also has the ability to directly modulate oligodendrocyte progenitor cells function (OPCs) to enhance differentiation and remyelination in MS lesions. Chronic demyelination is a major cause of neurodegeneration in MS patients. The CNS contains OPCs that have the potential to differentiate into mature oligodendrocytes and remyelinate denuded axons. However, myelin

debris lingering in the MS plaques inhibits the process of axon remyelination. Here we **demonstrate an immune independent action of GA and OPCs, through the enhancement of OPC differentiation and remyelination.** Our data shows that GA increases markers of myelination in CG4 cells, an OPC cell line. We have observed increased myelin basic protein (MBP) RNA and protein expression in CG4 cells treated with GA. Since myelin is known to inhibit the differentiation of OPCs, we will challenge OPCs with myelin, and then measure production of MBP in the presence or absence of GA. We expect to see increased MBP expression when GA is applied; this would indicate the ability of GA to overcome myelin inhibition and augment remyelination. We believe GA promotes OPC differentiation in MS, which could explain its beneficial effects as a therapeutic; further identification of a downstream signaling pathway could lead to the development of a novel remyelination therapeutic.

Disclosures: S. Cottrell-Cumber: None. **A. Rosen:** None. **A. Fernandez-Castaneda:** None. **A. Gaultier:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

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Program#/Poster#: 224.10/J5

Topic: C.09. Demyelinating Disorders

Support: DMRDP

National Mulitple Sclerosis Society

Title: CB2 receptor mediated cannabinoid signaling contributes to the therapeutic effect of 2-AG hydrolytic enzyme ABHD6 inhibition on EAE

Authors: J. WEN, M. TANAKA, R. RIBEIRO, *Y. ZHANG
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Abstract: Agents targeting both inflammation and neurodegeneration at the early stage might be desirable for the treatment of multiple sclerosis (MS). The endocannabinoid system, consisting of cannabinoid receptors, their endogenous ligands including anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and enzymes for their synthesis and degradation, is known to play a crucial role in controlling neuroinflammation and neurodegeneration. Studies have shown that increasing endogenous levels of 2-AG by inhibition of its principal hydrolytic enzyme

monoacylglycerol lipase (MAGL) can cause 2-AG overload, desensitization and behavioral tolerance despite of its demonstrated therapeutic effect. ABHD6 is a recently identified serine hydrolase and can compete with MAGL for 2-AG degradation. In this study, we investigated the role of ABHD6 inhibitor WWL70 in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. At 28 days after EAE induction, the expression of ABHD6 significantly increased and treatment with WWL70 (10 mg/kg, i.p.) starting from the disease onset and then once daily remarkably reduced mouse symptoms, inflammatory response and neuronal damage, demonstrated by significantly lower clinical scores, less expression of iNOS, IL-1β and TNF-α, decreased infiltration of T cells, increased number of mature oligodendrocytes and preserved myelination in EAE spinal cord. Interestingly, When EAE mice were treated with WWL70 together with CB2 antagonist AM630, mice displayed the similar symptoms and pathological changes to that seen in the EAE control mice. Co-treatment with the CB1 antagonist AM281 did not affect the efficacy of WWL70 in EAE mice. WWL70 also attenuated blood-brain barrier damage through inhibiting activated leukocyte cell adhesion molecule (ALCAM) expression. Furthermore, the findings that the increased expression of CB2 receptors in EAE mouse spinal cord and the lack of effects of WWL70 in CB2 knock out EAE mice provide strong evidence that CB2 mediated endocannabinoid signaling contributes to the therapeutic efficacy of ABHD6 inhibition. In addition, we found that treatment with WWL70 reduced the induction of COX-2 and the production of prostaglandin E2 (PGE2) in microglia activated by lipopolysaccharide, consistent with the recent notion that inhibition of 2-AG hydrolysis can enhance cannabinoid signaling and reduce eicosanoid signaling. These results suggest that selectively targeting the 2-AG hydrolytic enzyme ABHD6 might be used as a novel therapeutic strategy for MS.

Disclosures: J. Wen: None. **Y. Zhang:** None. **M. Tanaka:** None. **R. Ribeiro:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

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Topic: C.09. Demyelinating Disorders

Support: COBRE NIH 5P30RR0323135

NGMS8P30 GM 103498

Title: Fingolimod promotes primary sensory afferent growth through S1P1 receptor inhibition of cAMP

Authors: *M. MCNAMARA, A. GALVIN, T. CLASON, C. J. FOREHAND
Neurosci. Grad Program, Univ. of Vermont, Burlington, VT

Abstract: Fingolimod, a Sphingosine-1 phosphate (S1P1) agonist is an approved oral treatment for relapsing forms of multiple sclerosis. The primary therapeutic mechanism of Fingolimod is to bind to S1P1 receptors on the surface of lymphocytes, internalize the receptor and prevent S1P1 dependent egress from the primary lymphoid tissue where it would infiltrate the central nervous system and cause neurodegeneration (Gasperinin et al., 2012). In addition to its effects on immune cells, Fingolimod is known to increase brain derived neurotrophic factor (BDNF) secretion from neurons *in vitro* (Deogracias et al., 2012). During neural development, dorsal root ganglion (DRG) cells produce a peripheral and central process. The central process of DRG neurons extend into the spinal cord at the dorsal root entry zone. These processes branch and extend longitudinally along the rostral-caudal axis in the white matter prior to growth into the grey matter of the cord. Longitudinal growth is increased by BDNF and inhibited by blocking TrkB. We show here that Fingolimod increases the rate of extension of the DRG central process along the rostral-caudal axis ($p<0.0001$). Further, we have shown that application of Fingolimod significantly increases the release of BDNF into the media ($p<0.05$) suggesting that Fingolimod promotes BDNF release to accelerates axon outgrowth. The current experiments are designed to test the hypothesis that Fingolimod increases axon outgrowth through the S1P1 receptor to promote BDNF release. Application of an S1P1 antagonist W123 significantly decreased longitudinal growth while application of S1P1 agonist SEW2817 increased longitudinal growth. As activation of the S1P1 receptor is known to decrease cAMP we tested whether manipulation of cAMP affected axon outgrowth. We show here that application of Forskolin, a drug known to increase cAMP decreased axon outgrowth. In contrast, H89, a drug known to decrease PKA activation by cAMP increased axon outgrowth. Fingolimod and H89 together showed no greater increase in axonal extension than either alone. However, Fingolimod was able to decrease the inhibitory effect of Forskolin on axonal extension. These results suggest that Fingolimod acts by decreasing cAMP to stimulate axonal outgrowth. These results further suggest that a decrease in cAMP will lead to an increase in BDNF secretion in this system.

Disclosures: M. McNamara: None. **A. Galvin:** None. **T. Clason:** None. **C.J. Forehand:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

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Topic: C.09. Demyelinating Disorders

Support: NIH Grant 1R01NS081141

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Title: Optical coherence tomography (OCT) as a predictive and longitudinal *in vivo* biomarker of disease and estrogen receptor β agonist-induced repair in a mouse model of multiple sclerosis

Authors: *A. J. KHALAJ[1,2], P. KIM[3], M. SYED[2], S. HABIB[3], S. NUSINOWITZ[3], J. A. KATZENELLENBOGEN[4], S. K. TIWARI-WOODRUFF[1,2]
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Abstract: Identifying predictive and longitudinal *in vivo* biomarkers to assay therapeutic efficacy is integral to developing treatments for neurodegenerative diseases such as multiple sclerosis (MS). Optic neuritis (ON) is a common acute manifestation of MS onset; similarly, experimental autoimmune encephalomyelitis (EAE) mice, a rodent model of MS, exhibit ON. Optical coherence tomography (OCT) allows for direct visualization of retinal structure, including optic nerve head topography. Quantification of the retinal nerve fiber layer (RNFL) and ganglion cell layer (GCL) by OCT provides an indirect measure of axonal and neuronal loss in anterior visual pathways. Optic neuropathies and numerous neurological disorders, including MS, show abnormal RNFL thickness. Here, we investigated the effects of late myelin oligodendrocyte glycoprotein (MOG_{35-55})-induced chronic EAE and therapeutic treatment of chronic EAE mice with the highly selective estrogen receptor β agonist Indazole-Cl (Ind-Cl) on retinal and optic nerve health. Fundus imaging and serial high-resolution spectral domain optical coherence tomography (sdOCT), followed by immunohistochemistry and electron microscopy analysis, were performed on normal and chronic EAE mice therapeutically treated with Ind-Cl or vehicle (i.e., treatment was initiated after peak clinical disease). Image analysis using template-based marking of retinal layers, including RNFL, GCL, nuclear layers, and retinal pigment epithelium (RPE), was performed using Bioptigen software (Durham, NC). Significant differences in RNFL, GCL, and RPE between normal, vehicle-, and Ind-Cl-treated EAE groups were observed. Preliminary immunohistochemical and electron microscopy data indicate increased inflammation in the retina and optic nerve, and decreased axon myelination in the optic nerve, of EAE mice. As compared to vehicle treatment, therapeutic treatment with Ind-Cl decreased retinal and optic nerve inflammation and improved optic nerve axon myelination. These data support OCT and optic nerve analysis as strongly translational *in vivo* biomarkers with which to assess the efficacies of potential neuroprotective agents for the treatment of inflammatory neurodegenerative diseases. Further, these data support therapeutic Ind-Cl as a treatment capable of improving widespread, quality-of-life-diminishing MS-related retinal and optic nerve pathology.

Disclosures: **A.J. Khalaj:** None. **P. Kim:** None. **M. Syed:** None. **S. Habib:** None. **S. Nusinowitz:** None. **J.A. Katzenellenbogen:** None. **S.K. Tiwari-Woodruff:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.13/J8

Topic: C.09. Demyelinating Disorders

Title: Serial block face scanning electron microscopy (sbf-sem) approaches for assessing remyelination

Authors: G. KIDD[1], *S. MEDICETTY[2], B. TRAPP[1], E. BENSON[3], B. BAI[4], A. ROHOLT[4]
[1]Neurosciences, Cleveland Clin., Cleveland, OH; [2]Renovo Neural Inc., Cleveland, OH; [3]3D-EM, [4]Renovo Neural, Inc., Cleveland, OH

Abstract: Promoting generation of new myelin is an important therapeutic objective of treating demyelinating diseases such as multiple sclerosis (MS). Remyelination involves both lateral extension of oligodendrocyte processes along the axon to increase internodal length and radial growth to extend the myelin spiral. Experimental studies of remyelination most frequently utilized myelin thickness and g-ratio (axon:fiber diameter) measurements, while internodal lengths have been technically difficult to obtain. Serial blockface scanning electron microscopy (SBF-SEM) provides serial images of tissue with ultrastructural resolution and comparatively wide fields of view (0.5mm or more), which allows axonal reconstruction in 3 dimensions. To investigate rates of change in myelin thickness, internodal length, and g-ratio during remyelination, we used SBF-SEM datasets to evaluate remyelinating internodes in mouse corpus callosum at different time points after demyelination. Mice were subjected to a 12-week course of cuprizone chow and daily rapamycin injections in order to induce and maintain demyelination. They were subsequently allowed to recover for a period of either 0, 3, or 6 weeks. Myelin thickness increased to WT values by 6 wk recovery. Internodal length measurements were a more sensitive indicator of remyelination, increasing with 3 and 6 weeks recovery but not reaching WT values. Reduced internodal length also identified the few myelin internodes that had resisted demyelination in the demyelination-only treatment. In contrast, g-ratio values did not differ significantly between groups. In studies of remyelination *in vivo*, SBF-SEM-based measurements offer a sensitive metric of internodal regeneration.

Disclosures: **G. Kidd:** F. Consulting Fees (e.g., advisory boards); Renovo Neural, Inc.. **S. Medicetty:** None. **B. Trapp:** F. Consulting Fees (e.g., advisory boards); Renovo Neural, Inc.. **E. Benson:** None. **B. Bai:** None. **A. Roholt:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.14/J9

Topic: C.09. Demyelinating Disorders

Support: Drexel Start-Up Fund

Title: A comparison of therapeutic candidates for promoting oligodendrocyte differentiation and remyelination

Authors: *B. SHULTZ, Y. ZHONG
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Abstract: Demyelination occurs when oligodendrocytes are killed, often in response to injury or disease. Once neurons lose this myelin sheath, they lose the ability to readily transmit action potentials, resulting in a loss of patient function. To replace lost oligodendrocytes, neural stem/progenitor cells (NSPCs) can be transplanted to the injury or disease site, where they could differentiate into mature oligodendrocytes and promote new myelin formation. Alternatively, endogenous NSPCs may also be manipulated to differentiate into new oligodendrocytes. In practice, however, oligodendrocyte differentiation and maturation are often limited *in vivo* due to the abundance of inhibitory and pro-inflammatory signals that can be found in injured or diseased tissue. To overcome this hurdle, therapeutic molecules can be delivered to promote either endogenous or transplanted NSPCs to preferentially differentiate into mature, myelin-forming oligodendrocytes. A number of growth factors/molecules have been shown to promote NSPCs to differentiate into mature oligodendrocytes. However, few studies have been conducted to directly compare their potency. The objective of this study is to identify the molecule that is most effective in promoting oligodendrocyte differentiation and maturation. NSPCs were isolated from E17 rat brains, expanded in neurosphere suspension, plated down onto multiwell plates, and then differentiated in the presence of BDNF, NT-3, Sonic Hedgehog (SHH), or 3,3',5-triiodothyronine (T3), a thyroid hormone. After 10 days, the cells were fixed and stained with antibodies against O4, an early oligodendrocyte marker; myelin basic protein (MBP), a mature oligodendrocyte marker and myelin component; Tuj1, a neuronal marker; and glial

fibrillary acidic protein (GFAP), an astrocyte marker. Cells differentiated in media supplemented with 30 ng/mL T3 exhibited a significantly higher percentage of MBP+ cells than any other group, indicating that T3 is the most effective at promoting oligodendrocyte differentiation and maturation. Systemic T3 administration, however, is likely to cause patients to experience hyperthyroidism, resulting in a wide range of possible side effects including muscle weakness, nervousness, anxiety, and weight loss, among others. Thus, controlled local delivery is a much more suitable approach. Currently, our lab is developing an injectable hydrogel capable of providing sustained local release of T3, which is a promising therapeutic approach to promoting new myelin formation.

Disclosures: B. Shultz: None. **Y. Zhong:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.15/J10

Topic: C.09. Demyelinating Disorders

Support: US Dept. Veterans Affairs

Title: Young plasma treatment delays the onset and decreases the severity of chronic eae

Authors: A. C. MARCU[1], M. C. JOSYLN[1], P. J. YANNIE[1], R. CUTLER[1], J. L. DUPREE[2], *G. H. DE VRIES[1]
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Abstract: The severity and onset of multiple sclerosis (MS) is closely related to age: patients usually develop the disease after the age of 20 and when diagnosed in older patients (>40 years), the disease takes an unrelenting progressive course for which there is no therapy. Therefore, a therapeutic strategy would be to reverse aging and decrease susceptibility to disease. Previous studies reportedthat the decline in neurogenesis and cognitive impairments observed duringaging can be reversed when an older CNS is exposed to a young systemic milieucreated by injection of young plasma. Using the chronic experimental autoimmune encephalomyelitis (EAE) model for demyelinating disease, we investigated the possibility that young plasma treatment can render the CNS less susceptible to EAE by reversing the aging process. Cohorts of 12 week old mice were pre-treated with young plasma (4 weeks old) or homologous plasma (12 weeks old) every

other day for 10 days, followed by initiation of EAE. Plasma injections were continued every other day for another 20 days. Mice were monitored daily and scored for the onset, severity and incidence of disease. In the mice injected with young plasma, the onset of EAE symptoms was delayed by 3 days compared to the control group, reaching a maximum average EAE score of 1, while the homologous plasma injected group had a maximum average EAE score of 3. The incidence of disease was decreased in the young plasma injected group with only 5 out of 12 mice becoming sick (42%), while in the homologous plasma injected control group, 11 out of 12 mice (92%) exhibited clinical signs of EAE. Microglial infiltration and activation was dramatically decreased in the young plasma injected group. This group also showed dramatic axonal preservation as revealed by the ratio of phosphorylated to non-phosphorylated filaments. Further experiments are underway to evaluate the molecular basis for these results. In summary, young plasma treatment is a novel therapeutic strategy which delays the onset, decreases the severity and greatly decreases the incidence of demyelinating disease in mice.

Disclosures: A.C. Marcu: None. **M.C. Josyln:** None. **P.J. Yannie:** None. **R. Cutler:** None. **J.L. Dupree:** None. **G.H. De Vries:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.16/J11

Topic: C.09. Demyelinating Disorders

Title: 7, 8 dihydroxyflavone suppresses optic neuritis in experimental autoimmune encephalomyelitis

Authors: *D. TRISLER[1,2], V. K. C. NIMMAGADDA[1], B. TANDUKAR[1], S. I. V. JUDGE[1,2], C. T. BEVER[1,2], T. K. MAKAR[1,2]
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Abstract: Background: Optic neuritis (ON) is characterized by inflammation of the optic nerve, and is one of the first clinical signs of multiple sclerosis (MS). ON often manifests as an acute, self-limited episode with visual impairment that recovers over several weeks in the majority of patients. However, permanent visual symptoms can persist in a significant number of patients and repeat episodes of ON can lead to significant optic nerve atrophy. TrkB signaling pathways have been demonstrated to be a promising strategy to suppress inflammation and impart

neuroprotection in many neurodegenerative diseases including MS. 7, 8 dihydroxyflavone (DHF) is a small molecule has been reported to mimic the physiological actions of brain derived neurotrophic factor and to activate TrkB receptors in the brain when administered systematically. Objective: Determine the effect of DHF treatment on optic neuritis in experimental allergic encephalomyelitis (EAE), an animal model for MS. Methods: EAE was induced in C57Bl/6 female mice by immunization with myelin oligodendroglial glycoprotein peptide 35-55. DHF (5 mg/kg/day) was administered intraperitoneally from the day of disease induction. Mice were euthanized on day 28. Optic nerves were fixed in 10% formalin and embedded in paraffin. 5µm sections were used for histological analysis. Results: DHF significantly suppressed the clinical severity of EAE paralysis. Histological examination revealed decreased inflammation (H&E) and demyelination (Luxol Fast Blue) in optic nerves of DHF treated mice compared to untreated. There is a significant increase in TrkB activation in optic nerves of treated mice. Immunohistochemical analysis of optic nerves showed a significant decrease in CD45 (Common Leucocyte antigen), CD3 (T-Cell marker) and CD 20 (B-cell marker) expression in DHF treated mice compared to untreated. Conclusion: We showed that DHF treatment significantly reduced the clinical severity of EAE paralysis and also suppressed optic neuritis in these mice. DHF may prove to be a potential therapeutic approach in MS patients presenting with ON.

Disclosures: D. Trisler: None. **V.K.C. Nimmagadda:** None. **B. Tandukar:** None. **S.I.V. Judge:** None. **C.T. Bever:** None. **T.K. Makar:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.17/J12

Topic: C.09. Demyelinating Disorders

Title: Chondroitin sulfate proteoglycan scar formation after chronic nerve compression injury is attenuated by chondroitinase ABC administration

Authors: M. D. TAPADIA[1], M. LUU[1], J. JUNG[1], W. WANG[1], J. SU[1], T. MOZAFFAR[2], *R. GUPTA[1]
[1]Orthopaedic Surgery, [2]Neurology/Orthopaedic Surgery, Univ. of California, Irvine, Irvine, CA

Abstract: Spinal cord injury (SCI) stimulates glial scar formation rich in chondroitin sulfate proteoglycans (CSPGs). Previous work has shown that this scar can be digested with chondroitinase ABC (ChABC) to improve functional outcomes (1-2). Improvements in axonal

regeneration have also been noted with ChABC administration after peripheral nerve transection (3). Recent data from our lab confirms scar formation after CNC injury consisting of upregulation of ECM proteins such as laminin α2, collagen IV, and fibronectin. With this in mind, we sought to determine whether CSPG's are implicated in neural scar formation following chronic nerve compression (CNC) injury, and whether glycosaminoglycan digestion with ChABC might improve the dysfunction that persists in later stages of CNC despite surgery (4). Mouse sciatic nerves were harvested at 2- and 6-weeks after CNC injury for western blot (WB) and immunohistochemistry (IHC) of CSPGs including decorin, brevican, and NG2. A subset of mice then underwent intraneural injection of ChABC (0.2U/mL) at 6 weeks. Myelin degeneration and ultrastructural changes were examined with toluidine blue staining and electron microscopy. Electrophysiology studies were performed prior to each harvest. Of all CSPG's analyzed, decorin showed remarkable upregulation on both WB and IHC with 1.5 times greater expression in epi- and perineurium of compressed nerves at 6 weeks that was attenuated by ChABC injection. These data suggest that CNC injuries, similar to peripheral nerve transection and spinal cord injuries, result in proteoglycan scar that is reduced with ChABC. Further investigation is warranted into the relationship between proteoglycan scar and Schwann cell dysfunction following CNC injury, and the optimal dosing and treatment duration of ChABC. References: 1. Lee YS, Lin CY, Jiang HH, Depaul M, Lin VW, Silver J. Nerve regeneration restores supraspinal control of bladder function after complete spinal cord injury. J Neurosci. 2013; 33(26): 10591-606. 2. Barritt AW, Davies M, Marchand F, Hartley R, Grist J, Yip P, McMahon SB, Bradbury EJ. Chondroitinase ABC promotes sprouting of intact and injured spinal cord systems after spinal cord injury. J Neurosci. 2006; 26(42):10856-867. 3. Zuo J, Neubauer D, Graham J, Krekoski CA, Ferguson TA, Muir D. Regeneration of axons after nerve transection repair is enhanced by degradation of chondroitin sulfate proteoglycan. Exp Neurol. 2002; 176:221-28. 4. Early surgical decompression restores neurovascular blood flow and ischemic parameters in an in-vivo animal model of chronic nerve compression injury. Jung J, Hahn P, Choi B, Mozaffar T, Gupta R. J Bone Jt Surg. 2014; manuscript in press.

Disclosures: **M.D. Tapadia:** None. **J. Jung:** None. **W. Wang:** None. **M. Luu:** None. **J. Su:** None. **T. Mozaffar:** None. **R. Gupta:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.18/K1

Topic: C.09. Demyelinating Disorders

Support: Multiple Sclerosis Society of Canada

Title: A chondroitin sulfate proteoglycan synthesis inhibitor promotes myelin regeneration and immunomodulation in experimental models of demyelination

Authors: *M. B. KEOUGH, J. A. ROGERS, P. ZHANG, S. K. JENSEN, E. L. STEPHENSON, M. G. HURLBERT, J. R. PLEMEL, C.-C. LING, V. W. YONG
Univ. of Calgary, Calgary, AB, Canada

Abstract: Following demyelination, reactive astrocytes accumulate and generate a barrier of molecules known as the glial scar. One component of the scar, the chondroitin sulfate proteoglycans (CSPGs), are well established inhibitors of axonal regrowth. Our group and others have shown that CSPGs are also capable of inhibiting oligodendrocyte precursor cell (OPC) responses to injury, by reducing morphological differentiation *in vitro* and remyelination *in vivo*. Direct CNS injection of the enzyme chondroitinase ABC has been effective at reducing the CSPG burden following traumatic injuries, however practical limitations arise when considering this treatment approach for widespread demyelinating diseases such as multiple sclerosis. Here we tested the utility of a novel CSPG synthesis inhibitor, acetylated 4-fluoro-N-acetylglucosamine (fluorosamine), which prevents elongation of CSPG side-chains, to promote remyelination after injury. We show that CSPGs inhibit primary cultured murine OPCs in a side-chain dependent manner, confirming existing reports. Cultured murine astrocytes produce CSPGs in their media following cytokine stimulation; treatment with fluorosamine reduces both the amount of side-chains as well as total protein cores secreted into the media, while not affecting cell viability. Experimental demyelination via injection of the toxin lysolecithin into the mouse spinal cord white matter induces a glial scar with pronounced CSPG accumulation. Systemic treatment with fluorosamine reduces this CSPG burden, resulting in a greater extent of remyelination. We observe an additional benefit of fluorosamine in that it reduces the proliferation of stimulated T cells *in vitro*. Indeed, fluorosamine treatment following induction of experimental autoimmune encephalomyelitis in mice reduces disease severity when treatment is initiated at onset of clinical signs or peak disease. All available medicines for multiple sclerosis target only the aberrant immune component of the disease; we believe the next generation of therapeutics will require direct enhancement of myelin regeneration in addition to immunomodulation, and this may be achieved by altering the inhibitory microenvironment.

Disclosures: **M.B. Keough:** None. **J.A. Rogers:** None. **P. Zhang:** None. **S.K. Jensen:** None. **E.L. Stephenson:** None. **M.G. Hurlbert:** None. **J.R. Plemel:** None. **C. Ling:** None. **V.W. Yong:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.19/K2

Topic: C.09. Demyelinating Disorders

Support: NIH Grant 5R01NS082347-02

Title: Fingolimod therapy in multiple sclerosis is associated with an increase in inner nuclear layer thickness

Authors: O. A. S. AL-LOUZI[1], A. LANG[2], P. BHARGAVA[3], A. CARASS[2], J. PRINCE[2], S. SAIDHA[3], P. CALABRESI*[3]
[2]Dept. of Electrical and Computer Engin., [3]Dept. of Neuroimmunology and Neuroinfectious disorders, [1]Johns Hopkins Univ., Baltimore, MD

Abstract: **Background:** Fingolimod, a sphingosine-1-phosphate (S1P) receptor modulator, was the first oral medication approved for the treatment of relapsing-remitting multiple sclerosis (RRMS), and is a known cause of macular edema. Recently, an increase in macular volume, as measured by optical coherence tomography (OCT), has been described in MS patients following fingolimod initiation. However, it remains unclear within which retinal layers alterations occur, contributing to this increase in macular thickness. **Goal:** To determine whether initiation of fingolimod is associated with an increase in thickness of specific retinal layers. **Methods:** Spectral-domain OCT, with automated intra-retinal layer segmentation, was performed on 20 RRMS patients before and after initiation of fingolimod. OCT was also performed on a control group of 20 RRMS patients, not commenced on fingolimod. The control group was matched based on age, gender, and time interval between OCT exams. Macular segmentation measures were obtained by averaging the thickness values within 3 regions centered at the fovea: a circle of diameter 1mm (central macula), an annulus with an inner radius of 1mm and outer radius of 2.5mm (inner macula) and an annulus with an inner radius of 2.5mm and an outer radius of 5mm (outer macula). Retinal layer thickness changes within and between groups were analyzed using mixed-effects linear regression accounting for within-subject inter-eye correlation. **Results:** The average inner nuclear layer (INL) thickness increased by +0.19μm ($p<0.001$) in the group of patients who initiated fingolimod, over a mean follow-up duration of 7 months (SD 3) after starting the medication, versus a mean change of -0.06μm ($p=0.51$) in the control group. In the fingolimod-treated group, no statistically significant change was found in the average change of

the ganglion cell+inner plexiform layer thickness (GCIP; mean -0.12µm, p=0.33), the outer nuclear layer (ONL; mean +0.07µm, p=0.64), or the average macular thickness (AMT; mean +0.24µm, p=0.49). The increase in INL thickness was greatest in the central macula (mean change of +0.6µm, p<0.001), followed by the inner macula (mean change of +0.27µm, p=0.005), and was least in the outer macula (mean change of +0.14µm, p=0.025). **Conclusions:** Fingolimod therapy in patients with MS is associated with an early increase in INL thickness. This finding helps localize the retinal biological changes that occur in MS eyes as a result of S1P receptor modulation.

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Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

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Topic: C.09. Demyelinating Disorders

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NMSS Grant RG 4046-A6

NIH Grant NS079144-01

Title: Targeted growth arrest-specific protein 6 (Gas6) delivery to the CNS protects axons from damage during chronic experimental autoimmune encephalomyelitis (EAE)

Authors: *R. C. GRUBER, A. RAY, B. SHAFIT-ZAGARDO
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Abstract: Growth arrest-specific protein 6 (Gas6) is a soluble agonist of the Tyro, Axl, Mer, (TAM) family of receptor tyrosine kinases identified to have anti-inflammatory and promyelinating properties. During experimental autoimmune encephalomyelitis (EAE) wildtype (WT) mice demonstrate a significant induction of Gas6, Axl, and Mer but not Protein S or Tyro3 mRNA. We tested the hypothesis that intracerebroventricular (ICV) delivery of Gas6 directly into the CNS of WT mice during myelin oligodendrocyte glycoprotein (MOG)-induced EAE would improve the clinical course of disease relative to artificial cerebrospinal fluid (ACSF)-treated mice. Gas6 did not delay disease onset, but significantly reduced the clinical scores

during peak and chronic EAE. Alternate-day subcutaneous interferon-beta (INFβ) injection delayed the onset of EAE by ~3-days, but did not reduce clinical scores during acute or chronic EAE. ICV Gas6+INFβ injections marginally reduced clinical scores relative to ACSF+INFβ treatment; however, the dual treatment resulted in higher clinical scores relative to Ivc-Gas6. Mice receiving Gas6 for ≥30 days had preserved SMI31+ neurofilament immunoreactivity, significantly fewer SMI32+ axonal swellings in the ventral spinal cord and less demyelination, relative to ACSF-treated mice. $Gas6^{-/-}$ mice sensitized with MOG_{35-55} peptide exhibit higher clinical scores, with significantly increased Iba1+ glia, and enhanced expression of TNF-α, IL-17, and IL-6 mRNA relative to WT mouse spinal cords with scores for 8 consecutive days. Our data is consistent with a role for Gas6 in dampening the inflammatory response as well as preserving axonal integrity and myelination during EAE.

Disclosures: R.C. Gruber: None. **B. Shafit-Zagardo:** None. **A. Ray:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.21/K4

Topic: C.09. Demyelinating Disorders

Support: KAKENHI 25430056

Title: Exendin-4 promotes neurite outgrowth, neuronal survival and myelination *in vitro*

Authors: *K. SANGO[1], M. TSUKAMOTO[1,2], N. NIIMI[1], Y. KANAZAWA[1,2], K. WATABE[1], K. UTSUNOMIYA[2]
[1]Dept Sensory Motor Syst, Tokyo Met Inst. Med. Sci., Tokyo, Japan; [2]Div. Diabetes, Metab & Endocrinol, Dept Intrnl. Med., Jikei Univ. Sch. Med., Tokyo, Japan

Abstract: Glucagon-like peptide (GLP)-1 is an endogenous incretin hormone secreted from enteroendocrine L cells in response to oral nutrient ingestion. Besides its insulinotropic actions on pancreatic β-cells, the widespread distribution of GLP-1 receptors (GLP-1R) suggests pleiotrophic actions of GLP-1 on extrapancreatic tissues, including the nervous system. In the present study, we investigated the neurotrophic and neuroprotective properties of exendin (Ex)-4, a GLP-1R agonist, on adult rat dorsal root ganglion (DRG) neurons. Predominant localization of GLP-1R to small peptidergic and large DRG neurons *in vivo* and *in vitro*, as revealed by immunohistochemistry, suggests the involvement of GLP-1 in both small and large fiber

functions. Treatment of DRG neurons with Ex-4 at 100 nM significantly increased the average neurite length from 114.3 μm to 164.5 μm after 2 days in culture, and the average survival ratio from 55.7% to 67.4% after 7 days in culture. The enhancement of neurite outgrowth and neuronal viability induced by Ex-4 may be partially attributed to the inhibition of Rho kinase activity, as we observed in PC12 cells exposed to Ex-4 at 100 nM for 30 min. Moreover, Ex-4 promoted myelination in coculture of DRG neurons and immortalized adult rat Schwann cells IFRS1; treatment with Ex-4 at 10 and 100 nM accelerated the migration of IFRS1 cells toward the neurites extending from DRG neurons after 14 days of coculture, as seen under a phase-contrast microscope, and upregulated the expression of myelin proteins PMP22 and P0 after 21 days of coculture, as revealed by western blotting. These findings imply the efficacy of Ex-4 for the promotion of functional repair after axonal injury and the restoration of diabetic and other peripheral neuropathies.

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Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.22/K5

Topic: C.09. Demyelinating Disorders

Title: Reward-related decreases of mental fatigue in individuals with multiple sclerosis: an fMRI study

Authors: *E. DOBRYAKOVA[1], H. GENOVA[2], J. DELUCA[3], G. WYLIE[3]
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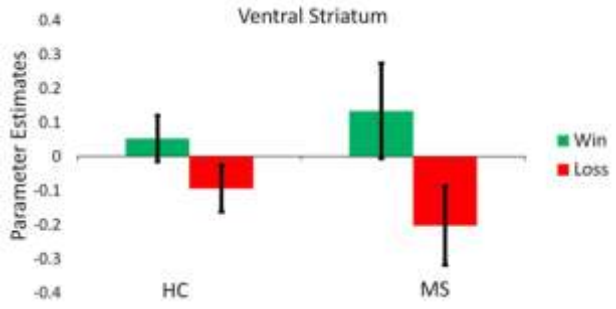
[2]Neuropsychology/Neuroscience Lab., [3]Kessler Fndn., West Orange, NJ

Abstract: Up to 95% of people with multiple sclerosis (MS) experience mental fatigue (Walker et al., 2012). Mental fatigue has been proposed to be associated with impairment of the striatum (Chaudhuri & Behan, 2000), a primary input nuclei of the basal ganglia that receives dopamine-projection neurons from the substantia nigra. The striatum has been shown to be involved in affective and cognitive processes, such as effort calculation and reward valuation. Thus, there might be a link between mental fatigue and reward processes. Hence, stimulating the striatum during a affective/cognitive task may lead to a modulation of the expression of mental fatigue in individuals with MS. To test this theory we recruited 16 individuals with MS who experience

mental fatigue and 16 healthy control subjects (HC). Participants in the MS group had higher levels of fatigue compared to the HC group, as per Fatigue Severity Scale and Modified Fatigue Impact Scale. During the fMRI scan, participants performed the card-guessing task (Delgado et al., 2000) previously shown to engage the striatum via presentation of positive and negative monetary feedback. The task consisted from a reward and a no reward conditions. During the reward condition, participants had an opportunity to win a monetary bonus, while during the no reward condition this opportunity was not presented to them. Self-reported fatigue ratings were also acquired during the scan. fMRI data showed that, compared to HCs, differential striatal activity to positive and negative feedback was greater in the MS group and was associated with lower levels of self-reported fatigue. This suggests that MS subjects who experience fatigue may judge a given amount of reward differently from HCs and that increased dopamine in the striatum may lead to a decrease of fatigue in



MS.

Disclosures: **E. Dobryakova:** None. **H. Genova:** None. **J. DeLuca:** None. **G. Wylie:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

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Topic: C.09. Demyelinating Disorders

Support: NIH Grant R37NS34467

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NIH Grant RO1AG039452

Title: White matter degeneration in pericyte-deficient mice

Authors: *G. L. SI, A. MONTAGNE, Z. ZHAO, A. P. SAGARE, A. M. NIKOLAKOPOULOU, B. ZLOKOVIC*
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Abstract: White matter degeneration is observed in a variety of neurological disorders, including Alzheimer's disease (AD). Previous studies have shown that patients with AD often exhibit demyelinated white matter, in some cases even prior to the formation of amyloid-β plaques and neurofibrillary tangles. Hypoxic and ischemic brain injuries are also strongly associated with white matter damage. Pericytes are critical for the maintenance of vascular integrity; however, whether pericyte loss contributes to white matter abnormalities is unknown. Here, we report that white matter brain regions in pericyte deficient-mice undergo accelerated degeneration with age. First, we observed reduced axonal projections in highly-myelinated brain regions, such as the corpus callosum and internal capsule, using AAV-GFP labeling of the axonal projections. Our subsequent DTI-MRI experiments showed that both young and aged pericyte-deficient animals have overall reduced white matter integrity, as well as axonal disorganization within myelinated regions. Histological data confirmed presence of disorganized fibers as well as reductions in myelin sheath thickness, relative abundance and volume in pericyte-deficient animals. Observed phenotypes may be attributed to vascular leakage and cerebral blood flow reductions, which are caused by pericyte loss. White matter and axonal changes correlate with behavioral deficits in pericyte-deficient animals. As neurovascular dysfunction and pericyte loss have been reported as a prominent feature of AD, these findings suggest that white matter injuries found in AD patients may, at least in part, stem from pericyte degeneration and vascular abnormalities.

Disclosures: G.L. Si: None. **A. Montagne:** None. **Z. Zhao:** None. **A.P. Sagare:** None. **A.M. Nikolakopoulou:** None. **B. Zlokovic*:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.24/K7

Topic: C.09. Demyelinating Disorders

Title: Correlation between iron deposition and increased microglia activation in MS: Subcortical grey matter and cortical white matter differences

Authors: *E. B. JOHNSON
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Abstract: Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system and microglia are implicated in its pathogenesis. The development of the disease is accompanied by the activation of microglia. Previous studies have shown Magnetic Resonance Imaging (MRI) scans displaying transverse relaxivity R_2* and frequency shifts that characterize gradient echo signal decay which are closely correlated with iron and myelin occurrences within brain tissue. A trend of iron accumulation in the core and perilesional areas of MS has been reported while increased density of activated microglia is observed in areas showing iron accumulation. However, to our knowledge, neither a direct link between regions of MRI signal change and lesional iron accumulation, nor the causal relationship between iron accumulation and microglia activation within MS lesions have been fully determined. To investigate the link between MRI signal intensity and increased microglia activation as a result of iron accumulation tissue was analyzed from MS patients whose brain were previously scanned *in situ* with high field quantitative MRI (R_2* mapping, phase susceptibility imaging). *In situ* MRI was correlated with Perl's staining for ferric iron, Weil's staining for myelin, and immunohistochemistry to detect iron deposition and microglial morphology, within patient tissues including lesions in white matter and regions of iron-rich deep grey matter. Additional analyses were used to quantify microgliosis and regions of iron accumulation. Findings indicate two discrete patterns of iron deposition, which may suggest distinct pathological mechanisms of iron accumulation based on the location of the lesions within cortical white matter as well as within non-lesional deep grey matter. The existence of intra- and inter-patient variations of iron accumulation within lesions and within non-lesional deep grey matter may be found to act as an important marker for disease progression and pathology.

Disclosures: E.B. Johnson: None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.25/K8

Topic: C.09. Demyelinating Disorders

Support: NIH Grant R01NS60822

Heep Fellowship, Texas A&M University

Title: The effect of social disruption stress on pain behaviors and cognitive deficits in chronic phase TMEV infection

Authors: *H. R. LINSENBARDT[1,2], J. L. COOK[2], E. E. YOUNG[2], E. G. VICHAYA[2], C. R. YOUNG[2], N. REUSSER[2], R. STORTS[2], C. J. WELSH[2,1], M. W. MEAGHER[2,1]
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Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS, with symptoms including motor impairments, sensory deficits, pain, and cognitive impairments. Pain and cognitive deficits affect over half of MS patient, but little is know about the underlying mechanisms. The present study used the Theiler's murine encephalomyelitis virus (TMEV) model to characterize changes in pain and memory during late disease and to determine whether social disruption stress (SDR) exacerbates these impairments. Although previous TMEV studies have relied on withdrawal reflex measures (Lynch et al., 2008), these measures do not reflect the affective and motivational experience of pain. Alternative measures of pain, such as fear conditioning, that reflect the aversive motivational significance of pain, have started to be used in pain research but have not been applied to models of MS. Likewise, previous EAE studies of MS-like memory impairments have relied on the Morris water maze task (D'Intino et al., 2005), however performance may be confounded by motor impairments. Thus, alternative measures of memory, such as the novel object recognition task, that minimize motor performance demands should be explored. To this end, we examined the effects chronic TMEV infection and SDR have on behavioral measures of pain, cognition, and motor impairments. Male SJL mice were assigned to 3 groups: stress + TMEV, unstressed + TMEV, unstressed + vehicle. Following the SDR stressor, mice were injected with TMEV or vehicle after which assessments were taken until day 177 post-injection. Behavioral measures included fear conditioning to assess the affective dimension of pain and the novel object recognition to assess recognition memory impairments. Consistent with prior studies, TMEV infection led to impaired motor function during the acute and chronic phases. However, the effects of stress on motor function were mixed during the acute phase. Importantly, stress exacerbated the effect of infection on sensitivity to mechanical stimuli, and fear conditioning to footshock was enhanced in the stressed infected mice, suggesting that the affective dimension of pain was enhanced. Furthermore, stress impaired memory in the novel object recognition test in infected mice. Histological analyses revealed that the unstressed uninfected controls had significantly fewer lesions than infected mice. Infected mice also had increased hippocampal CA1 lesions which was negatively correlated with decreased memory consolidation. Our results support the use of fear conditioning and novel object recognition in the study of MS-induced impairments.

Disclosures: H.R. Linsenbardt: None. **J.L. Cook:** None. **E.E. Young:** None. **E.G. Vichaya:** None. **C.R. Young:** None. **N. Reusser:** None. **R. Storts:** None. **C.J. Welsh:** None. **M.W. Meagher:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.26/K9

Topic: C.09. Demyelinating Disorders

Support: Frontiers Clinical Pilot Funding

Title: Do Glyoxalase 1 enzyme levels differ with people peripheral neuropathy?

Authors: *S. ALOTHMAN[1], N. WILSON[2], J. RYALS[2], L. D'SILVA[1], L. HERBELIN[3], M. PASNOOR[3], P. KLUDING[1], D. WRIGHT[2]
[1]Physical Therapy and Rehabil. Sci., [2]Anat. & Cell Biol., [3]Neurol., The Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Introduction: Glyoxalase 1 (Glo1) is a key enzyme that breaks down reactive dicarbonyls and helps prevent the buildup of advanced glycation end-products (AGE). AGE accumulation, combined with reduced neurotropic and insulin support, glucose toxicity, and oxidative stress, contributes to the development of diabetic peripheral neuropathy (DPN). The purpose of this study is to determine the levels of Glo1 in blood and skin in people with DPN or cryptogenic (idiopathic) neuropathy. We hypothesize that Glo1 levels are lower in people with DPN compared to people without neuropathy. Methods: Participants in this pilot study were assigned to one of four groups based on their diabetic and neuropathy status: 1) no diabetes and no DPN, 2) diabetes without DPN, 3) diabetes with DPN, and 4) cryptogenic neuropathy and no diabetes. Nerve conduction studies, quantitative sensory testing, and clinical examinations were used to calculate a Total Neuropathy Score, a measure of DPN severity. The primary outcome measure is fasting blood Glo1 levels. Glo1 levels will be correlated with the severity of DPN. Results: Preliminary results demonstrate a trend of lower blood Glo1 activity in people with DPN and in people with cryptogenic neuropathy compared to normal controls. Complete results on Glo1 levels in blood and skin are pending. Between groups statistical comparison will be completed with an ANOVA. Discussion: If our hypothesis is supported, targeting AGEs through increased Glo1 enzyme activity might be a new treatment option to lessen the symptoms of DPN. Understanding the relationship between Glo1 and neuropathy severity is the first step in establishing clinical trials investigating the therapeutic effects of agents regulating Glo1 level in patients with DPN.

Disclosures: S. Alothman: None. **N. Wilson:** None. **J. Ryals:** None. **L. D'Silva:** None. **L. Herbelin:** None. **M. Pasnoor:** None. **P. Kluding:** None. **D. Wright:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.27/L1

Topic: C.09. Demyelinating Disorders

Title: Immunohistochemical characterization of multiple sclerosis plaques in human brain

Authors: *J. BAUN[1], B. TIPTON[1], C. ZURHELLEN[1], C. SEGOVIA[1], R. C. SWITZER III[1], S. O. AHMAD[2]
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Abstract: Multiple Sclerosis (MS) is a demyelinating disease with a complex pathological profile that includes myelin degeneration, neuronal damage, and immune cell infiltration in the areas containing plaques. We evaluated the pathology associated with MS in the brain of a 39 year old female whose cause of death was unrelated to the disease. In acute plaques the amino cupric silver method (de Olmos) revealed a dense core of degenerating nerve cells and fibers. Chronic lesions had little staining of cells or fibers and were devoid of staining by the Nissl counterstain, Neutral Red. Another silver stain, the silver nucleolar stain (AgNOR) was developed to reveal the nucleolar organizing regions in cancerous cells. We utilized the stain here to reveal the differences in interior cellularity between acute and chronic plaques. This is useful in getting accurate counts of the cell populations present in brain regions undergoing demyelination, and has proven to be a useful tool for stereological purposes. Weil-Myelin staining revealed roughly spherical plaques devoid of myelin staining. Nissl staining with Thionine distinguished acute and chronic lesions. Acute lesions appeared to be surrounded by a dense band of cells while the interior of the plaque had a normal distribution of cells. In chronic lesions the core was much lighter suggesting a loss of cells. The Perl's iron stain revealed a paucity of staining in acute lesions. Chronic lesions were surrounded by iron positive cells, some of which appeared to be phagocytic and filled with debris. Iba-1 immunoreactivity in acute plaques was observed both in the center of the plaque and in a dense ring of immunoreactive microglia surrounding the plaque. In chronic lesions the central immunoreactivity was diminished, but the ring of cells surrounding the plaque appeared thicker and more dense. Staining of near adjacent sets of serial sections reveals the chemoarchitechtural differences between acute and chronic states in MS lesions.

Disclosures: **J. Baun:** None. **B. Tipton:** None. **C. Zurhellen:** None. **C. Segovia:** None. **R.C. Switzer III:** None. **S.O. Ahmad:** None.

Poster

224. Demyelinating Disorders: Human Studies and Therapeutics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 224.28/L2

Topic: C.09. Demyelinating Disorders

Support: MS society 978/12

Title: The role of chronic meningeal pro-inflammatory cytokine expression in grey matter pathology in multiple sclerosis

Authors: ***R. JAMES**, E. J. BROWNE, R. M. SCHALKS, N. D. MAZARAKIS, R. REYNOLDS
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Abstract: The secondary progressive phase of multiple sclerosis (SPMS) is characterized by accumulating axonal loss and grey matter pathology that at present cannot be adequately treated. Subpial demyelinated lesions in cerebral cortical grey matter are suggested to result from the diffusion of pro-inflammatory cytokines from areas of meningeal inflammation into the brain parenchyma. The presence of tertiary lymphoid organ-like (TLO) structures within the meninges has been reported in a large proportion of SPMS cases and is associated with immune cell recruitment, greater cortical demyelination, substantial neuronal loss and shorter disease duration. We have shown a 2-fold increase in lymphotoxin-α (LTα) gene expression by RT-qPCR in human postmortem MS meningeal tissue compared to controls. To test the hypothesis LTα might drive TLO formation in MS we have developed an animal model of cortical demyelination driven by meningeal inflammation involving acute or chronic delivery of cytokines into the subarachnoid space (SA) of the rat brain. In acute studies, recombinant LTα and IFNγ were stereotaxically injected into cortical SA of DA rats immunised 21 days previously with low dose MOG sufficient to induce an anti-myelin humoral response without CNS infiltration and clinical symptoms of EAE. 7 days following cytokine delivery there was a substantial increase in CD4+/CD8+ T-cells and microglial inflammation along with focal B cell-rich areas within dense SA cellular aggregates in IFA- and MOG-immunised animals compared to PBS injected controls. In MOG-immunised animals, extensive subpial demyelination was

observed underlying these cellular aggregates in a pattern highly reminiscent of subpial lesions observed in MS cortex. No demyelination was observed in IFA-immunised animals injected with LTα + IFNγ, suggesting demyelination is not a direct cytotoxic effect of the cytokines but required the development of a specific anti-myelin autoimmune response. To study the effects of chronic cytokine expression we injected a high titre VSV-G pseudotyped lentiviral vector (LV) expressing either GFP or human LTα under the control of a CMV promoter into the SA. The LV vector is able to induce long-term GFP expression up to 12 weeks after injection that is localised in epithelioid cells of the meninges, subpial astrocyte end-feet and a few pyramidal neurons. Aggregates of cells including CD8+ T-cells were observed in the sagittal sulcus and SA together with cells expressing human LTα at 28 days post injection. Our results suggest that LTα is a potent inducer of meningeal inflammation and thus may contribute to subpial demyelination lesion formation and clinical progression in MS.

Disclosures: R. James: None. **E.J. Browne:** None. **R.M. Schalks:** None. **N.D. Mazarakis:** None. **R. Reynolds:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.01/L3

Topic: C.10. Trauma

Support: AHA 13GRNT15730001, NIH K01AG031926, R01AT007317, and R01NS078026

Title: Mouse models of intracerebral hemorrhage in ventricle, cortex, and hippocampus

Authors: *Y. GAO[1,2], W. ZHU[2], C.-F. CHANG[2], J.-R. WAN[4], S.-S. ZHU[3], J. WANG[2]
[1]The First Clin. Col., Wuhan Univ. Renmin Hosp., Hubei, China; [2]Anethesiology & Critical Care Med., [3]Psychiatry and Behavioral Sci., Johns Hopkins University, Sch. of Med., Baltimore, MD; [4]Biol. Sci., Illinois Inst. of Technology, Col. of Sci., Chicago, IL

Abstract: Intracerebral hemorrhage (ICH) is a devastating condition. Existing preclinical ICH models focus largely on striatum but neglect other brain areas such as ventricle, cortex, and hippocampus. Clinically, however, hemorrhagic strokes do occur in these other brain regions. In this study, we established mouse hemorrhagic models that utilize stereotactic injections of autologous whole blood or collagenase to produce ventricular, cortical, and hippocampal injury. We validated and characterized these models by histology, immunohistochemistry, and

neurobehavioral tests. In the intraventricular hemorrhage (IVH) model, C57BL/6 mice that received unilateral ventricular injections of whole blood (25 microliters) demonstrated bilateral ventricular hematomas, ventricular enlargement, and brain edema in the ipsilateral cortex and basal ganglia at 72 h. Unilateral injections of collagenase (150 U/ml, 0.4 or 0.2 microliter, respectively) caused reproducible hematomas and brain edema in the frontal cortex in the cortical ICH (c-ICH) model and in the hippocampus in the hippocampal ICH (h-ICH) model. Immunostaining revealed cellular inflammation and neuronal death in the periventricular regions in the IVH brain and in the perihematomal regions in the c-ICH and h-ICH brains. Locomotor abnormalities measured with a 24-point scoring system were present in all three models, especially on days 1, 3, and 7 post-ICH. Locomotor deficits measured by the wire-hanging test were present in models of IVH and c-ICH, but not h-ICH. Interestingly, mice in the c-ICH model demonstrated emotional abnormality, as measured by the tail suspension test and forced swim test, whereas h-ICH mice exhibited memory abnormality, as measured by the novel object recognition test. All three ICH models generated reproducible brain damage, brain edema, inflammation, and consistent locomotor deficits. Additionally, the c-ICH model produced emotional deficits and the h-ICH model produced cognitive deficits. These three models closely mimic human ICH and should be useful for investigating the pathophysiology of ICH in ventricle, cortex, and hippocampus and for evaluating potential therapeutic strategies. Key words: cognition; emotion; forced swim test; intracerebral hemorrhage; motor; tail suspension test; wire-hanging test Support Contributed By: Grants from AHA and NIH

Disclosures: Y. Gao: None. **W. zhu:** None. **C. Chang:** None. **J. Wan:** None. **S. Zhu:** None. **J. Wang:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Program#/Poster#: 225.02/L4

Topic: C.10. Trauma

Support: DoD grant W81XWH-10-1-0578

Sanford School of Medicine Summer Research Program

UDiscover undergraduate research program

Title: The effects of mild traumatic brain injury and psychosocial stress on limbic anxiety-like behaviors are mediated by glucocorticoid receptors

Authors: L. FOX, D. DAVIES, J. SCHOLL, D. MEYER, *M. J. WATT, G. FORSTER
Basic Biomed. Sciences, Sanford Sch. Med., Univ. of South Dakota, VERMILLION, SD

Abstract: Mild traumatic brain injury (mTBI) occurs in settings ranging from sports to warfare, often associated with heightened arousal or stress. Consequences of mTBI include generalized anxiety and symptoms of posttraumatic stress disorder. We developed an animal model of mTBI with psychosocial stress to better simulate realistic conditions for mTBI acquisition, and sought to determine the consequences of this perturbation on anxiety-like and fear behaviors. Adult male Sprague-Dawley rats were subjected to social defeat or control conditions immediately prior to mTBI (induced by weight-drop) or sham surgery. Peripheral corticosterone levels were measured at the time of surgery using ELISA, and behavioral testing was conducted 7 days following surgery. The experience of social defeat immediately prior to surgery significantly increased plasma corticosterone levels. Either mTBI or social defeat alone increased anxiety-like behaviors in the elevated plus maze (EPM), but the combination of mTBI and psychosocial stress further increased anxiety states. Furthermore, mTBI, social defeat or the combination of mTBI with social defeat increased fear conditioning as compared to controls, but only mTBI with social defeat impaired extinction of the fear response. Next, we determined whether corticosterone receptors (glucocorticoid or mineralocorticoid) underlie the effects of mTBI with psychosocial stress on anxiety-like behaviors. Adult male Sprague-Dawley were injected (sc.) with spironolactone (50 mg/kg), mifepristone (20 mg/kg), or a propylene-glycol vehicle, 40 minutes prior to the social defeat or control conditions, which were immediately followed by mTBI or sham surgery. Rats were tested in the EPM 8-9 days post-surgery. Mifepristone, but not spironolactone, was able to prevent heightened anxiety-like behaviors induced by mTBI combined with social defeat, suggesting that glucocorticoid receptors play a role in modulating anxiety-like behavior following mTBI with psychosocial stress. Therefore, early targeting of glucocorticoid receptors may provide a novel direction in preventing mTBI-associated affective symptoms.

Disclosures: L. Fox: None. **D. Davies:** None. **J. Scholl:** None. **D. Meyer:** None. **M.J. Watt:** None. **G. Forster:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.03/L5

Topic: C.10. Trauma

Support: NINDS Grant HD061944-01

Title: The effect of levetiracetam treatment on gene expression and functional behavior using a controlled cortical impact model of traumatic brain injury

Authors: *B. E. ELMORE[1], G. D. ANDERSON[2], E. D. KANTOR[2], F. M. FARIN[3], T. K. BAMMLER[3], J. W. MACDONALD[3], M. R. HOANE[1]
[1]Psychology, Southern Illinois Univ. - Carbondale, Carbondale, IL; [2]Pharm., [3]Envrn. and Occup. Hlth. Sci., Univ. of Washington, Seattle, WA

Abstract: Traumatic brain injury (TBI) is one of the leading causes of death and disability in the United States, affecting more than 1.7 million Americans each year. Phenytoin has been the standard of care for prevention of acute seizures for 7 days after TBI. However, phenytoin, a broad-spectrum enzyme inducer, can decrease the concentrations and effect of other drugs and has been shown not to decrease the incidence of post-traumatic epilepsy (PTE). Unlike phenytoin, levetiracetam (LEV), has antiepileptogenic effects in animals models and has been identified as a potential candidate for treating not only PTE, but also in enhancing recovery function following TBI. It is currently approved for use as an adjunct therapy for seizure disorders, and contains a unique pharmacological profile belonging to the pyrrolidine class of drugs. Unlike phenytoin, LEV has been well tolerated in clinical populations without significant side effects, and the need for serum monitoring. The current understanding of its mechanisms is incomplete, but past research has found that it reduces high voltage activated calcium currents, up-regulates the expression of glutamate transporters, and decreases regional IL-1β expression. The purpose of the current study was to evaluate the effect of LEV on gene expression and functional recovery in a CCI model of TBI using a clinically relevant dose and regimen. The total sample consisted of 65 male Sprague-Dawley rats in two experiments. Animals were randomly assigned to one of three treatment conditions: TBI + vehicle (0.9% sterile saline), TBI + Levetiracetam (14 mg/hr/Kg), and Sham TBI + vehicle. A moderate to severe unilateral parietal injury was induced (AP: -2.4, ML: 2.4, ID: 2.5) at a velocity of 3 mm/s using a 4 mm tip. Treatment with LEV was administered for 7 days using osmotic infusion pumps, with an i.p. loading dose of 50 mg/Kg. This dosing regimen was determined to provided serum concentrations associated with clinically relevant doses. Microarray-based transcriptional profiling was done to access gene expression. Recovery of function was assessed using the locomotor placing task, rotor-rod task, and the Morris water maze. The results showed no evidence of any functional detrimental effects from LEV treatment, but changes in gene expression were observed. With the need to provide AED prophylaxis in TBI, it will be important to determine the effect of LEV in combination with other potential neuroprotective agents (i.e. nicotinamide, progesterone).

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Poster

225. Traumatic Brain Injury: Animal Studies

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.04/L6

Topic: C.10. Trauma

Support: NIH Grant R01NS081189

Title: Exosomes derived from multipluripotent mesenchymal stromal cells improve functional recovery and promote brain remodeling in rats after traumatic brain injury

Authors: Y. ZHANG[1], M. CHOPP[2,3], Y. MENG[1], M. KATAKOWSKI[2], H. XIN[2], A. MAHMOOD[1], *Y. XIONG[1]
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Abstract: Object. Transplanted multipotent mesenchymal stromal cells (MSCs) improve functional recovery in rats after traumatic brain injury (TBI). Here, we test a novel hypothesis that systemic administration of cell-free exosomes generated from MSCs promotes functional recovery and neurovascular remodeling in rats after TBI. Methods. Wistar rats were subjected to TBI followed by tail vein injection of 100 μg protein of exosomes derived from MSCs or an equal volume of vehicle phosphate-buffered saline (n = 8/group) 24 hours later. To evaluate cognitive and sensorimotor functional recovery, the modified Morris water maze, neurological severity score and footfault tests were performed. Animals were sacrificed at 35 days after TBI. Histopathological and immunohistochemical analyses were performed for measurements of lesion volume and neurovascular remodeling (angiogenesis and neurogenesis). Results. Compared with saline-treated controls, exosome-treated TBI rats showed significant improvement in spatial learning at 34-35 days measured by the Morris water maze test ($p < 0.05$), and sensorimotor functional recovery, i.e., reduced neurological deficits and footfault frequency, observed at 14-35 days post injury ($p < 0.05$). Exosome treatment significantly increased the number of newborn endothelial cells in the lesion boundary zone and dentate gyrus, and significantly increased the number of newborn immature and mature neurons in the dentate gyrus. Conclusions. We, for the first time, demonstrate that MSC-generated exosomes significantly improve functional recovery, at least in part, by promoting endogenous

angiogenesis and neurogenesis in rats after TBI. Thus, MSC-generated exosomes may provide a novel cell-free therapy for TBI and possibly other neurological diseases.

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Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.05/L7

Topic: C.10. Trauma

Support: Department of Neurology, Beth Israel Deaconess Medical Center

University of Connecticut Office of Undergraduate Research

Title: Are there strain differences to anatomical and behavioral sequelae of repetitive mild traumatic brain injury in mice?

Authors: *G. D. ROSEN[1], G. C. JOHNSON[1], T. KEHINDE[1], K. O'CONNELL[2], A. RENDALL[2], R. H. FITCH[2]
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Abstract: Scientific interest on the effects of repetitive mild TBI (RmTBI) has grown in the past decade, undoubtedly propelled by the increased incidence and awareness of concussions sustained by participants in ongoing military conflicts as well as those who play contact sports. RmTBI can produce a constellation of acute and chronic symptomology (post-concussive syndrome, PCS), and the serious consequences of RmTBI_chronic traumatic encephalopathy (CTE)_are associated with cognitive and psychiatric disturbances, increased co-morbidity of neurodegenerative disorders, as well as an increase in tau deposition in selected regions of the brain. Despite the dramatic increase in attention to this serious problem, our understanding of the risk factors that can predict outcome_CTE, PCS, etc._is severely limited. The research presented here will use a mouse model to address the following question: Why is it that some individuals can sustain multiple concussions with no lingering effects, whereas others with fewer injuries are devastated? We aim to determine the biological and/or genetic markers than can help us predict outcome following RmTBI, specifically examining two important risk factors_age of injury and

genetics. We used a weight drop injury model that effectively mimics the physiological and cognitive effects of RmTBI, but without the severe brain damage that is typically associated with rodent models (Kane et al., J Neurosci Methods. 2012;203(1):41-9). In this experiment, we exposed adolescent (5 weeks of age) and adult (11 weeks of age) male mice from two strains (C57BL6/J and DBA/2J) to 5 successive days of mild TBI or a sham procedure. One cohort of mice were sacrificed 30 days after the last injury and their brains removed, and the remaining mice were given a battery of behavioral tests. Brains were assayed for phospho-tau by Western Blot. In addition, we used highly efficient and accurate stereological procedures to estimate the number of GABAergic neurons in the cerebral cortex, in order to assess changes in GABAergic tone associated with RmTBI. Behavioral outcome measures included assessments of anxiety and social behaviors, as well as measures of sensory processing and memory. Understanding the effects of age and genetics (and, importantly, their interaction) on RmTBI susceptibility could lead to ameliorative or even preventative treatments.

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Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: NIH T32 DK007052

NIH R01 DK007052

NIH R01 NS079061

Title: Central and systemic levels of pigment epithelium-derived factor are altered in the rat following traumatic brain injury

Authors: *V. L. REEVES[1], S. W. CARLSON[2], E. E. KERSHAW[1], C. E. DIXON[2]
[1]Medicine, Div. Endocrinol. and Metabolism, [2]Neurosurg., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Background: Pigment epithelium-derived factor (PEDF), a multifunctional protein found in multiple tissues, is associated with metabolic effects as well as anti-angiogenic and neurotrophic properties. These effects may be protective in the setting of brain injury and offer a

potential biological therapeutic target for the treatment of traumatic brain injury (TBI). Changes in the systemic and brain abundance of PEDF in the context of TBI are unknown. Methods: To examine the effect of TBI on serum and brain PEDF concentrations, anesthetized male Sprague-Dawley rats were subjected to controlled cortical impact (CCI) brain injury (4m/sec, 2.7mm deformation) or sham injury and euthanized 1 week post-injury. The abundance of PEDF in the serum, white and brown adipose tissue, muscle, pancreas, liver, and multiple brain regions were assessed by ELISA. To determine the localization of PEDF in cell types in the brain, the incidence of colocalization between PEDF and markers of neurons, astrocytes, endothelial cells, microglial, and oligodendrocytes were evaluated by immunohistochemistry. Results: Brain-injured rats exhibited a significant increase in the concentration of PEDF in brown adipose tissue one week post-injury, compared to sham-injured rats. In the brain, CCI-injured rats exhibited a significant increase in PEDF in the contused cortex, hippocampus, and striatum, compared to sham-injured rats. PEDF immunoreactivity appeared to colocalize with markers of astrocytes, vasculature, endothelial cells, neurons, and oligodendrocytes in contused brain regions following brain injury. Conclusions: Following CCI-injury, an elevation in PEDF abundance was observed in multiple systemic and central tissues. Our findings highlight that not only does CCI-injury result in regional specific elevations in PEDF concentrations in glial, endothelial, and neuronal cells following TBI, but also CCI-injury results in peripheral changes in PEDF concentrations in metabolically active tissues. These results suggest that PEDF is modulated by TBI both in the peripheral tissue and in brain tissue.

Disclosures: V.L. Reeves: None. **S.W. Carlson:** None. **E.E. Kershaw:** None. **C.E. Dixon:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.07/L9

Topic: C.10. Trauma

Support: NSC 102-2314-B-038-024

Title: Knockout of androgen receptor decreases the expression of aquaporin-4 following traumatic brain injury

Authors: Y.-H. CHEN[1], C.-C. TSENG[2], *L.-Y. YANG[3]
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Abstract: Traumatic brain injury (TBI) is one leading global health issue and has been estimated to affect more than 10 million people each year. The pathophysiology of TBI is quite complex and is still poorly understood. Androgens have been shown to exert a beneficial effect on injured neurons and to reduce the expression of microglia and reactive astrocytes following brain injury. It is generally accepted that androgen receptor mediates the neuroprotective effect of androgens. Our recent data show that deletion of androgen receptor enhances the TBI-induced expression of glial fibrillary acidic protein (GFAP). Evidence has shown that the water-channel protein aquaporin-4 (AQP4) has been involved in the regulation of water balance in the brain. However, it remains undetermined if deletion of androgen receptor affects the expression of AQP4 following TBI. In this study, we investigated the impact of deletion of androgen receptor on the expression of AQP4 following TBI using male androgen receptor knockout (ARKO) mice and male control littermates. We induced brain injury using the cortical impact device and evaluated the expression of AQP4 4 and 24 h after the injury. Our preliminary results showed that knockout of androgen receptor decreased the expression of AQP4 4 and 24 h following TBI. Moreover, the AQP4 expression in the injured brain was significantly lower in male ARKO mice than in male controls. Because deletion of AQP4 has been shown to decrease the water efflux in vasogenic edema and to aggravate the edema in the obstructive hydrocephalus, our findings strongly suggest that deletion of androgen receptor worsens brain edema following TBI.

Disclosures: Y. Chen: None. **L. Yang:** None. **C. Tseng:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.08/L10

Topic: C.10. Trauma

Support: NFL Charities Medical Research Grant

Title: Evaluation of behavioral paradigms to assess functional deficits following single and multiple mild traumatic brain injuries in a murine model

Authors: *J. N. NICHOLS, T. L. NIEDZIELKO, S. A. EPPS, C. L. FLOYD
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Abstract: Mild and repeated mild traumatic brain injury (mTBI) is a major health concern, particularly for military personnel and individuals participating in contact sports. In the United

States, it is estimated that 1.7 million persons sustain a TBI each year, of which approximately 75% are mTBI. Individuals that acquire an mTBI typically experience cognitive and neuropsychiatric deficits with individuals sustaining multiple mTBIs having a worse prognosis. Cognitive deficits include impairments of attention and learning/memory, while neuropsychiatric changes can consist of sleep disturbances, obsessive compulsive behaviors, depression, anxiety, and aggression. The use of animals to elucidate the underlying mechanisms of mTBI-associated deficits is critical for understanding of the pathophysiology and development of novel therapeutics. Although there have been behavioral assessments established to evaluate moderate-severe TBI, elucidation of robust behavioral tests that are sensitive to mTBI has been challenging and inconclusive. Consequently, the goal of this study was to evaluate the behavioral changes incurred after single and multiple instances of mTBI utilizing a panel of behavioral tests. We utilized an impact-acceleration model to induce single or multiple (3) mTBI(s) in adult male C57BL/6 mice with an inter-injury interval of 24 hours. Acutely following injury or sham surgery, mice were subjected to an array of tasks to assess the extent of functional deficits. Attention and learning/memory was assessed using Bussey chambers and the reversal Morris water maze (rMWM). An automated vibration platform (LABORAS chamber) was used to track ethological behaviors such as immobility and grooming. Neuropsychiatric symptoms were evaluated using the Porsolt swim test (depression), elevated plus maze (anxiety), and resident intruder task (aggression). No significant differences in cognitive performance tests were observed between sham, mTBI, and multiple mTBI groups. Data from the LABORAS chambers revealed decreased immobility duration and increased grooming duration in the multiple mTBI mice suggesting insomnia and obsessive behavior. When gauging despair behavior, we found that multiple mTBI mice spent less time immobile which could represent disinhibition. We also found that mTBI mice exhibited a reduction in aggressive behavior indicating a social impairment. Classically, the field has focused on tests of cognitive function to assess functional impairments after mTBI, but our results suggest that deficits might be better evaluated using tasks that target neuropsychiatric symptoms. Supported by NFL Charities Medical Research Grant.

Disclosures: **J.N. Nichols:** None. **T.L. Niedzielko:** None. **S.A. Epps:** None. **C.L. Floyd:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.09/L11

Topic: C.10. Trauma

Title: Acute inflammatory response in rodent brain and blood following a blast-induced traumatic brain injury

Authors: *C. C. TENN, N. CADDY, M. GARRETT, L. MCLAWS, C. VAIR
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Abstract: Introduction: The predominant cause of neurotrauma among the military population is exposure to a blast wave. The shockwave produced by an explosive device can travel through the brain causing mild brain damage without visible signs of injury. Traumatic brain injuries (TBI) are known to trigger a neurinflammatory cascade which contributes to the brain injury as well as long term neuronal damage and cognitive impairment. A number of inflammatory cytokines are released in response to TBI and their temporal profile could provide information about the injury severity. This study examined the central and systemic inflammatory response to TBI by comparing cytokine levels in rat brain and blood at various time points after a shockwave exposure. Methods: To create a blast-induced TBI, a custom designed shocktube capable of simulating a blast insult was used. Anaesthetized rats received a side-on, head only exposure to the shockwave at a pressure of 138 kPa. Controls underwent the same treatment except for the shockwave exposure. Following exposure, serum and brain tissues (hippocampus and cerebellum) were collected either at 6, 24 or 48 hours. The samples were assayed for several inflammatory cytokines (IL- 1α, IL-1β, IL-6, IL-10 and TNF-α) and chemokine levles (MCP-1 and MIP-1α) using a multiplex cytokine array system. Results: An acute cytokine response was observed in brain tissues and serum of animals exposed to the shockwave. Levels of IL-1α, IL-1β and IL-6 significantly increased in the brain at 6h post exposure as compared to controls. Serum levels of IL-1α and IL-6 significantly increased at the 6h time point only while IL-1β levels were elevated at the 24h and 48h time period. No differences were observed in TNF-α levels between the groups at any of the time points measured. The anti-inflammatory cytokine, IL-10 levels in brain samples showed an increase at 24h post-exposure and remained elevated up to 48h while serum levels for this cytokine did not show any difference from the controls until the 48h time point. The chemokines, MCP-1 and MIP-1α levels showed an early increase in both serum and brain tissues and persisted for hours following exposure. Conclusion: The results are consistent with TBI triggering an acute response of pro- and anti-inflammatory cytokines and chemokines. The early increase in several of these inflammatory cytokines observed in the brain before blood would suggest there is no clear relationship between the central and systemic inflammatory response to the brain injury induced by shockwave exposrue. Determining a cytokine profile inducedby TBI could provide insights into neuroprotective approaches to treating this type of injury.

Disclosures: C.C. Tenn: None. **N. Caddy:** None. **M. Garrett:** None. **L. McLaws:** None. **C. Vair:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.10/L12

Topic: C.10. Trauma

Support: Veterans Administration Merit funding

Title: CRMP2 modulation for treatment of TBI-induced cognitive dysfunction and pathology

Authors: *M. E. HARRIS-WHITE[1], A. POTESHKINA[1], M. F. JOHNSON[1], P. ESLAMI[1], K. M. VENKOVA-HRISTOVA[2], A. M. HRISTOV[2], K. HENSLEY[2]
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Abstract: Widespread traumatic damage to axons, termed diffuse axonal injury (DAI), may be a central contributor to functional/behavioral deficits following traumatic brain injury (TBI). Studies suggest axonal injury is not limited to time of injury and that progressive axonal injury continues many days post injury. What may not be readily apparent are the longer-term consequences of TBI that may manifest in this population. Risk for chronic neurodegenerative disease, including Alzheimer's disease (AD), is increased by TBI. Although a complex problem, science must identify safe and effective treatments that can be used both acutely and chronically to assist short-term recovery and prevent long-term neurodegenerative disease risk. Dysregulation of tau appears to be common across the different types of TBI. Chronic traumatic encephalopathy (CTE) is a progressive tau protein-linked disease associated with repetitive concussive injury in athletes and in military blast TBI. The frequent association of CTE with other disorders suggests that repetitive brain trauma and hyper phosphorylated tau protein deposition promote accumulation of other abnormally aggregated proteins including amyloid beta protein (Aβ). Although currently unclear how Aβ deposition and tau dysregulation following TBI are related, a destabilized proteostasis network (i.e., imbalanced axonal transport and autophagy) is a likely culprit. Similar to tau, CRMP2 is a microtubule associated protein though CRMP2 is functionally distinct from tau. CRMP2 stabilizes microtubules and facilitates protein trafficking important to maintenance of synapse stability. Manipulating CRMP2-dependent processes may affect development of AD pathology, specifically, APP processing and microtubule stability and may also be a useful TBI target. In the present study we show that lanthionine ketimine ester (LKE), a bioavailable derivative of a natural brain sulfur amino acid

metabolite, lanthionine ketimine, which we have previously shown to alleviate pathology and slow cognitive decline in the 3xTgAD mouse model, can spare cognition and pathology following DAI through mechanisms involving CRMP2. Methods: C57Bl6 mice underwent craniotomy plus moderate central fluid percussion injury (cFPI). Sham surgery (craniotomy) was performed on controls. Barnes maze was performed five weeks post surgery followed by histological analyses (APP, phospho c-Jun, Iba-1) and biochemical analysis (CRMP2, autophagy markers). cFPI mice fed 100 mg/kg/d LKE had significantly better Barnes maze performance and reduced phospho c-Jun staining in the corpus callosum and hippocampus compared to cFPI mice on control diet.

Disclosures: **M.E. Harris-White:** None. **A. Poteshkina:** None. **M.F. Johnson:** None. **P. Eslami:** None. **K.M. Venkova-Hristova:** None. **A.M. Hristov:** None. **K. Hensley:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Xonovo, Inc..

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.11/M1

Topic: C.10. Trauma

Support: Combat Casualty Care Research Program

Title: Energy-related biochemical changes in the peri-lesion area of striatum of conscious rats following penetrating ballistic-like brain injury

Authors: *L. LEUNG, M. WINTER, Y. DENG-BRYANT, D. SHEAR, F. TORTELLA
Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: This study utilized microdialysis in awake, freely moving rats to evaluate cerebral interstitial levels of glucose, lactate and pyruvate following unilateral frontal penetrating ballistic-like brain injury (PBBI). Pre-injury baseline measures were taken 2h prior to PBBI, from 1 to 4h post-injury and at 1, 2, 3, 7 and 14 days post-injury (DPI). The results were compared to those obtained from sham control group (received craniotomy only; n=9/group). Prior to injury, glucose concentrations in both groups were similar (PBBI = 2.82±0.4 mg/dL and Sham = 2.09±0.28 mg/dL). The glucose level following PBBI dropped to 1.32±0.48 mg/dL at 3h post-injury. It remained low for the first 72h, followed by an increase from 7 to 14 DPI. The

glucose concentration of control group remained near baseline levels with a graduate increase evident at 14 DPI. Interstitial lactate levels were significantly increased in the PBBI group during the first 24h following injury (baseline = 0.37±0.09 mmol/L; 24h post-PBBI = 0.88±0.19 mmol/L) and remained elevated out to 7 DPI (0.65±0.13 mmol/L). No significant changes in lactate concentration were detected over time in control group (baseline = 0.35±0.07 mmol/L; 24h post-PBBI = 0.48±0.07 mmol/L; 7 DPI = 0.29±0.08 mmol/L). Pyruvate levels did not differ between the sham (17.51±5.06 μmol/L) and PBBI animals (20.91±5.44 μmol/L) during the first 48h following injury but were significantly decreased at 3 DPI (13.64±6.29 in PBBI group vs. 24.18±6.87 μmol/L in sham control group; $p < .05$) and resolved by 7 DPI. Following PBBI, the lactate/pyruvate ratio increased significantly within the first 3h (2h post-PBBI = 132.04±29.39 vs. 2h-Sham = 53.90±22.74) and then dropped to levels comparable to sham control by 24h. The acute reduction in interstitial glucose levels following PBBI may be due to the increase in glucose utilization immediately after injury. Reduced metabolic activity near the lesion at later time points might account for the increased interstitial glucose concentration. Increased lactate levels within brain tissue are suggestive of dysfunctional oxidative metabolism and may be attributed to reduced cerebral blood flow and increased glycolysis following PBBI. The lactate/pyruvate ratio reflects the cellular redox state. Accordingly, increased lactate/pyruvate ratio suggests an impaired cellular respiration which may be related to mitochondrial dysfunction following PBBI. Overall, this study identified the temporal profile of energy dysregulation induced by PBBI in the peri-lesion area. These time-dependent alterations in brain metabolism may serve as therapeutic targets for treating brain trauma.

Disclosures: **L. Leung:** None. **M. Winter:** None. **Y. Deng-Bryant:** None. **D. Shear:** None. **F. Tortella:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

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Topic: C.10. Trauma

Support: NIH grant R01MH05190

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NIH NS049545

NIH NS030291

Miami Project to Cure Paralysis

Title: Traumatic brain injury induces astrocytic serine racemase expression and D-serine localization *in vivo*

Authors: *D. T. BALU[1], E. J. PEREZ[2], S. TAKAGI[1,3], J. T. COYLE[1], D. J. LIEBL[2,4]
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Abstract: D-serine, a co-agonist at the NMDA receptor (NMDAR), is synthesized from L-serine by the enzyme serine racemase (SR), which is heavily expressed in the forebrain. SR and D-serine were originally thought to be expressed in astrocytes. However, data from our laboratory and others has shown that SR and D-serine are primarily expressed in neurons *in vivo*. The original work describing SR and D-serine localization utilized primary astrocyte cultures derived from neonatal brains, which were recently shown not to be representative of mature astrocytes *in vivo* and display a gene expression profile more akin to reactive astrocytes. Therefore, to reconcile this discrepancy we examined the expression of SR and D-serine after controlled cortical impact (CCI), a model of traumatic brain injury known to induce reactive astrocytes. We found that CCI increased the amount of glial fibrillary acidic protein (GFAP)-positive astrocytes in the hippocampus, particularly in the ipsilateral hemisphere, in a time-dependent manner with peak expression occurring between 3 and 7 days post injury. Remarkably, CCI also induced the expression of SR in GFAP+ astrocytes and reduced neuronal SR expression in the ipsilateral hippocampus. However, SR expression remained primarily neuronal in the contralateral hemisphere, particularly at early time-points following CCI. D-serine localization also switched to GFAP+ astrocytes following CCI, although its transition occurred later than SR (7-14 days post-CCI). Our findings demonstrate that SR and D-serine can be found in astrocytes *in vivo*, but only under reactive conditions.

Disclosures: **D.T. Balu:** None. **E.J. Perez:** None. **S. Takagi:** None. **J.T. Coyle:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A patent owned by Massachusetts General Hospital for the use of D-serine as a treatment for serious mental illness could yield royalties for Dr. Coyle. F. Consulting Fees (e.g., advisory boards); served as a consultant for EnVivo, and Abbvie in the last 2 years. **D.J. Liebl:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.13/M3

Topic: C.10. Trauma

Support: FDA Medical Countermeasures Initiative (MCMi)

Title: Modification of somatosensory evoked potentials following focal traumatic brain injury in the mouse

Authors: *J. A. FISHER[1,2], S. HUANG[2], M. YE[2], M. NABILI[2], E. F. CIVILLICO[2], V. KRAUTHAMER[2], M. MYERS[2], C. G. WELLE[2]
[1]Dept. of Physiol., New York Med. Col., Valhalla, NY; [2]Ctr. for Devices and Radiological Hlth., U.S. Food and Drug Admin., Silver Spring, MD

Abstract: Traumatic brain injury (TBI) presents a significant challenge to civilian and military medicine. In the U.S., each year there are over 1.5 million TBIs, resulting in 50,000 deaths. Non-fatal brain injuries can also cause impairment that lead to long-term disability. Even mild TBI can have severe long-term consequences, such as cognitive deficits, psychiatric morbidities, epilepsy and increased risk for Alzheimer's and Parkinson's disease. Whereas clinical neuroimaging, namely CT and MRI, is an important tool for diagnosing TBI, such imaging is typically performed at a much later time relative to the initial injury, precluding the ability to monitor the progression of the injury and ensuing sequelae. Identifying easily obtainable, quantitative electrophysiological biomarkers could therefore represent a breakthrough for the diagnosis of TBI. Both early- and late-onset components of the somatosensory-evoked potential (SEP) elicited by mild electrical stimulation of the median nerve have been shown to reflect brain injury and are sensitive to even mild neural perturbations such as low-intensity ultrasound. We utilized high-intensity focused ultrasound (HIFU) to induce focal, calibrated brain injuries in anesthetized mice. Using micro-electrocorticography (μECoG) arrays, we recorded SEPs triggered by median nerve stimulation and mapped the triggered average responses across primary somatosensory and motor cortices. In preliminary experiments, focal HIFU insult reduced the amplitude and altered the temporal profile of late-onset (>50 ms post stimulus) SEPs. Adjusting the intensity and position of the injury also affected the signals recorded at the primary somatosensory cortex. These results represent a significant step toward establishing a quantitative electrophysiological biomarker for diagnosing TBI. From a regulatory standpoint, additionally, quantitative physiological metrics, such as electrophysiological signals, could improve the consistency and rapidity of the scientific evaluation of neurological devices used for the diagnosis of brain injury.

Disclosures: **J.A. Fisher:** None. **S. Huang:** None. **M. Ye:** None. **M. Nabili:** None. **E.F. Civillico:** None. **V. Krauthamer:** None. **M. Myers:** None. **C.G. Welle:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Program#/Poster#: 225.14/M4

Topic: C.10. Trauma

Support: FDA MCM2JXXXXX274MJ

Title: Electrophysiological signatures of brain injury in the mouse primary motor cortex

Authors: ***M. YE**[1], M. NABILI[1], J. FISHER[1,2], S. HUANG[1], Y. KIM[3], E. CIVILLICO[1], V. KRAUTHAMER[1], M. R. MYERS[1], C. WELLE[1]
[1]The Office of Sci. and Engin. Labs/CDRH, FDA, Silver Spring, MD; [2]Physiol., New York Med. Col., Valhalla, NY; [3]Ctr. for Neurosci. and Regenerative Med., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Diagnosis of mild brain injury is a clinical challenge due to the lack of objective biomarkers. Electrophysiological indices, such as quantitative EEG (qEEG), have the potential to serve as biomarkers for neural damage. Recent advances in EEG detection technology may soon allow for rapid, non-invasive signal detection on inexpensive and portable platforms. However, the signature changes in neural activity that accompany mild brain injury have not been well established. In our study, we identify electrophysiological signatures of mild brain injury in a novel mouse model. Brain activities are recorded with a 16-channel epidural micro-electrocorticography (μECoG, Neuronexus) array implanted on the primary motor cortex. Controllable high-intensity focused ultrasound (HIFU) pulses that mimic blast overpressure waves are used to produce brain injury in mouse (McCabe JT, et al. 2014). HIFU-induced brain injury is evaluated by immunohistochemical markers for microglial activation (Iba-1), astrocyte reactivity (GFAP), blood-brain barrier disruption (IgG), overexpression of phosphorylated-tau (p-tau), and assessed by the open field and rotarod behavioral tests. μECoG recordings and behavioral tests from freely moving animals are performed once per week for 4 consecutive weeks prior to the HIFU exposure for baseline characterization. The acute electrophysiological and behavioral responses following injury are collected immediately and 24 hours after the HIFU exposure. Chronic changes are monitored weekly, up to 8 weeks or until the electrophysiological signal patterns stabilize. The frequency content of all electrophysiological correlates is quantified

by multitaper fast fourier transform (FFT) following artifact rejection (Chronux toolkit and custom MATLAB code). At the completion of the chronic electrophysiological recording period, animals are sacrificed for histological evaluation of brain injuries. A long-term reduction in the lower frequency band of μECoG signals following HIFU exposure was observed in the pilot experiments. Also, preliminary data demonstrate an elevated expression of Iba-1 and GFAP in the corpus callosum and hippocampus 24 hours after HIFU exposure, in agreement with a previous report (McCabe JT, et al. 2014). These preliminary results indicate the potential of quantitative EEG as a brain injury biomarker, as well as the possibility for rapid brain injury detection using portable technologies.

Disclosures: **M. Ye:** None. **M. Nabili:** None. **J. Fisher:** None. **S. Huang:** None. **Y. Kim:** None. **E. Civillico:** None. **V. Krauthamer:** None. **M.R. Myers:** None. **C. Welle:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.15/M5

Topic: C.10. Trauma

Title: Cellular mechanisms underlying the baroreflex dysfunction in a rat model of traumatic brain injury

Authors: ***J. OH**[1], C.-K. LEE[2], K. WHANG[1], S.-W. JEONG[2]
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Abstract: The arterial baroreflex is one of the homeostatic mechanisms for maintaining blood pressure. In human patients, traumatic brain injury (TBI) is known to lower baroreflex sensitivity (BRS) which is associated with a high mortality rate. To date, cellular mechanisms underlying the TBI-blunted BRS remain unknown. With a hypothesis that the baroreflex dysfunction arises from autonomic imbalance, we tested whether TBI differentially modulates excitability of cardiac sympathetic and parasympathetic neurons. In this regard, TBI was induced in 8-week-old male Sprague-Dawley rats using a head impactor (TBI-0310, PSI, USA). Four week after TBI, the baroreflex was assessed by the phenylephrine pressor test. Compared with control rats (1.1 ± 0.09, n=4), BRS was significantly reduced in TBI group (0.57 ± 0.08, n=4). Under the gramicidin-perforated configuration of the current clamp, action potentials (AP) were recorded in sympathetic stellate ganglion (STG) and parasympathetic intracardiac ganglion (ICG) neurons. In response to current injection (1X, 2X, and 3X), the frequency of action potentials (AP) was

significantly increased in the STG neurons, while decreased in the ICG neurons of TBI group. TBI altered rheobase and AP duration in the opposite direction in the STG and the ICG without affecting other passive and active properties. Real-time PCR analysis revealed that expression of the K_A 3.3, K_A 3.4, K_A 4.1, K_A 4.2, and K_A 4.3 was down-regulated in the STG of TBI group. Consistent with this finding, A-type K^+ currents was significantly attenuated, which may increase excitability of the STG neurons. Interestingly, N-type Ca^{2+} channels were significantly down-regulated in the ICG neurons, which may possibly cause hypoexcitability. Taken together, these data suggest that TBI-blunted BRS arises from the imbalanced cardiac autonomic motor activities which is caused by the ionic mechanisms including down-regulation of either A-type K^+ or N-type Ca^{2+} channels.

Disclosures: **J. Oh:** None. **C. Lee:** None. **K. Whang:** None. **S. Jeong:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.16/M6

Topic: C.10. Trauma

Support: ERA-NET NEURON

Title: Increased expression of uPAR after traumatic brain injury in uPA deficient mice

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Abstract: Rational: The urokinase-type plasminogen activator (uPA) together with its reseptor, urokinase-type plasminogen activator receptor (uPAR) constitutes a proteolytic system associated with tissue remodelling after various epileptogenic insults, such as traumatic brain injury (TBI). The regulation of post-injury expression of different components of uPAR-interactome (ligands and receptors) is, however, poorly understood. In the present study we assessed the effect of uPA deficiency on the expression of its receptor, uPAR, after TBI. Methods: TBI was induced by controlled cortical impact (CCI, velocity 5m/s, depth 0.5 mm) on 12 wk old male Wt (n=8) and uPA (n=10) mice. After 4 days post-CCI mice were decapitated for qRT-PCR. Expression of uPAR and uPA genes were assessed in Ipsilateral and contralateral perilesional cortex, hippocampus and thalamus. Results: The expression of uPA mRNA in the

perilesional cortex was elevated 4-fold in the Wt-CCI group as compared to that in controls ($p<0.05$). The expression of uPAR mRNA was similarly upregulated in the perilesional cortex both in the Wt CCI (4-fold, $p<0.05$) and uPA -/- CCI groups (4-fold, $p=0.01$). There was no difference between the injured groups. In the uPA -/- CCI group uPAR mRNA was elevated also in ipsilaterally in the hippocampus (6-fold, $p=0.01$) and thalamus (4-fold, $p<0.05$). Conclusion: The present study shows that expression of uPAR mRNA after TBI is not compromised by genetic deficiency of its ligand uPA. Therefore, uPAR may stay functional for mediating the signaling initiated by other components of uPAR-interactome.

Disclosures: T. Bolkvadze: None. **A. Pitkänen:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Program#/Poster#: 225.17/M7

Topic: C.10. Trauma

Title: A neuroprotective effect of Salubrinal in traumatic brain injury

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Abstract: We have previously reported that mild traumatic brain injury (mTBI) induced various short and long term cognitive deficits as well as apoptosis in the mice brains. Apoptosis may be caused by prolonged and severe accumulation of misfolded proteins and protein aggregation in the endoplasmic reticulum (ER stress). In addition, we reported that mTBI activated the pro-apoptotic arm of the unfolded protein response (UPR). The main goals of the present study were to test the involvement of the eIF2α/ATF4 pathway (the translational/adaptive arm of the ISR (integrated stress response) in mTBI brains and to suggest this pathway as a potential therapeutic target. In order to do so we directly activated the translational/adaptive arm of the ER stress by salubrinal, which is a selective phosphatase inhibitor of p-eIF2α. We induced head injury with a non-invasive closed-head weight drop (30 gr). Salubrinal was injected to mTBI mice immediately and 24h after injury (1mg/kg, ip). The Y-maze and the Novel Object recognition tests (to assess spatial and visual memories, respectively) were conducted either 7 or 30 days post trauma. Salubrinal administration significantly reversed memory deficits following mTBI either 7 or 30 days after injury. In order to understand the mechanism that underlies salubrinal's neuroprotection, Western blot analysis was conducted: mTBI induced a significant reduction in

eIF2α phosphorylation, which was prevented by Salubrinal. While ATF4 (activating transcription factor 4), the immediate target molecule of eIF2α, was not altered in Salubrinal mTBI brains (with or without salubrinal), the phosphorylated ATF4 was significantly reduced in mTBI brains. This reduction was prevented by salubrinal administration. Our results show that targeting the adaptive arm of ER stress with Salubrinal may serve as a potential therapeutic strategy for brain damage.

Disclosures: C.G. Pick: None. **V. Rubovitch:** None. **S. Barak:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.18/M8

Topic: C.10. Trauma

Support: CDMRP Grant G170172513

Title: Development of a ferret model of traumatic brain injury; preliminary imaging, behavioral and immunohistochemical findings

Authors: *S. C. SCHWERIN[1], E. HUTCHINSON[3], K. NGALULA[1], C. PIERPAOLI[3], S. L. JULIANO[1,2]
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Abstract: In recent years, multiple preclinical models of traumatic brain injury (TBI) have emerged to investigate mechanisms of damage and plasticity following injury as well as to test novel therapeutic approaches. Mouse and rat models of TBI provide important insights into the brain's response to injury. However, the low relative volume of white matter and their lissencephalic cortex reduce their relevance to study hallmark human pathologies such as diffuse white matter injury. In contrast, the ferret's brain surface is highly folded and has a ratio of white to gray matter that is comparable to humans. These similarities may facilitate the translation of basic science discoveries to clinical research. In this study we developed a ferret model of TBI using controlled cortical impact (CCI). Methods. 9 adult male ferrets were included in the development of the surgical protocol. We then studies the effects of CCI in 4 additional ferrets (control, sham, mild, severe) using MRI, behavioral and histological outcomes with a 7 day survival. The mild CCI parameters were: bit diameter = 3 mm, penetration depth = 1 mm, impact

velocity = 3 m/s, and dwell time = 100 ms. For the severe CCI the depth was increased to 4 mm and the velocity was increased to 5 m/s. We acquired MRI in-vivo before and 1 day after surgery and ex-vivo. We tested behavioral abilities before and after CCI (6 hours; 1, 3, 7 days) including beam walk, gait analysis, open field, novel object recognition, righting reflex, and adhesive removal tests. Results. Surgical protocol improvements: 1. Retraction, not incision, of the large temporalis muscle covering the skull. 2. Closing of the cranial window is necessary to avoid direct pressure by the muscle on the brain. 3. Pre-surgical MRI for placement of the cranial window is advantageous due to considerable heterogeneity in cranio-cerebral geometry across ferrets. In-vivo MRI findings: At 1 day post injury we found T2W hyperintense regions in both injured ferrets, but greater in extent for the severe case. Ex-vivo MRI findings: At 1 week, we found increased T2 values in the perilesional cortex and white matter. DTI abnormalities included disrupted orientation near the lesion, reduced FA and abnormal MD diffusely in the body of the white matter. Behavioral findings: Motor, but not sensory deficit that was recovered by the seventh day. Histopathology revealed increased GFAP, microglia and extracellular matrix protein immunoreactivity in the injured brain. In conclusion, we have developed a CCI protocol for use in the ferret and our initial observations suggest the utility of this model as a sensitive and translationally relevant approach to the study of TBI.

Disclosures: S.C. Schwerin: None. **E. Hutchinson:** None. **K. Ngalula:** None. **C. Pierpaoli:** None. **S.L. Juliano:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: NIH Grants NS060005 and HD069620 (AEK)

Title: Lorazepam does not negatively impact neurobehavioral outcome after experimental brain trauma

Authors: K. E. FREE[1], J. B. LEARY[1], A. M. VOZAR[1], B. S. KIM[1], S. D. STEVENS[1], C. M. EDWARDS[1], J. P. CHENG[1], C. O. BONDI[1], *A. E. KLINE[2]
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Abstract: **Introduction:** Traumatic brain injury (TBI) affects 10,000,000 people worldwide, making it a significant health concern. In addition to motor and cognitive dysfunction, TBI also induces aggression and agitation, which hampers acute care and rehabilitation. To manage these behavioral dysfunctions, antipsychotic drugs (APDs) are administered. Studies from our laboratory have shown that chronic administration of APDs impedes the acquisition of spatial learning. Despite the negative effects, some form of sedation is necessary so that physicians can assess and treat disruptive patients. **Hypothesis:** Lorazepam, a benzodiazepine, will not produce deleterious effects on motor and cognitive outcome after experimental TBI. **Methods:** Twenty-eight anesthetized adult male rats received either a controlled cortical impact (2.8 mm tissue deformation at 4 m/sec) or sham injury and then were randomly assigned to 4 groups where a TBI and corresponding sham group received either lorazepam (1.0 mg/kg; i.p.) or saline vehicle (1.0 mL/kg; i.p.) once daily for 19 days. Motor function and cognition were assessed using established tests on days 1-5 (beam-walk) and 14-19 (water maze), respectively. **Results:** No significant motor and cognitive differences were revealed between the TBI + lorazepam and TBI + vehicle groups ($p = 0.60$ and $p = 0.09$, respectively). **Conclusions:** These results suggest that daily administration of lorazepam (1.0 mg/kg) does not impair motor and cognitive outcome after experimental TBI, which is unlike that reported for APDs. **Significance:** Lorazepam should be considered as an alternative treatment to control clinical TBI-induced agitation and aggression as it will allow for patient assessment and treatment without impairing subsequent recovery. Ongoing studies are determining the range of doses that can be safely administered.

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Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: NIH Grant NS060005 (AEK)

NIH Grant HD069620 (AEK)

Title: Attentional set-shifting after brain trauma is restored by a preclinical model of neurorehabilitation

Authors: *C. O. BONDI[1], J. P. CHENG[1], H. M. TENNANT[1], N. LAJUD[3], K. E. FREE[1], C. M. MONACO[1], J. B. LEARY[1], A. E. KLINE[2]
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Introduction: Cognitive impairment associated with prefrontal cortical dysfunction is a major component of disability in traumatic brain injury (TBI) survivors. Specifically, deficits in executive function and behavioral flexibility are present across all injury severities. While impairments in spatial learning have been extensively reported, experimental models of TBI investigating more complex cognitive disabilities are relatively scarce. We have begun to employ the attentional set-shifting test (AST), a complex cognitive paradigm analogous to the Wisconsin Card Sorting Test, which is used to measure strategy-switching deficits in patients with frontal lobe damage, TBI, and psychiatric disorders. Previously, we demonstrated that a controlled cortical impact (CCI) injury produced significant impairments in executive function and cognitive flexibility in the AST. **Hypothesis:** Environmental enrichment (EE), a preclinical model of neurorehabilitation, will restore cognitive performance post-injury in the AST. **Methods:** Thirty-one isoflurane-anesthetized male rats received a CCI (2.8 mm cortical deformation at 4 m/s) or sham injury and then were randomly assigned to TBI and sham groups that were further divided into EE and standard (STD) housing (n=6-10/group). At four weeks post-surgery, rats were tested on the AST, which involves a series of increasingly difficult discriminative tasks to obtain food reward, including simple and compound discriminations, stimulus reversals, and intra-and-extradimensional (ED) shifts. **Results:** TBI impaired ED set-shifting and stimulus reversal learning and increased total response errors and set loss errors (i.e., after 50% or more of the contingency rule has been achieved) ($p < 0.05$). Moreover, EE significantly attenuated the detrimental effects of TBI on cognitive performance in the AST ($p < 0.05$). **Conclusions:** EE exposure as a model of preclinical neurorehabilitation significantly attenuated CCI injury-induced impairments in executive function and behavioral flexibility, which supported the hypothesis. **Significance:** These novel findings demonstrate that executive function and behavioral flexibility deficits in our CCI model are sensitive to the beneficial effects of EE, which may be viewed as a viable preclinical model of cognitive rehabilitation. **Future directions:** Evaluation of pharmacological and cognitive rehabilitation therapies as a clinically relevant combinational paradigm, as well as elucidating mechanisms underlying the neuropsychological deficits.

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Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: NIH Grants NS060005 and HD069620 (AEK)

Title: Manipulating initiation time and duration of environmental enrichment exposure after traumatic brain injury to more accurately mimic clinical rehabilitation

Authors: *A. M. GREENE[1], V. V. MATTIOLA[1], J. B. LEARY[1], L. J. CARLSON[1], J. P. CHENG[1], C. M. MONACO[1], C. O. BONDI[1], A. E. KLINE[2]
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Abstract: Environmental enrichment (EE) consists of increased living space, complex stimuli, and social interaction that promotes exploration and confers improvements in behavioral outcome and histopathology after experimental traumatic brain injury (TBI) vs. standard (STD) housing. However, as a model of rehabilitation, continuous EE is not clinically relevant due to the timing parameters of the typical EE and thus translatability could be limited. Specifically, TBI patients typically receive rehabilitation only after critical care has been provided and then only for 3-6 hours per day. Thus, to mimic the clinic, the goal of this study was to determine whether delaying EE by three days and providing only six hours per day would provide benefits similar to continuous EE. To address this rehabilitation relevant issue, isoflurane-anesthetized male rats were subjected to a controlled cortical impact (2.8 mm depth at 4 m/s) or sham injury and randomly assigned to TBI+EE (continuous), TBI+EE (3 day delayed, 6 hr day), and respective sham controls. Motor function (beam-balance/beam-walk) was assessed on post-operative days 1-5. Spatial learning/memory (Morris water maze) was evaluated on days 14-19. The data showed that EE, regardless of timing, improved motor and cognitive function compared to STD housing ($p < 0.0001$). Moreover, there were no differences between the TBI+EE (continuous) and TBI+EE (3 day delayed, 6 hr day), $p > 0.05$. These data demonstrate that delayed and abbreviated EE produces motor and cognitive benefits similar to continuous EE after TBI and thus further supports EE as a preclinical model of neurorehabilitation. Ongoing studies are evaluating the effects of longer delays in implementing EE after TBI.

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Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: NSC 102-2410-H-431-005-MY3

NSC 101-2410-H-431-007

NSC 100-2410-H-431-003

Title: Examines of depressive responses for posttraumatic stress disorder-like rats in forced swimming task

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Abstract: Patients with posttraumatic stress disorder (PTSD) are often associated with the depressive behavior. With regard to pharmacological interventions, the opiate system and the hippocampus-pituitary-adrenal gland (HPA) stress system has been shown to respectively involve in the symptoms of PTSD. However, whether opiate and HPA systems govern the depressive comorbidity of PTSD in animal model remains unknown. The purpose of the present study addresses this issue. At the beginning of the experiment, all of rats encountered footshock (3 mA, 10 second) to form PTSD effects and were intraperitoneally administrated with 10 mg/kg of morphine, 1 mg/kg of naloxone, and its vehicle normal saline on Day 1. Later, rats were subjected to the situational reminder procedure where rats were placed 2 minutes each trial in the footshock compartment on Days 2, 7, and 13. On Day 14, all rats received a forced swimming test for 5 minutes to assess the depressive behavior, including floating, swimming, and struggling responses. The results indicated that non-significant differences occurred at the factors of opiate drugs, dexamethasone, and the interaction of opiate drugs and dexamethasone for floating behavior. For swimming, non-significant differences occurred at opiate drugs and the interaction of opiate drugs and dexamethasone. However, there was a significant difference in dexamethasone treatments. For struggling behavior, a significant difference occurred at dexamethasone treatments but non-significant differences occurred at opiate drugs and the interaction of opiate drugs and dexamethasone. Taken together, the opiate system does not govern the depressive responses of PTSD; however, a high-dose of dexamethasone probably increases the swimming responses; indicating the high dose reduces depressive responses. The high-dose of dexamethasone decreases the struggling responses when compared to the low-dose

of dexamethasone; indicating the high dose of dexamethasone facilitates depressive responses. Thus, the low- and high-doses of dexamethasone show an inconsistent result between swimming and struggling responses. The paradoxical findings need to be scrutinized in the further studies.
Keywords: depression, posttraumatic disorder, opiate, hypothalamus-pituitary-adrenal gland system, footshock stress

Disclosures: L.H. Yeh: None. **F. Wu:** None. **A. Huang:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: USAMRCC W81XWH-10-2-0171

Title: A single head impact in adult mice produces two patterns of neurological and behavioral deficits

Authors: *N. M. GRINKINA, Y. LI, A. M. EL SEHAMY, M. HABER, P. J. BERGOLD
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Abstract: In clinical traumatic brain injury, a single closed head impact (CHI) produces a heterogeneous pattern of behavioral and neurological deficits. We have developed a CHI animal model that reliably produces two injury syndromes (CHI-1 and CHI-2) after a single impact to a closed head of an adult C57/Bl6 mouse. Within minutes after impact, mice could be separated into CHI-1 and CHI-2 groups based upon the recovery from apnea and righting reflex. CHI-1 mice spontaneously reinitiated breathing and regained a righting reflex in 312 ± 42 seconds. CHI-2 mice had apnea lasting more than 30 seconds and required cardiopulmonary resuscitation with 100% O_2. CHI-2 mice regained righting reflex in 528 ± 72 seconds. At seven days, the sham, CHI-1 and CHI-2 groups were trained on an active place avoidance task in which mice avoid a stationary shock zone on a rotating arena by attending to relevant distal spatial cues while ignoring proximal olfactory cues. CHI-1 and CHI-2 mice had different behavioral deficits. Sham and CHI-1 animals acquired the task while CHI-2 animals did not. Also, sham-CHI mice lowered time to 1st entrance, a parameter of task retention, while CHI-1 mice did not. The inability of CHI-1 mice to lower time to 1st entrance was also seen on the next day on the same task with the shock zone location rotated 180^{o}. These data suggest that CHI-1 mice are deficient

in task retention and CHI-2 mice are deficient in task acquisition. These behavioral deficits of CHI-1 and CHI-2 mice did not differ one month after injury. CHI-1 and CHI-2 mice also had different patterns of grey and white matter injury (Sangobowale, et al., accompanying poster). Minocycline (MINO) and N-acetycysteine (NAC) limited behavioral deficits in rats injured in the controlled cortical impact model (Abdel-Baki, et al., 2010; Haber, et al., 2014). We therefore tested whether the drugs also limited deficts in the CHI-injured mice. Treatment with MINO plus NAC greatly diminished the acquisition deficit of CHI-2 mice. These data suggest that MINO plus NAC has an effect in two models of traumatic brain injury (controlled cortical impact and CHI) and in two species (mouse and rat). We are now testing whether the drug combination has an effect on the retention deficit of CHI-1 mice.

Disclosures: N.M. Grinkina: None. **M. Haber:** None. **P.J. Bergold:** None. **Y. Li:** None. **A.M. El Sehamy:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: USAMRCC Grant W81XWH-10-2-0171

Title: A single head impact to adult mice produces two patterns of white matter injury

Authors: *M. A. SANGOBOWALE, N. GRINKINA, Y. LI, M. HABER, P. BERGOLD
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Abstract: The inherent heterogeneity of TBI (traumatic brain injury) has been a barrier to finding effective therapies. To better understand the heterogeneity of TBI, we report a closed head injury (CHI) animal model that reliably produces two injury syndromes (CHI-1 and CHI-2) after a single closed head impact to adult mice. CHI-1 and CHI-2 mice differ in righting reflex, and cognitive and memory deficits (Grin'kina, et al., accompanying poster). The two injuries also differ in structural damage to the brain. At 14 days, NeuN staining shows little difference between the hippocamppi of CHI-1 and CHI-2 mice. In contrast, at one month, there was significantly less NeuN immunoreactivity in the hilus, CA3 and CA1 regions. No change was observed in the granule cell layer of the dentate gyrus at either 14 days or one month. At one month, CHI-1 and CHI-2 mice show a significant loss of MAP2 immunoreactivity in the hilus,

CA3 and CA1 regions. White matter injury was assessed in cerebellum, cingulum, corpus callosum, fimbria and splenium. Myelin was assayed using luxol fast blue and axon density measured using a pan neurofilament antibody. CHI-2 mice had widespread myelin and neurofilament loss in cerebellum, cingulum, corpus callosum, and fimbria while white matter injury in CHI-1 mice was limited to cingulum and corpus callosum. CHI-2 mice have a more severe injury than CHI-1 mice, thus, CHI provides a model for the clinical heterogeneity of human TBI. In a rat controlled cortical impact model, the combination of the FDA-approved drugs minocycline (MINO) and N-acetylcysteine (NAC) repair white matter through remyelination. One month after injury, MINO plus NAC treatment increased luxol staining in CHI-2 mice. We are testing whether remyelination is responsible for the increase in luxol staining.

Disclosures: M.A. Sangobowale: None. **N. Grinkina:** None. **Y. Li:** None. **M. Haber:** None. **P. Bergold:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: NINDS Grant RO1070512

Title: Minocycline and N-acetylcysteine protects oligodendrocytes and promotes remyelination after mild controlled cortical impact

Authors: M. HABER[1], J. Y. KIM[2], J. JAMES[3], M. SANGOBAWALE[1], N. M. GRIN'KINA[1], A. RAMADANI[3], *P. J. BERGOLD[4]
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Abstract: Mild controlled cortical impact (mCCI) demyelinates white matter. This white matter damage can potentially be repaired through remyelination. The combination of minocycline (MINO) plus n-acetylcysteine (NAC) limits white matter damage after mCCI. White matter was examined using antibodies against antigenic markers on oligodendrocyte precursor cells (PDGFR-α, platelet derived growth factor receptor α), oligodendrocyte soma (APC, adenomatous polyposis coli protein) or myelinating processes (PLP, phospholipid protein;

CNPase, 2',3'-Cyclic-nucleotide 3'-phosphodiesterase). Myelin lipid was assessed with luxol fast blue and axons were assessed with a pan-neurofilament antibody. After mCCI, the corpus callosum of saline-treated rats lowered luxol binding and APC, CNPase, and PLP immunoreactivity. Neurofilament immunoreactivity was unchanged. Thus, mCCI induced oligodendrocyte, but not axonal, loss. Two days after injury, PDGFR-α immunoreactivity was high and decreased over subsequent days. Over the next 14 days, APC immunoreactivity increased in saline-treated injured rats, but CNPase and PLP expression and luxol fast blue staining remained significantly below sham-CCI values. Semithin sections of corpus callosum showed an absence of myelin sheaths. These data suggest that, despite production of new oligodendrocytes, remyelination fails following mCCI. MINO plus NAC treatment resulted in similar demyelination as saline treatment. There was no drug effect on axons. MINO plus NAC treated rats showed PDGFR-α and APC expression levels similar to sham-mCCI rats. MINO plus NAC also increased expression of CNPase and PLP. Luxol fast blue staining also increased and myelin sheaths were seen in semi-thin sections of corpus callosum. These data suggest that MINO plus NAC repairs white matter by protecting oligodendrocytes acutely after injury and promotes remyelination. We are studying later time points to test if there is a long-lasting failure to remyelinate after mCCI. MINO and NAC are also being applied as individual drugs to understand the contribution of each drug to remyelination. The repair of white matter by MINO plus NAC is a novel mechanism to treat traumatic brain injury.

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Poster

225. Traumatic Brain Injury: Animal Studies

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DoD and VA (PV)

Title: Assessment of the Time Dependent changes in the amygdala of rats exposed to Blast-Induced Neurotrauma

Authors: *F. GHODDOUSSI[1], S. V. SAJJA[2], M. P. GALLOWAY[1], P. VANDEVORD[2]
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Abstract: Introduction: Traumatic brain injury (TBI) induced by blast waves (blast TBI, bTBI) is a significant clinical problem in soldiers returning from combat. Psychiatric illnesses such as depression, anxiety, sleep disturbances, memory impairment, and PTSD have a delayed expression after the bTBI event. Reliable biomarkers are non-existent for bTBI which is under diagnosed partly due to the absence of criteria in morphology based neuroimaging modalities (e.g. CT and MRI). Therefore using other neuroimaging modalities such as MRS and DTI may provide an avenue for diagnostic biomarkers of the pathology. Central processing of fear-related memories and emotions occurs in the amygdala. Multiple clinical studies support a critical role for amygdalar nuclei in the disordered emotional reactions of patients with PTSD. Methods: We used proton magnetic resonance spectroscopy (1H-MRS) *ex vivo* to assess the neurochemical profiles in the amygdala (AMYG) of male rats 3 hours, 1, 2, 3 and 7 days, 1 and 3months after exposure to a calibrated blast overpressure (117kPa). Immunohistochemistry assessed the progression of cell death and astrogliosis. Short-term and working memory impairment as well as anxiety-like behaviors was assessed by novel object recognition and light/dark box respectively. Results: In the bTBI group, anxiety-like behavior was evident from 2 d thru 3 mo and impaired memory tasks evident at 7 d thru 3 mo post insult. MRS analysis showed that GABA, GLU [and their ratio to creatine (CRE)] and the NAA/CRE ratio was decreased in the AMYG 2 d after blast. The ratio of myo-inositol (INS)/CRE and N-acetyl-aspartate-glutamate (NAAG) was significantly increased after 3m. Caspase-3 was elevated in the acute (3h-7d) but not at the chronic stages (1-3m) after bTBI. Microglial activity (Iba1 staining) was elevated at 1 & 3mo. Fluorojade B (neurodegeneration) and GFAP (astrogliosis marker) were increased over the entire experimental period. Increased microglial activity in amygdalae was positively correlated with anxiety-like behaviors. Discussion: Decreased NAA/CRE, GLU, and GABA observed 2 d after blast is consistent with compromised mitochondrial (oxidative) stress and energy metabolism and may herald the activation of cellular death pathways. Since INS is primarily present in astrocytes, the increased level of INS/CRE ratio after 3m may reflect the increased astrogliosis, a pathology that was positively correlated to the anxiety-like behavior. Increased levels of INS were also correlated with impaired memory in the novel object recognition task. These results highlight the utility of 1H-MRS for assessment of the status of the brain after bTBI and during treatment

Disclosures: F. Ghoddoussi: None. **S.V. Sajja:** None. **M.P. Galloway:** None. **P. VandeVord:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

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Topic: C.10. Trauma

Support: ONR Work Unit 601152N.0000.0001.A1308

Title: The kinetics of intracranial pressure changes following exposure to blast and the effect of n-acetylcysteine amide on recovery after blast in a rat model

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Abstract: Background - Blast-induced traumatic brain injury (bTBI) has been a leading cause of neurocognitive impairment in the military population. Blood brain barrier (BBB) integrity has been shown to be compromised in traumatic brain injury including bTBI. However, the long term kinetics of BBB changes and its consequences remain unclear. In this study, the effects of blast overpressure (BOP) on intracranial pressure (ICP), its correlation with BBB breakdown, and the effect of N-acetylcysteine amide (NACA) on the BOP induced changes were explored in a rat model. Methods - Animals were exposed to either one or three BOPs (110 kPa) in frontal orientation with animal's head facing the blast. ICP was monitored by a telemetric device over a period of 7 days. BBB permeability and integrity were determined by Evans Blue staining and occludin immunoreactivity, respectively. NACA was administered either pre- or post-blast by single i.p injection (500 mg/kg). Results - A significant elevation in ICP was observed after single or repetitive exposures to BOP. The amplitude of ICP increase was higher in the repetitive exposure group. The degree of ICP elevation in the groups treated with NACA pre- or post-blast was reduced. A two-way repeated measures ANOVA followed by Bonferroni post-hoc analysis was performed to compare the groups. In the repetitive group, the effect of pre-blast injection was significant ($p<0.001$) when compared to a placebo treated group. All injured, NACA treated groups were significantly different ($p<0.001$) than naïve control group. In a single blast group, ICP immediately declined after post-blast NACA administration and returned to near pre-blast levels. The BBB permeability after blast, examined by Evans Blue (EB) dye, was increased in the brain two hours after single or multiple exposures of blast, indicating a compromise in the integrity and function of the BBB. The increase in BBB permeability could result from a

decreased immunoreactivity of occludin, which is one component of tight junctions in BBB after blast. Conclusions - These results demonstrate that BBB breakdown may play an important role in the mechanism of mild TBI. The subsequent ICP increase can be used as one of the markers of brain damage and the antioxidant NACA may be useful as a new therapeutic modality for ameliorating BOP-induced brain damage.

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Poster

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Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.28/N6

Topic: C.10. Trauma

Support: Center for Neuroscience and Regenerative Medicine 60855-300600-7.01

Title: High speed videographic analysis of controlled cortical impact (CCI) devices

Authors: Y. KIM[1,2], J. OZL[1,2], A. FU[1,2], L. TUCKER[1,2], *J. T. MCCABE[3,1]
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Abstract: In pre-clinical traumatic brain injury (TBI) research, a critical element is to validate instrument performance in relation to biomechanical, physiological, histological, and behavioral effects of the injury. The purpose of this study was to characterize instrument performance of one of the most widely used pre-clinical models of TBI, by using high speed videography. Two electromagnetically-driven CCI impact actuators of the same model (Impact One™ Stereotaxic Impactor for CCI, Leica Biosystems) were used with a 3mm short impactor tip, a dwell time of 100ms, and impact penetration depth controlled by changing the position of the stereotaxic frame. Poron cushioning material and a mouse brain with craniotomy were used to record CCI impactor tip with a high speed digital camera (Y3-S1, IDT Inc.). Motion analysis indicated that in the initial ~15ms after activation, the impactor tip makes two or three distinctive advances before it reaches the desired depth setting for full extension. The velocity of the impactor tip on the 1st extension was normally faster than the preset values. The velocity of the tip on the subsequent extension was significantly slower. The amount of over-speed was greater during the

1st extension when the velocity was set at slower values. In the 1st and 2nd advances, the tip extended less than the targeted distance. The terminal location of the tip when it was stationary during final extension was near the desired depth for most of the impact time. Excluding movement of the tip for the first 15ms, the velocity and location of the tip were essentially identical regardless of settings. A similar phenomenon was observed when impact testing was performed on the mouse brain with craniotomy. The analysis suggests that device velocity setting plays little, if any, role as a variable in CCI injury. Depth performance was reproducible and closely matched stereotaxic setting, suggesting this variable (as well as tip diameter and shape; not studied here) may be the most significant factor in the application of CCI in pre-clinical TBI research.

Disclosures: Y. Kim: None. **J. Ozl:** None. **A. Fu:** None. **L. Tucker:** None. **J.T. McCabe:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.29/N7

Topic: C.10. Trauma

Support: Pediatric Brain Injury Modeling - University of Calgary

Title: Caloric intake affects behavioural outcomes following pediatric concussion in rats

Authors: *R. M. MYCHASIUK[1], H. HEHAR[2], A. FARRAN[3], I. MA[2], M. J. ESSER[2]
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Abstract: Recent statistics released by the World Health Organization indicate that in industrialized countries like the United States and Canada, over 60% of the population is at risk for being overweight (as determined with a BMI $\geq$ 25). These findings are alarming as compelling evidence suggests that excessive caloric intake negatively affects brain plasticity. This research also indicates that there is a link between obesity and adverse neurological outcomes such as reduced cognitive functioning, stroke, and Alzheimer ’s disease. Conversely, dietary restriction and lowered caloric intake have been associated with increased synaptic plasticity, reduced neurodegeneration, improved brain health, and increased life span. Although pediatric concussion and mild traumatic brain injury (mTBI) are very common and the initial

symptoms can be very distressing, the majority of children have a complete acute recovery. However a significant proportion may suffer permanent impairment and the long-term implications of these early life injuries is not fully known. Currently, most research has focused on treatment strategies after the injury, rather than the factors that may predict differential susceptibility or resilience to outcomes. The current study was designed to determine if exposure to a high fat diet (HFD) or caloric restriction (CR) throughout life (including the prenatal period) would differentially alter behavioural outcomes following a mTBI/concussion in early childhood. Eight dams were fed a HFD, CR, or the standard rat chow (STD) (total dams; n = 24) for the duration of pregnancy and weaning at which point rat pups were maintained on the same diet as their mothers. At postnatal day 30 (P30) half of the rat pups received an mTBI/concussion (n =44) and the other half received a sham injury (n =44). Rats underwent a behavioural test battery including: beam-walking, the elevated plus maze (EPM), and the Morris water task (MWT). Results from this study indicate that caloric intake prior to a mTBI/concussion does alter behavioural outcomes on the three tasks discussed above. mTBI animals on the HFD tended to have poorer motor outcomes on the beam walking task, increased anxiety on the EPM, and altered executive functioning in the MWT probe trial. On the contrary, mTBI rats maintained on the calorically restricted diet exhibited heightened recovery on these tasks and were often indistinguishable from their sham counterparts. This study therefore indicates that daily routines, such as dietary intake, may influence mTBI/concussion related outcomes in early childhood.

Disclosures: R.M. Mychasiuk: None. **H. Hehar:** None. **A. Farran:** None. **I. Ma:** None. **M.J. Esser:** None.

Poster

225. Traumatic Brain Injury: Animal Studies

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 225.30/N8

Topic: C.10. Trauma

Support: CURE Taking Flight Award

VA RRD Merit

Title: Hippocampal excitability after diffuse brain injury in swine

Authors: *A. ULYANOVA, M. R. GROVOLA, P. F. KOCH, J. P. HARRIS, V. E. JOHNSON, D. K. CULLEN, J. A. WOLF
Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Hippocampal excitability and the development of epileptogenesis after diffuse brain injury were studied using a model of closed-head rotational acceleration in swine. We utilized *in vivo* electrophysiological recordings to investigate changes in hippocampal circuitry after mild traumatic brain injury in sham versus injured animals. Male Yucatan swine (age 6 months) were injured by rotational acceleration of the head (180-260 radians per second) in the coronal plane. There was no prolonged loss of consciousness (< 15 min) or subdural bleeds, however axonal pathology was present. In order to appropriately place the 32-channel probe, cell layers of the swine hippocampi were mapped via single unit activity in each animal prior to probe insertion. Field potential activity and neuronal firing rates in the dorsal hippocampus were recorded using high-density electrode arrays with simultaneous afferent stimulation in sham (n=5) or injured animals (n=10) one or two weeks post injury. Single and paired pulse stimulations in the Schaffer collateral and a theta burst paradigm in the entorhinal cortex were utilized. Hippocampal responses were analyzed for changes in baseline activity, firing rates and response to stimulation. Changes in hippocampal excitability were also analyzed in repetitively injured animals (180 rad/sec, 7 days apart) and in minocycline-treated animals. There were significant changes in baseline activity and in responses to stimulation at all levels of injury. Return to baseline after paired pulse stimulation took at least 100 msec after single-injury and was further increased after repetitive injury. At the same time, responses to stimulation recorded at 15 days post injury were similar to that in sham. At higher injury levels (260 rad/sec), recurrent paired pulse stimuli rendered hippocampal circuitry unresponsive and induced epileptiform activity such as paroxysmal depolarizing shifts and high-frequency oscillations. Theta burst stimulation induced epileptiform activity at higher injury levels but not in sham or lower injury levels, however firing rates were reduced at all injury levels tested. These alterations suggest an increased excitability, or a shift in the excitation-inhibition balance of the local hippocampal circuitry. These data suggest that single diffuse brain injury induces hippocampal axonal dysfunction and hippocampal excitability changes such as baseline oscillatory activity and neuronal firing rates. Throughout the post injury period, these alterations lead to circuit-level changes in the hippocampus that will elicit sub-clinical epileptiform activity.

Disclosures: A. Ulyanova: None. **J.A. Wolf:** None. **M.R. Grovola:** None. **P.F. Koch:** None. **J.P. Harris:** None. **V.E. Johnson:** None. **D.K. Cullen:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.01/N9

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

Support: CNPq

FAPERJ

Title: Neuronal death induced by endoplasmic reticulum stress is blocked by overexpression of co-chaperone E3 ubiquitin ligase CHIP in hippocampus

Authors: *F. CABRAL MIRANDA, J. ADAO NOVAES, H. PETRS SILVA, L. CHIARINI
UFRJ, RIO DE JANEIRO, Brazil

Abstract: INTRODUCTION: The unfolded protein response (UPR) is triggered after endoplasmic reticulum (ER) stress and is involved with mechanisms regarding cellular stress adaptation and death. Here we show for the first time that E3 ubiquitin ligase and co-chaperone carboxyl terminus HSP70/90 interacting protein (CHIP) prevents neuronal death in hippocampus after severe ER stress. **MATERIAL AND METHODS**: We use organotypic hippocampal slice cultures of male Lister Hooded rats (post natal day 7) cultured for 13 days. After that tissue slices are exposed to tunicamycin, an inhibitor of N-glicosylation, to induce ER stress. Overexpression of CHIP was performed by adeno-associated virus (AAV8-CHIP) infection. Quantification of cellular death was performed by propidium iodide (PI) uptake, chromatin condensed nuclei counting. Slices were processed for western blot or immunofluorescence in order to analyze activation of UPR. **RESULTS:** Overexpression of CHIP decrease the PI uptake, indicating that CHIP blocks necrosis induced by tunicamycin in hippocampus. In addition we found that overexpression of CHIP blocked both chromatin condensation and DNA fragmentation, indicating that CHIP blocks apoptosis induced by tunicamycin in hippocampus. Tunicamycin induces upregulation of CHOP expression. We found that overexpression of CHIP prevent the upregulation of CHOP induced by tunicamycin. In addition, phosphorylation of eIF2alfa induced by tunicamycin was prevented by overexpression of CHIP. Thus, we found that CHIP prevents activation of death pathways associated with the Unfolded Protein Response. **CONCLUSIONS**: Our results points to a connection between CHIP and ER stress death triggering. Our data suggest a neuroprotective role for CHIP in harmful cellular stimuli and even to neurodegenerative diseases related to UPR and endoplasmic reticulum stress. **Financial Support: CNPQ / FAPERJ**

Disclosures: F. Cabral miranda: None. **J. Adao novaes:** None. **H. Petrs silva:** None. **L. Chiarini:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.02/N10

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

Title: Cyclin-dependent kinase 5 promotes truncated apoptosis inducing factor-mediated neuronal cell death via phosphorylation of stub1/chip

Authors: *C. KIM, W. H. SHIN, K. C. CHUNG, Y. J. OH
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Abstract: Cyclin-dependent kinase 5 (CDK5) is a proline-directed Ser/Thr kinase and plays critical roles in many cellular pathways associated with neurodegenerative diseases. Likewise, it has been established that C-terminus of Hsp70-interacting protein (*STUB1*/CHIP), a co-chaperone as well as one of the E3 ubiquitin ligases, also exerts a pathophysiological role on autophagy and neurodegeneration. However, the possibility and/or mechanisms underlying regulation of CHIP function via CDK5 are not examined. Here, we report that CHIP is a novel CDK5 substrate in various models of neurodegeneration established by using neuronal cell lines and mouse cortical neurons. More specifically, CDK5 phosphorylates CHIP via direct binding with highly charged domain of CHIP as determined by *in vitro* and *in vivo* assays. Moreover, the phosphorylation of CHIP by elevated CDK5 activation significantly attenuates CHIP-mediated ubiquitination of endogenous substrates, but not its ligase activity. The degradation of truncated AIF (tAIF), alpha-synuclein, and p53 among CHIP substrates is negatively regulated by CDK5-mediated phosphorylation of CHIP. For example, CHIP-mediated tAIF degradation via ubiquitin-proteasomal system (UPS) is significantly inhibited through disruption of binding between CHIP and tAIF as a consequence of CDK5-mediated phosphorylation of CHIP. Consistently, the phosphorylation of CHIP by elevated CDK5 activation following neurotoxic stimuli generates and stabilizes tAIF, leading to caspase-independent neuronal cell death. Thus, we propose a novel regulatory loop by which phosphorylation of CHIP via CDK5 eventually leads to tAIF-mediated neuronal cell death.

Disclosures: C. Kim: None. **W.H. Shin:** None. **K.C. Chung:** None. **Y.J. Oh:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.03/N11

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

Support: University Start-up Funding #R9321

NIH (NS71022)

Title: Activation of β-catenin/TCF signaling in p25-overexpressing cells induces trans-neuronal loss of β-catenin and activated apoptosis in p25-free neurons by secreted factor Notum

Authors: *H. CHOW[1], K. HERRUP[1,2]
[1]Div. of Life Sci., The Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong;
[2]Rugters Univ., Piscataway, NJ

Abstract: The cyclin-dependent kinase 5 (CDK5) activators, p35 and p25, have a proposed role in the pathogenesis of neurodegenerative disorders. We have recently shown that glycogen synthase kinase 3β (GSK3β) also binds to p25 (but not p35). When it does so, GSK3β hyperphosphorylates tau; yet the phosphorylation of β-catenin is reduced, increasing its stability. This stabilized β-catenin increases in the cell nucleus and should protect neurons from apoptosis. Intrigued by observations that tangle-free neurons close to tangle-bearing neurons display degenerative morphologies and markers, we hypothesized that the tangle-bearing neurons, though themselves protected from cell death, might produce a factor that damaged otherwise healthy cells nearby. To test this idea we analyzed the bi-transgenic CK-p25 Tg mouse in which the expression of p25 is under the control of CamKII promoter and can be switched on or off with doxycycline. After inducing p25 expression for 8 weeks, we found that neurons in frontal cortex showed robust p25 expression and these cells had enhanced nuclear β-catenin. We found a one-third increase in the number of cleaved caspase-3 positive neurons, yet only half of these cells were also GFP-p25 positive. Intriguingly, nearby neurons with limited induction of GFP-p25 (and low levels of nuclear β-catenin), showed substantially elevated cell death markers. We observed the same phenomenon *in vitro*; cleaved caspase-3+ N2a cells or primary cortical neurons were significantly more frequent in close proximity to cells ectopically expressing p25; nuclear β-catenin was not present in the majority of these cells. A possible mechanism for this result is that Notum - a secreted Wnt antagonist and a β-catenin downstream target - was significantly elevated in conditioned medium from p25-overexpressing cells. Notum immunostaining was also elevated in brain sections of CK-p25 Tg mice. Our findings suggest that persistent aberrant activation of β-catenin by p25-expressing neurons triggers a trans-neuronal inhibition of Wnt signaling by Notum that leads in turn to the degeneration of

p25/tangle-free neurons. The existence of this neuron-killing-neuron scenario has significant implications for our understanding of neurodegenerative disease.

Disclosures: H. Chow: None. **K. Herrup:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.04/N12

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

Support: RFBR grants 10-04-01770 and 11-04-01961

Title: Study on ATP concentration changes in cytosol and mitochondria of individual cultured neurons during glutamate-induced deregulation of calcium homeostasis

Authors: *V. G. PINELIS[1], A. M. SURIN[2,3], L. R. GORBACHEVA[5], I. G. SAVINKOVA[1,6], R. R. SHARIPOV[4], B. I. KHODOROV[4]
[1]membranology, [2]molecular genetic and cellular biology, Scientific Ctr. For Children's Health, Russian A, Moscow, Russian Federation; [3]Lab. of ionic transport, [4]Inst. of Gen. Pathology and Pathophysiology, RAMS, Moscow, Russian Federation; [5]Dept. of Human and Animal Physiol., [6]Lomonosov Moscow State Univ., Moscow, Russian Federation

Abstract: For the first time, simultaneous monitoring of changes in the concentration of cytosolic and mitochondrial ATP ([ATP]c and [ATP]mit accordingly), pH (pHc), and intracellular free Ca2+ concentration ([Ca2+]i) of the individual neurons challenged with toxic glutamate (Glu) concentrations was performed. To this end, the ATP-sensor AT1.03 for cytosol and mitochondria, which binds to ATP and therefore enhances the efficiency of resonance energy transfer between blue fluorescent protein (energy donor) and yellow-green fluorescent protein (energy acceptor), was expressed in cultured hippocampal neurons isolated from 1-2-day-old rat pups. Excitation of fluorescence in the acceptor protein allowed monitoring changes in pHc. Cells were loaded with fluorescent low-affinity Ca2+ indicators Fura-FF or X-rhod-FF to register [Ca2+]i. It was shown that Glu (20 μM, glycine 10 μM, Mg2+-free) produced a rapid acidification of the cytosol and decrease in [ATP]c and [ATP]mit. An approximately linear relationship (r2 = 0.56) between the rate of [ATP]c decline and latency of glutamate-induced delayed calcium deregulation (DCD) was observed: higher rate of [ATP]c decrease corresponded to shorter DCD latency period. DCD began with a decrease in [ATP]c of as much as 15.9%. In

the phase of high [Ca2+]i, the plateau of [ATP]c dropped to 10.4% compared to [ATP]c in resting neurons (100%). In the presence of the Na+/K+-ATPase inhibitor ouabain (0.5 mM), glutamate-induced reduction in [ATP]c in the phase of the high [Ca2+]i plateau was only 36.6%. Changes in [ATP]c, [Ca2+]i, mitochondrial potential, and pHc in calcium-free or sodium-free buffers, as well as in the presence of the inhibitor of Na+/K+_ATPase ouabain (0.5 mM), led us to suggest that in addition to increase in proton conductivity and decline in [ATP]c, one of the triggering factors of DCD might be a reversion of the neuronal plasma membrane Na+/Ca2+ exchange. We thank Dr. H. Imamura for kindly providing the plasmid of the ATP-sensors. This work was supported by Russian Foundation for Basic Research grants 10-04-01770 and 11-04-01961

Disclosures: V.G. Pinelis: A. Employment/Salary (full or part-time):; full time. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Contracted Research/Research Grant. **A.M. Surin:** A. Employment/Salary (full or part-time):; Employment/Salary (part-time). B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Contracted Research/Research Grant. **L.R. Gorbacheva:** A. Employment/Salary (full or part-time):; part-time. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants. **I.G. Savinkova:** A. Employment/Salary (full or part-time):; part-time. **R.R. Sharipov:** A. Employment/Salary (full or part-time):; part-time. **B.I. Khodorov:** A. Employment/Salary (full or part-time):; full-time. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research Gran.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.05/O1

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

Support: IN204213 PAPPIT grant to LM

Title: Early activation of autophagy during glucose deprivation contributes to cortical neuronal death

Authors: *C. GERÓNIMO, L. MASSIEU
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Abstract: A decrease of blood glucose levels or insufficient supply to the brain results in impairment of neuronal function. Whenever blood glucose decreases below 20 mg/dl, the hypoglycemic coma and brain injury take place. Hypoglycemia can occur as a complication of insulin treatment in diabetic patients, leading to brain glucose deprivation (GD). Neuronal damage induced by hypoglycemia is initiated by an excitotoxic mechanism triggered by the release of glutamate and aspartate. However, several molecular and cellular mechanisms are activated that ultimately lead to cellular death. A variety of stress stimuli are able to induce autophagy including nutrient and energy deprivation. Autophagy is a lysosome-mediated intracellular catabolic mechanism characterized by the appearance of double- or multiple-membrane cytoplasmic vesicles and the lipidation and redistribution of the cytoplasmic protein LC3-I, towards vesicles. The main role of autophagy is to maintain the cellular homeostasis and supply of building blocks for protein synthesis and energy in response to starvation. However, excessive autophagic degradation can lead to cell death, known as autophagic cell death. The aim of the present study is to analyze the role of autophagy in GD-induced neuronal death. Cortical cultures of 7-8 DIV were exposed for different times to GD followed by glucose reintroduction (GR) to evaluate morphological (the presence of double membrane vesicles) and biochemical (LC3-I/LC3-II, beclin1 and p62 protein levels) features of the autophagic pathway. To evaluate the role of autophagy in neuronal death by the MTT reduction and LDH release assays, cultures were exposed for 2 h to GD and 22 h to GR. We found a rapid activation of autophagy and accumulation of autophagosomes during the GD period. However, the autophagic flux was completed until the first stages of GR. Autophagy inhibition by 3-MA incubation during or after GD prevented neuronal death. Conversely, inhibition of autophagic degradation with chloroquine (CQ) during the GD period resulted in aggravation of neuronal death. Nonetheless, CQ treatment during GR improved cell viability, suggesting that regulated activation of autophagy during GD is beneficial for cortical cell survival. This study was supported by IN204213 PAPPIT grant to LM.

Disclosures: C. Gerónimo: None. **L. Massieu:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.06/O2

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

Title: Astrocytes provide a neuroprotective effect that counteracts rapid glutamate-receptor mediated neurotoxicity

Authors: D. MCEWEN, T. GARAY, J. BROWN, O. MCMANUS, D. ROCK, *V. GROPPI
Essen Biosci., Ann Arbor, MI

Abstract: Glutamate is the primary excitatory neurotransmitter in the central nervous system. Signaling through glutamatergic receptors, such as NMDA, AMPA, and kainate, is important in synaptic plasticity and cognitive function, such as learning and memory. While glutamate signaling is critical to neuronal function, excess glutamate can cause an influx of calcium through NMDA receptors, resulting in excitotoxicity, mitochondrial damage, and upregulation of pro-apoptotic genes. In injury or disease, such as ALS, Alzheimer's disease, and stroke, accumulation of extracellular glutamate results in neural damage and even cell death. *In vitro*, stimulators of glutamate receptors, such as glutamate and kainate, show a potent and rapid toxic effect on cortical and forebrain neurite outgrowth when neurons are cultured alone. However, *in vivo*, it has been suggested that glial cells, such as astrocytes, contribute significantly to overall neuronal health and survival. Here, we developed a quantitative, kinetic *in vitro* model of forebrain neurons with astrocytes to investigate neurotoxicity in a co-culture setting. The forebrain neurons are lentivirally infected to express a fluorescent protein and enable real-time neurite quantitation, using IncuCyte Zoom live-content imaging system. When placed in culture, the forebrain neurons extend neurites and form complex neural networks over the first seven days, after which the networks continue to develop and mature. In a monoculture setting, kainite produces a concentration- and time-dependent decrease in neurite length. However, in the presence of astrocytes, kainate treatment shows low toxicity compared to untreated cultures and can modestly stimulate neurite outgrowth at low kainite concentrations at early times *in vitro*. These *in vitro* findings agree with *in vivo* evidence suggesting that kainate-stimulated astrocytes secrete trophic factors such as BDNF and NGF. Interestingly, however, in the co-culture setting continued kainate treatment eventually results in a concentration-dependent toxicity causing neurite retraction. This neurotoxic effect may be due to maturation of the cultures, leading to the development of synaptic connections and the upregulation of glutamate receptors. Together, these data demonstrate the importance of the glial contribution to neuronal cell health and neuropharmacology in an *in vitro* co-culture setting.

Disclosures: **D. McEwen:** A. Employment/Salary (full or part-time):; Essen BioScience. **T. Garay:** A. Employment/Salary (full or part-time):; Essen BioScience. **J. Brown:** A.

Employment/Salary (full or part-time):; Essen BioScience. **V. Groppi:** A. Employment/Salary (full or part-time):; Essen BioScience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Essen BioScience. **D. Rock:** A. Employment/Salary (full or part-time):; Essen BioScience. **O. McManus:** A. Employment/Salary (full or part-time):; Essen BioScience.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.07/O3

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

Support: NSERC Grant 418489

CIHR Grant 126127

Title: Glutamate toxicity changes expression profile of gangliosides in neurodegenerating rat primary cortical neurons

Authors: *D. PARK[1], G. A. LAJOIE[2], S. N. WHITEHEAD[1]
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Abstract: Neurons within different brain regions have varying levels of vulnerability to external stress and therefore respond differently to injury. A potential reason to explain this may lie within a key lipid class of the cell's plasma membrane called gangliosides. These glycosphingolipid species have shown to play various roles in the maintenance of neuronal viability. The purpose of this study is to use electrospray ionization mass spectrometry (ESI-MS) technique and immunohistochemistry to evaluate the temporal and structural changes in the expression profiles of various ganglioside species during the course of neurodegeneration in rat primary cortical neurons exposed to glutamate toxicity. Primary embryonic (E18) rat cortical neurons were cultured to DIV14. Glutamate toxicity was induced for 1, 3, 6 and 24 hours and immunohistochemistry was used to stain for GM1 and GM3 species. ESI-MS was used to quantify the ganglioside species expressed within these injured neurons, which were compared to expression profiles of healthy neurons. Immunohistochemistry revealed that at early stages of glutamate toxicity, level of GM1 was highly expressed along the neuronal projection. At later time points, these levels decreased but still showed high expression relative to significant reduction in these projections. GM3 were undetectable in uninjured control neurons, but the

expression was initiated by 1 h and remained elevated throughout neurodegeneration. ESI-MS data shows abundant detection of GD1a and GT1a in healthy neurons. Other species such as GM1, GM2, and GM3 still remains to be quantified. These data suggests that different gangliosides play diverse roles in the process of neurodegeneration.

Disclosures: D. Park: None. **G.A. Lajoie:** None. **S.N. Whitehead:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

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Program#/Poster#: 226.08/O4

Topic: C.10. Trauma

Support: NIH Grant P01NS058484

NIH Grant P30NS051220

Kentucky Spinal Cord and Head Injury Research Trust

Title: Calpain 5 carries dual nuclear localization signals

Authors: *J. W. GEDDES[1], R. SINGH[2], M. K. BREWER[2], C. B. MASHBURN[2], D. LOU[2], V. BONDADA[2], B. GRAHAM[2]
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Abstract: The calpain family of Ca^{2+}-dependent cysteine proteases has 15 members in mammals. Most investigations have focused on the classical calpains 1 and 2 which bind to a common small subunit through penta EF hand domains. Other ubiquitous calpains are also expressed in the CNS, although little is known regarding their localization and function. Calpain 5 (CAPN5) is a non-classical member of the calpain family. It lacks the EF hand motif characteristic of classical calpains but retains catalytic and Ca^{2+} binding domains, and contains a unique C-terminus domain. Tra-3, an ortholog of CAPN5, has been shown to be involved in necrotic cell death in *C. elegans*. CAPN5 is expressed throughout the CNS, but its expression relative to other calpains and subcellular distribution has not been investigated previously. Based on relative mRNA levels, Capn5 is the second most highly expressed calpain in the CNS, with Capn2 mRNA being most abundant. Unlike classical calpains which are cytosolic, CAPN5 is predominantly localized to the nucleus and was not detected in the cytosol. CAPN5 possesses

two nuclear localization signals (NLSs): an N-terminal monopartite NLS and a unique bipartite NLS closer to the C-terminus. The C-terminal NLS contains a SUMO-interacting motif that contributes to nuclear localization, and mutation or deletion of both NLSs renders CAPN5 exclusively cytosolic. Dual NLS motifs are common among transcription factors. Interestingly, CAPN5 is found in punctate domains associated with promyelocytic leukemia (PML) protein within the nucleus. PML nuclear bodies are implicated in transcriptional regulation, cell differentiation, cellular response to stress, viral defense, apoptosis, cell senescence, as well as protein sequestration, modification, and degradation. The roles of nuclear CAPN5 remain to be determined.

Disclosures: **J.W. Geddes:** None. **R. Singh:** None. **M.K. Brewer:** None. **C.B. Mashburn:** None. **D. Lou:** None. **V. Bondada:** None. **B. Graham:** None.

Poster

226. Excitotoxicity and Metabolic Stress

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Program#/Poster#: 226.09/O5

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

Support: Canadian Institutes of Health Research

Title: Upregulation of the energy sensor AMPK inhibits mTOR function and promotes neuronal death in glaucoma

Authors: ***N. A. BELFORTE**, J. CUEVA VARGAS, A. DI POLO
Dept. of Neurosci., Univ. of Montreal Hosp. Res. Ctr., Montreal, QC, Canada

Abstract: Background: The AMP-activated protein kinase (AMPK) is an intracellular energy sensor within cells. AMPK is activated under conditions of energy stress when intracellular ATP levels decline and intracellular AMP increases, as occurs during nutrient deprivation and hypoxia. Activation of AMPK leads to the inhibition of mammalian target of rapamycin (mTOR), a key regulator of cell growth and protein synthesis. Our preliminary data show that mTOR activity in retinal ganglion cells (RGCs) is markedly reduced soon after acute optic nerve lesion. Mitochondrial dysfunction leading to ATP deficiency has been proposed to play a role in RGC death in glaucoma. Here, we investigated the role of the AMPK-mTOR axis in experimental glaucoma. Methods: Ocular hypertension was induced in C57BL/6 mice by injection of polystyrene magnetic microbeads (8×10^8 beads/ml) in the anterior chamber. AMPK

or mTOR activity in RGCs was assessed by retinal immunostaining using phospho-AMPK antibodies$^{(Thr172)}$ or phospho-S6 antibodies, respectively, in combination with RGC-specific markers. Inhibition of AMPK was achieved by intraperitoneal injection of the compound C, a cell-permeable and selective AMPK blocker, while mTOR was inhibited with rapamycin. RGC soma or axons were quantified by Brn3a immunostaining on flat-mounted retinas or using toluidine blue-stained optic nerve cross sections, respectively. Results: Our data show marked upregulation of AMPK activity that occurred concomitant with a decrease in mTOR function at one and two weeks after induction of ocular hypertension and prior to RGC death. Blockade of AMPK activity with compound C restored mTOR function and promoted robust RGC soma and axon survival. Administration of rapamycin inhibited the compound C-mediated neuroprotection suggesting that this response is mTORC1-dependent. Conclusion: Our study provides evidence that activation of the energy sensor AMPK contributes to RGC death in experimental glaucoma, suggesting that the AMPK-mTOR pathway plays a role in neuronal loss during metabolic stress.

Disclosures: **N.A. Belforte:** None. **J. Cueva Vargas:** None. **A. Di Polo:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.10/O6

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

Support: Swiss National Science Foundation Grant CR3213_132993

Title: L-lactate as a neuroprotective agent against excitotoxicity: implication of an energy-dependent process

Authors: ***J. PASCAL**[1], I. ALLAMAN[1], P. MARQUET[2], P. J. MAGISTRETTI[3]
[1]EPFL, Lausanne, Switzerland; [2]Dept. of Psychiatry, Univ. Hosp. of Lausanne, Lausanne, Switzerland; [3]KAUST, Thuwal, Saudi Arabia

Abstract: The development of neuroprotective strategies is a key issue in translational neuroscience. In this context, several reports have described a neuroprotective action of L-lactate (Izumi *et al.*, 1994; Ros *et al.,* 2001; Berthet *et al.*, 2009, 2012). We have recently undertaken an in-depth investigation of the neuroprotective properties of L-lactate taking advantage of Digital Holographic Microscopy (DHM), a new imaging technique able to detect early signs (within minutes) of cell death in culture (Jourdain *et al.*, 2011; Pavillon *et al.*, 2012). In this study,

excitotoxicity-induced neuronal death in primary cultures of mouse cortical neurons was used as a model. In these experimental conditions early signs of neuronal death were observed in 65% of cells following application of glutamate (100 μM; 2min). This process is mediated by the activation of NMDA receptors since APV (50 μM) and L689.560 (10 μM), respectively an antagonist of NMDA receptor and a blocker of glycine site of NMDA receptors, significantly decreased the extent of cell death induced by glutamate (only 15% cell death under these conditions). When neurons were exposed to glutamate in the presence of 10 mM L-Lactate the percentage of neuronal death was significantly decreased to 28 % instead of 65%. The neuroprotective action of L-Lactate was mimicked by L-pyruvate (10 mM) but not by D-Lactate (10 mM), a non-metabolized enantiomer of L-lactate, suggesting that neuroprotection induced by L-lactate and L-pyruvate relies on increases in energy substrates availability. Consistently, in the presence of UK5099 (1 μM), a specific blocker of L-Lactate transport, L-lactate-mediated neuroprotection was fully prevented (63% cell death). Finally, we observed that in the presence of Xanthine Amine Congener (10 μM), a nonselective adenosine receptor antagonist, the neuroprotective effect of L-Lactate was abolished (58% cell death), strongly suggesting that the effect of L-Lactate involves activation of adenosine receptors, through mechanisms to be elucidated, stimulation of adenosine release being one possibility. As a whole these results demonstrate that L-lactate confers neuroprotection to excitotoxic insults through a mechanism relying on an increase neuronal energy substrates availability and activation of adenosine receptors.

Disclosures: J. Pascal: None. **I. Allaman:** None. **P. Marquet:** None. **P.J. Magistretti:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.11/O7

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

Support: Indian Council of Medical Research (ICMR), Government of India

Title: Effect of ATP7B knockdown on cellular morphology and viability in human neuroblastoma (SH-SY5Y) cells

Authors: *R. KUNJUNNI[1], M. K. SRIWASTVA[1], S. SATHIANATHAN[1], P. CHATTOPADHYAY[2], M. BEHARI[3], V. SUBBIAH[1]
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Abstract: BACKGROUND In human beings copper export is mediated by two copper transporting ATPases - ATP7A and ATP7B. The protein ATP7B is expressed in liver, brain, placenta and kidneys where it is localized at the trans-Golgi network and vesicles. Mutations in ATP7B causes hepato-lenticular degeneration or Wilson Disease (WD) which is characterized by copper accumulation mainly in liver and brain. Incidence of WD is reported between 1 in 5000 to 1 in 30,000 live births worldwide. The present study aims to reveal the changes in morphology and viability brought about by ATP7B silencing in SH-SY5Y cells. METHOD Cells were transfected with specific siRNA to silence the human ATP7B gene and its downregulation was confirmed by quantitative real-time PCR. These cells were studied by phase contrast microscopy for various morphological parameters and the results were statistically evaluated by Mann Whitney test. Cell viability was studied by MTT assay and the mitochondrial membrane potential ($\Delta\Psi m$) was assessed by fluorescence microscopy with JC-1 staining. RESULTS Knockdown of ATP7B resulted in significant decrease in mean area, length and perimeter ($P<0.05$), indicating a gross decrease in cell size. The increase in shape factor and circularity ($P<0.01$) indicates variation in cell morphology. When cells were silenced for ATP7B and then treated with copper; and in reverse order, the resultant cellular morphology was same. When cells were treated only with copper, there was no significant change in morphological parameters. The cell viability was decreased to 62.5% in ATP7B silenced cells ($P<0.05$). The mitochondrial membrane potential, $\Delta\Psi m$ also showed significant decrease on ATP7B silencing. CONCLUSION We observed significant changes in cellular morphology on silencing of copper transporter gene ATP7B and not on mere copper treatment. The mitochondrial membrane potential, $\Delta\Psi m$ and cell viability was decreased in ATP7B silenced cells indicating a destructive stress response. This might possibly be due to intracellular copper accumulation which may also be found in WD pathology.

Disclosures: R. Kunjunni: None. **M.K. Sriwastva:** None. **S. Sathianathan:** None. **P. Chattopadhyay:** None. **M. Behari:** None. **V. Subbiah:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.12/O8

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

Support: NIH grant P20MD00988

NIH grant P20MD001632

NIH grant R25GM060507

Montgomery Street Foundation

Title: Expression and distribution of the scavenger receptor CD36 after Spinal Cord injury and its potential modulation by an O3PUFA and Vitamin E enriched diet

Authors: *K. CORDERO, J. D. FIGUEROA, M. DE LEÓN
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Abstract: Spinal cord injury (SCI) remains a devastating cause of lifetime medical comorbidities in modern society. Spasticity and hyperreflexia are prevalent comorbidities observed in spinal cord injury and multiple sclerosis patients. Because the neuronal membrane lipid composition and metabolism are markedly altered during the first few days after injury, increasing attention is now being paid to understand lipid transport and metabolism under these conditions. Evidence supports that aberrant neural metabolism occur within the spinal monosynaptic H-reflex in animal models of spasticity. Although lipid trafficking and metabolism changes after SCI, the functional role for these perturbations has not been studied. Previous findings from our lab have shown that dietary omega-3 polyunsaturated fatty acids (O3PUFAs) confer strong prophylaxis against SCI morbidities.There is inadequate understanding on the molecular mechanisms involved in their transport and metabolism after SCI. The fatty acid translocase membrane cluster of differentiation 36 (FAT/CD36) is a B class scavenger receptor, which has been implicated in the uptake and signaling of hydrophobic molecules. FAT/CD36 in humans has multiple polymorphisms associated with the risk of metabolic dysfunction and stroke. The purpose of this study was to 1) investigate the expression and roles of CD36 in the context of SCI and dietary O3PUFAs and Vitamin E; 2) Determine protective roles of hydrophobic molecules (O3PUFAs and Vitamin E) after SCI. We hypothesized that trauma to the spinal cord will result in a reduction in the protein levels of this protein. Further, we proposed that dietary O3PUFAs and Vitamin E may regulate the expression of CD36 after SCI. Here we determined the protein expression of CD36 after SCI and expanded the knowledge of a potential protective diet enriched with Vitamin E in addition to O3PUFAs. In particular, we analyzed locomotion recovery, H-reflex depression, bladder recovery, and the temporo-spatial expression of CD36 after SCI using behavioral and electrophysiogical studies, Credé's maneuver, immunoblotting and double-labeling immunofluorescent experiments respectively. Our data demonstrates that Vitamin E and O3PUFAs accelerate locomotor and bladder recovery, improves H-reflex depression, and that acute SCI results in a marked downregulation of CD36. This protein was predominantly observed in both neuronal and glial cells. Our next step is to determine if dietary lipids may be implicated in the regulation of this important protein. This protein may represent an important therapeutic target to reduce the burden of SCI in humans.

Disclosures: K. Cordero: None. **J.D. Figueroa:** None. **M. De León:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.13/O9

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

Support: NIH intramural funds

Title: The Cdk5 modulator TFP5 displays anti-inflammatory and anti-oxidative actions in neurons

Authors: *J. P. STEINER[1], M. BACHANI[1], K. MATHER[1], E. FENNELL[1], N. CHESTER[1], A. POPESCU[2], V. SHUKLA[3], N. AMIN[3], B. BALACHANDRANKRISHNAMMA[3], H. C. PANT[3]
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Abstract: Alzheimers Disease brains have increased activity of cyclin-dependent kinase 5 (Cdk5) and higher levels of p25, an abnormal activator derived as a truncated form of the normal p35 activator. Recently, we have found that the Cdk5 modulator TFP5 is able to selectively reduce the p25-induced hyperphosporylation of tau and neurofilament (NF H/M) and avert the biochemical, neuropathological and behavioral deficits *in vivo* in 5XFAD mice (Shukla et al *FASEB J. 27:* 174-186, 2013). In order to address the molecular mechanisms of the TFP5, we explored the effects of neuroinflammation and oxidative stress in addition to beta amyloid on rat and human neurons in culture. We found that TFP5 not only protected neurons and promoted survival from Ab_{1-40} neurotoxicity, but also prevented decreases in neurite length, cross sectional area and neuritic caliber to their media control levels. TFP5 was also effective against LPS-mediated inflammatory lesions to the neurons, blocking decreases in neurite length and increased axonal fragmentation. Effects of TFP5 on inflammatory cytokine levels in these cultures are being evaluated. TPF5 also prevented neuronal damage from oxidative stress injury in a concentration dependent manner, with an EC_{50} of 10 nM. Thus, TFP5 not only provides neuroprotective actions to these neurons, but also an anti-inflammatory action as well. Targeting multiple facets of the neurodegenerative process may help underlay the robust effects of TFP5 observed in multiple neurodegenerative disease models.

Disclosures: J.P. Steiner: None. **M. Bachani:** None. **K. Mather:** None. **E. Fennell:** None. **N. Chester:** None. **A. Popescu:** None. **V. Shukla:** None. **N. Amin:** None. **B. BalachandranKrishnamma:** None. **H.C. Pant:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.14/O10

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

Title: Tumor Necrosis Factor Related Apoptosis Inducing Ligand reduces the expression of the neuroprotective Na+/Ca2+ exchanger isoform 3 in human neuronal cells *in vitro*

Authors: O. VALERIO[1,2], G. DI BENEDETTO[2,3], G. CANTARELLA[2], V. LARICCIA[1], S. AMOROSO[1], *R. BERNARDINI[2]
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Abstract: Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) is a pro-apoptotic cytokine belonging to the tumour necrosis factor/nerve growth factor (TNF/NGF) superfamily. The protein Na+/Ca2+ exchanger isoform 3 (NCX3) protects neurons from damage and death following ischemic injury and plays important roles in regulating intracellular calcium. Following aberrant proteolytic cleavage of NCX3, such as for example, by calpain, neuroprotective function of the former is significantly reduced. NCX3 expression may be induced by nerve growth factor (NGF), a neurotrophin which binds the tyrosine kinase receptor TrkA and activates downstream kinases, such as Erk1/2, and the survival-related kinase Akt. Here, we verified the hypothesis that TRAIL can influence the expression of NCX3 via modulation of the NGF/TrkA system. Retinoic acid-differentiated human neuroblastoma SH-SY5Y cells were incubated 48 h with appropriate concentrations of TRAIL. NCX3 protein expression was then studied in SH-SY5Y cell lysates by means of Western blot analysis after 6, 16, 24 and 48 h of incubation. NCX3 expression decreased in a time-dependent fashion in the presence of TRAIL. Moreover, protein analysis of the phosphorylated forms of TrkA, Erk1/2 and Akt, performed at the same time intervals, revealed that p-TrkA expression was initially increased in SH-SY5Y cells treated with TRAIL after 6 and 16h, whilst it declined thereafter, until it almost disappeared after 48h. A similar pattern was observed for both p-Erk1/2 and p-Akt. Results indicate that the documented increase of TRAIL expression occurring during neuronal damage parallels with down-regulation of NGF-related kinases and substantial attenuation of NCX3 expression. As a result, neuroprotective properties of NCX3 are

significantly reduced. The TRAIL system could thus represent a potential target for treatment of neural damage related to down regulation of NCX3.

Disclosures: **O. Valerio:** None. **G. Di Benedetto:** None. **G. Cantarella:** None. **V. Lariccia:** None. **S. Amoroso:** None. **R. Bernardini:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.15/O11

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

Support: NIH Grant NS081149

Title: Ligand binding to the GluN2B subunit is required for NMDA-induced superoxide production independent of ion flux

Authors: ***A. M. BRENNAN**[1], J. A. GRAY[2], X. JIANG[3], R. A. SWANSON[1]
[1]UCSF, VA Med. Ctr., SAN FRANCISCO, CA; [2]Neurol., UC Davis, Ctr. for Neurosci., Davis, CA; [3]Pediatrics, UCSF, San Francisco, CA

Abstract: Emerging evidence suggests that NMDA receptor dependent signaling may not simply be a product of ion flux, but involves conformational changes in receptor subunits following ligand binding. Sustained activation of NMDA receptors leads to excitotoxic cell death in stroke, trauma, and neurodegenerative disorders. These effects are, in part, attributable to superoxide produced by the enzyme NADPH oxidase (NOX2). We report here that NMDA receptor dependent activation of NOX2 requires two independent events; (1) ligand binding to the NR2B subunits of NMDA receptors and (2) increased intracellular calcium. We find that increasing intracellular calcium with ionomycin to similar levels as following NMDA application is not sufficient to induce superoxide production in neurons. However, significant increases in superoxide were detectable following ionomycin application when neurons were co-treated with NMDA in the presence of the NMDA receptor glycine site antagonist 7-Chlorokynurenic acid to prevent channel opening. Treatment with NMDA, ionomycin, and the NMDA receptor glutamate site antagonist AP5 showed no increase in superoxide or cell death. Additional studies using genomic and mutational approaches demonstrated that the c-terminus of NR2B, but not NR2A, is required for NMDA induced NOX2 superoxide production. Taken together our work suggests that NMDA receptor activation of NOX2 superoxide production is

not simply an effect of NMDA-induced changes in intracellular calcium, but also requires agonist binding to the NR2B-subunit.

Disclosures: **A.M. Brennan:** None. **J.A. Gray:** None. **X. Jiang:** None. **R.A. Swanson:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.16/O12

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

Support: SFI 08/INV1/1949

Title: Investigation on the role of the BH3-only protein Bid in promoting excitotoxic neuronal injury

Authors: ***B. D'ORSI**, H. P. BONNER, H.-G. KÖNIG, J. H. M. PREHN
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Abstract: Excessive Ca^{2+} entry though NMDA receptors (excitotoxicity) has been implicated in several neurological disorders, including Ischemic Stroke, Epilepsy, Alzheimer's disease and amyotrophic lateral sclerosis. Bid is a pro-apoptotic BH3-only member of the Bcl-2 family of proteins which has been implicated in promoting the mitochondrial apoptosis pathway. Studies in excitotoxic-injury models have shown that following increased Ca^{2+} levels in the cytosol, Bid translocates to the mitochondrial membrane triggering mitochondrial outer membrane permeabilization (MOMP) and the release of apoptogenic intermembrane space proteins. In this study, we investigated the role of Bid in mediating neuronal death using combined models of excitotoxic/Ischemic injury, in mouse neocortical neurons *in vitro* and cerebral ischemic injury *in vivo*. *Bid*-deficient and WT cortical neurons were similarly sensitive to NMDA-induced excitotoxic tolerance and apoptosis, however, our data also suggested that Bid may actually play a critical role in contributing to excitotoxic injury under conditions that are associated with immediated calcium deregulation (ICD) and excitotoxic necrosis. Furthermore, detailed analysis of intracellular neuronal Ca^{2+} dynamics, using time-lapse confocal microscopy, revealed that neurons deficient in *bid* showed markedly reduced Ca^{2+} levels during the NMDA excitation period. Surprisingly, we also observed similar infarct size after middle cerebral artery occlusion in *bid*-deficient and WT mice *in vivo*. However, we here show that deficiency in *bid* exerted neuroprotection in cultured neurons when exposed to oxygen/glucose deprivation (OGD). In

conclusion, our data suggest that Bid may rather play a role in excitotoxicity in pathways associated with necrotic cell death and that the proposed role of Bid as a crucial mediator of cell death after focal cerebral ischemia may be need to be reconsidered.

Disclosures: B. D'Orsi: None. **H.P. Bonner:** None. **H. König:** None. **J.H.M. Prehn:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.17/P1

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

Support: NIH-CounterACT U01NS063555

Title: The inactivation of M current is the link between acetylcholinesterase inhibition by diisopropylfluorophosphate and excitotoxicity

Authors: *P. FERCHMIN, D. PEREZ, V. A. ETEROVIC
Biochem., Univ. Central Del Caribe, Bayamon, PR

Abstract: Poisoning with organophosphorous inhibitors of acetylcholine esterase (AChE) causes an accumulation of acetylcholine and overstimulation of the muscarinic receptors. The supraphysiological muscarinic activity causes glutamatergic seizures and excitotoxicity responsible of the majority of the brain injury caused by organophosphates. Current literature suggests that the activation of muscarinic M1 receptors coupled to Gq protein activates phospholipase C (PLC), which depletes the pool of membrane phosphatidylinositol 4,5-bisphosphate required for the activity of the Kv7.2-7.5 channels. Kv7.2-7.5 (KCNQ2-5) voltage gated potassium channels mediate the hyperpolarizing M-current. Decreased activity of these channels leads to neuronal membrane depolarization that result in glutamate release and excitotoxicity. Here we test the hypothesis that preserving or reactivating the activity of M currents prevents the excitotoxic neuronal injury. We used *ex vivo* acute hippocampal slices to measure excitotoxic damage and neuroprotection. The excitotoxic effect of diisopropylfluorophosphate was measured as the decrease of population spikes, and neuroprotection was assessed as preservation or recovery of population spikes. This parameter is appropriate for measuring early neurotoxicity and neuroprotection because it is proportional to the number of functionally active pyramidal neurons. This method was validated by us and other investigators. Using pharmacological tools, we explored the pathway between DFP inhibition of

AChE and neural damage. Atropine and the more selective M1 antagonists, pirenzepine, prevented the neurotoxic effect of DFP. Two inhibitors of PLC, which prevent the depletion of membrane phosphatidylinositol 4,5-bisphosphate abolished the effect of DFP. Retigabine and flupirtine, positive modulators of Kv7.2-7.5 (KCNQ2-5) channels, were also effective in preventing the noxious effect of DFP. The evidence that excitotoxicity was the final neurotoxic effect of DFP was shown by the neuroprotective efficacy of APV a reversible antagonist of the NMDA receptor. In conclusion, we hypothesize that DFP and most likely, other irreversible inhibitors of AChE cause neural damage by an overstimulation of muscarinic receptors, which activate PLC. PLC depletes membrane phosphatidylinositols leading to inhibition of the M current. The resulting depolarization is followed by glutamate release and excitotoxicity. Therefore, most of the neurotoxic effect of irreversible inhibitors of AChE can be prevented by preservation of the M currents.

Disclosures: **P. Ferchmin:** None. **D. Perez:** None. **V.A. Eterovic:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.18/P2

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

Title: Developing a model of spreading neurotoxicity

Authors: *A. J. SAMSON, C. N. CONNOLLY
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Abstract: The spreading progressive deterioration of the penumbra that surrounds a brain lesion is a major therapeutic target for the treatment of stroke and head injury. However, the mechanism(s) by which this spreading toxicity occurs is unknown. A major contributor to spreading toxicity is thought to be glutamate excitotoxicity. This is based on the fact that increased levels of local glutamate are released from dying cells (Rossi, et al 2000). Unfortunately, neuronal cell death is enhanced by use of NMDA receptor antagonists. This is thought to reflect a requirement for basal NMDA receptor synaptic activity in pro-survival signalling (Hardingham et al 2002, Hardingham & Bading 2003, Soriano et al 2006). Our lab has developed and characterised a novel primary rat neuronal culture model of spreading toxicity, and investigating morphological (synapse loss, dendritic beading and mitochondrial collapse) and functional (mitochondrial depolarisation, Na+/K+ -ATPase dysfunction and a block in

neurotransmitter release) events following a neuronal insult, the role these early neuronal events play in neuronal survival, both locally within the site of insult, and their consequences to neurons at a distance. Understanding the mechanisms of spreading penumbral toxicity will provide novel approaches to limit the effects of brain injury.

Disclosures: A.J. Samson: None. **C.N. Connolly:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.19/P3

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

Support: Innovative Research and Development Program (Texas State)

Title: The combination of retinoic acid and basic fibroblast growth factor enhances neuroprotection against proteasome inhibition-induced cytotoxicity in SH-SY5Y cells

Authors: *B. CHENG[1], J. LONGORIA[3], K. OVALLE[4], A. KUANG[4], V. SCOFIELD[2], J. GARCIA[3]
[2]Microbiology and Immunol., [1]Univ. Texas Hlth. Sci. Ctr., Edinburg, TX; [3]UT-Health Sci. Ctr. at San Antonio - Regional Academic Heath Ctr. at Edinburg (E-RAHC), Edinburg, TX; [4]Biol., Univ. of Texas - Pan American, Edinburg, TX

Abstract: Inhibition of proteasome activity and the resulting protein accumulation are known to be important events in the development of many neurological disorders, including Alzheimer's and Parkinson's diseases. In the present study, we demonstrate that pretreatment with all-trans-retinoic acid (RA) and basic fibroblast growth factor (bFGF) protects human neuroblastoma SH-SY5Y cells from programmed cell death caused by the proteasome inhibitor MG132, which initiates apoptotic pathways through the accumulation of ubiquitinated proteins. Since ubiquitinated protein aggregates appear in both unprotected and protected cells after MG132 insult, we sought to determine how RA and bFGF are able to slow or prevent the toxic effects of protein accumulation. In unprotected cells, MG132 treatment activates stress pathways, identified as increased levels of P53, phosphorylation of JNK, and a reduction in β-catenin expression. This results in cell death, which is preceded by cytoskeletal damage and by the appearance of apoptotic markers, including an increase in PARP cleavage, cytochrome c release from mitochondria, and a decrease in caspase-3 activity. Co-Treatment with RA and bFGF

markedly reduces PARP and caspase 3 cleavages, the expression of P53, and JNK phosphorylation, and the protected cells have normal β-catenin levels. We conclude that growth factors and other neuroprotectants, used in combination, could enhance neuron protections from toxic insults encountered in the microenvironment of the aging brain. In turn, this approach could replace or augment the efficacy of current therapies under study for the treatment of Parkinson's and Alzheimer's disease.

Disclosures: B. Cheng: None. **J. Longoria:** None. **K. Ovalle:** None. **A. Kuang:** None. **V. Scofield:** None. **J. Garcia:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.20/P4

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

Support: the Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, USA (MH 002762-16)

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the Fundamental Research Funds for the Central Universities, P.R.China (JKZD2013006)

Title: Telmisartan protects from glutamate-induced neuronal injury: Roles of AT1 receptor blockade

Authors: *T. PANG[1], J. WANG[3], R. HAFKO[4], J. BENICKY[4], E. SANCHEZ-LEMUS[4], H. LIAO[2], J. M. SAAVEDRA[5]
[1]New Drug Screening Ctr., China Pharmaceut. Univ., Jiangsu, China; [2]China Pharmaceut. Univ., Nanjing, China; [3]Dept. of Neuroscience, Georgetown Univ. Med. Center,, Washington, DC, DC; [4]Natl. Inst. of Mental Hlth., Bethesda, MD; [5]Georgetown Univ. Med. Ctr., Washington, DC, DC

Abstract: Glutamate excitotoxicity is a common cause of neuronal injury and apoptosis. At present, there are no pharmacological treatments to ameliorate glutamate excitotoxicity and provide neuroprotection for the related neuronal injury. Sartans (Angiotensin II AT1 Receptor Blockers, ARBs), which is a class of compounds commonly used for the treatment of cardiovascular and metabolic disorders, is one of such emerging therapeutic targets. They are

powerful neuroprotective agents *in vivo* and protect against IL-1β neurotoxicity *in vitro*. The purpose of our research was to determine the extent of sartans neuroprotection against glutamate excitotoxicity. LDH release assay, TUNEL staining and DNA fragmentation assay results show that sartans significantly reduces glutamate-induced neuronal injury and apoptosis in cultured rat primary cerebellar granule cells (CGCs). Telmisartan was the most potent sartan studied, with an order of potency telmisartan > candesartan > losartan > valsartan. Mechanisms were further studied. Reduction of pro-apoptotic caspase-3 activation, protection of the survival PI3K/Akt/GSK-3β pathway, and prevention of glutamate-induced ERK1/2 activation were involved. Participation of AT1A receptor was supported by glutamate-induced upregulation of AT1A gene expression and AT1 receptor binding. However, glutamate-induced neuronal injury and the neuroprotective effect of telmisartan were not abolished in CGCs obtained from AT1A knock-out mice. This indicates that part of the neuroprotective effect of telmisartan is independent of AT1 receptor blockade. Since PPARγ activation was reported as a pharmacological profile of telmisartan, we also determined if it was involved in the neuroprotective effects of telmisartan. PPARγ nuclear translocation was enhanced by telmisartan and the PPARγ antagonist GW9662 partially reversed the neuroprotective effects of telmisartan. The present results substantiate the therapeutic use of sartans, in particular telmisartan, in neurodegenerative diseases and traumatic brain disorders where glutamate neurotoxicity plays a significant role.

Disclosures: T. Pang: None. **J. Wang:** None. **R. Hafko:** None. **J. Benicky:** None. **E. Sanchez-Lemus:** None. **H. Liao:** None. **J.M. Saavedra:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.21/P5

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

Title: Chemokine CXCL12 increases CaMKII synaptic localization and glutamate-induced cell death

Authors: R. M. BRAGG, III, *J. K. ROSE
Psychology, Western Washington Univ., BELLINGHAM, WA

Abstract: Cerebral ischemia is known to induce cell death through hypoxia and oxidative stress following reperfusion. However, cell death can spread beyond the ischemic core when toxic

glutamate levels act on NMDA receptors of surrounding regions. Levels of CXCL12, a chemokine primarily released from glia, reportedly elevate following ischemia. Acute activation of its receptor, CXCR4, appears to serve a neuroprotective function while prolonged activation results in cell death and this cell death was reported to be dependent on the combined release of calcium from intracellular stores as well as calcium influx through NMDARs (Shepherd et al. 2012). Calcium influx through NMDA receptor channels leads to activation of CaMKII, and it has also been shown that intracellular calcium can be sufficient to activate CaMKII (McCord et al., 2013). Thus, it is of interest to determine if cell death following CXCL12 application is mediated by CaMKII; and further, if exposure to CXCL12 primes neurons for glutamate-induced toxicity thus increasing cell death. In the present study, it was confirmed that acute (30 min) delivery of CXCL12 is not detrimental, as uptake of a cell death indictor dye was not different from controls ($p > 0.01$), while sustained activation (3 hr) resulted in a significant increase in cell death compared to controls ($p < 0.01$). In addition, both acute and sustained CXCL12 induced translocation of CaMKII to synapses, measured as an increase in colocalization with PSD-95 ($p < 0.001$ and $p < 0.01$). Exposure to a strong excitatory stimulus (500 μM glutamate + 10 μM glycine) following either acute or sustained CXCL12 application appeared to elevate susceptibility to glutamate-induced cell death ($p < 0.001$). Interestingly, pre-exposure to CXCL12 also induced cell death following delivery of a more physiologically relevant concentration of glutamate (100 μM glu + 10 μM gly) ($p < 0.001$). Peptide inhibitors that act to block CaM or ATP binding to CaMKII both appeared to reduce CXCL12-induced increases in cell death. These results suggest that both acute and sustained CXCR4 activation increase neuron susceptibility to excitotoxicity and this priming is at least partially mediated by CaMKII.

Disclosures: **R.M. Bragg:** None. **J.K. Rose:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.22/P6

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

Support: CNPq

FAPERGS

Title: Evaluation of neurobehavioral and genotoxic parameters in rats treated with vigabatrin

Authors: *J. N. PICADA, SR[1], K. SOUSA[1], T. PIRES[1], M. AMBROZIO[1], C. VIEIRA[2], L. SOUZA[2], R. ANTUNES[2], V. COELHO[2], M. LEAL[2], P. PEREIRA[2]
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Abstract: Vigabatrin (VGB) is an Antiepileptic Drug used to treat Infantile Spasms and Refractory Epilepsy. This drug is able to increase brain GABA levels through irreversible inhibition of GABA-transaminase, the GABA catabolic enzyme. Vigabatrin also may produce an increase in GABA concentration, by inhibiting the glial uptake or stimulating its release. Currently, the benefits of VGB use may outweigh the risks in some conditions; however, previous studies have shown that VGB may be neurotoxic and should be used with caution. The aim of this study was to evaluate neurotoxicological effects of VGB measuring motor activity and genotoxic and mutagenic effects. Methods: Male Wistar rats were divided into 4 groups and given saline, VGB 50 mg/kg, 100 mg/kg or 250 mg/kg by gavage in both acute and sub-chronic treatments and evaluated in rotarod. For acute treatments, the administrations were conduced 24 h after the initial training and the latency to fall from the rotarod (one of maximum 60 sec) was determined 30, 60, 90, 120 min after administrations. For the sub-chronic treatments, animals received one administration a day during 14 days. Twenty four hours after the last administration, the animals were tested in the rotarod and the latency to fall from the apparatus (one of maximum 60 sec) was determined. The genotoxicity was evaluated using the alkaline version of comet assay in samples of blood, liver, hippocampus, and brain cortex. The mutagenicity was evaluated by micronucleus test in bone marrow of the same animals used in the sub-chronic treatment. Results: The groups treated with VGB showed similar performance in rotarod in comparison to saline group. Regarding the acute treatment, it was observed that VGB was able to induce DNA damage in blood and hippocampus only in higher doses. However, in the sub-chronic treatment, VGB did not show genotoxic or mutagenic effects. Conclusions: VGB did not impair motor activities in rats after acute and sub-chronic treatments. It showed a repairable genotoxic potential on central nervous system, since the genotoxicity was observed in the acute treatment.

Disclosures: J.N. Picada: None. **K. Sousa:** None. **T. Pires:** None. **M. Ambrozio:** None. **C. Vieira:** None. **L. Souza:** None. **R. Antunes:** None. **V. Coelho:** None. **M. Leal:** None. **P. Pereira:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.23/P7

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

Support: NINDS 5R01NS071076-04

Title: Functional alterations of motor cortical circuits in Arginase I knockout mice

Authors: *G. CANTERO[1], C. HU[1], M. T. LAZARO[2], P. GOLSHANI[3], G. S. LIPSHUTZ[4]
[1]Dept. of Surgery, [2]Neurol., David Geffen Sch. of Medicine, UCLA, Los Angeles, CA; [3]Neurol., David Geffen Sch. of Medicine, UCLA. West Los Angeles VA Med. Ctr., Los Angeles, CA; [4]Dept. of Surgery, Medicine, Psychiatry, David Geffen Sch. of Medicine, Semel Inst. for Neurosci. and IDDRC at UCLA, Los Angeles, CA

Abstract: Complete arginase I deficiency is the least severe urea cycle disorder, characterized by hyperargininemia and episodic hyperammonemia. Patients suffer from neurological impairment with cortical (Oldham, MS. et al., 2010) and pyramidal tract deterioration, spasticity, loss of ambulation, seizures, and intellectual disability (Prasad AN. et al., 1997). These neurologic abnormalities may arise from the accumulation of arginine and its metabolites or may result from hyperargininemia, and the resultant increase of several neurotoxic guanidino compounds. In a mouse model of the disorder, onset is heralded by weight loss beginning around postnatal day 15; gait instability soon follows, progressing to inability to stand and development of tail tremor with seizure-like activity and death. Previous results from our lab demonstrated that AAV-based therapy for hyperargininemia is effective and prevents development of neurological abnormalities and cognitive dysfunction in a mouse model of hyperargininemia (Lee EK. et al., 2013). Yet the mechanisms underlying neurological dysfunction caused by arginase deficiency is not understood. This level of understanding will be essential for development of other therapeutic strategies. To determine whether arginase deficiency alters the intrinsic excitability of cortical circuits, we performed whole-cell recordings from L5 excitatory neurons in slices of primary motor cortex of arginase I knock-out mice at postnatal day 13-16. We found strikingly decreased intrinsic excitability of regular spiking neurons, with fewer action potentials elicited at each current step injected. Moreover, we found that both rising and repolarization phases of action potentials are slower in knockout mice. The other measured parameters as resting membrane potential, threshold, input resistance, membrane time constant and capacitance were not altered. These results suggest that arginase I knockout mice display functional property deficits. Future studies will address whether synaptic function is also altered and whether these deficits can be rescued by gene therapeutic strategies.

Disclosures: G. Cantero: None. **C. Hu:** None. **M.T. Lazaro:** None. **P. Golshani:** None. **G.S. Lipshutz:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.24/P8

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

Title: Sensitivity, specificity and limitation of *in vitro* rodent hippocampal brain slice assay for assessment of drug-induced seizure liability

Authors: *J. ZHAI[1], Y.-Y. ZHOU[2], H. ZENG[2], A. LAGRUTTA[2], F. SANNAJUST[2]
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Abstract: Drug-induced seizures are common adverse drug reactions that can result in the failure of drugs to be licensed for clinical use. An effective screen to detect seizure liability in preclinical development can contribute to better lead molecule optimization prior to candidate selection. Compared to conventional *in vivo* experimental animal models the *in vitro* rat brain slice assay can provide a higher throughput, and overcome *in vivo* exposure margin limitations. However, it remains uncertain how well an *in vitro* brain slice can recapitulate the complex process of seizure generation *in vivo*. We examined the effects of 27 reference pharmacological agents (including GABA, glutamate, M_3, D_2 and adenosine receptor ligands, K-channel blockers, β-lactam antibiotic and acetylcholinesterase inhibitor) acting through different mechanisms on a conventional electrophysiological rat hippocampal brain slice assay, to define its sensitivity, specificity, and potential limitations. Population spikes (PS) were evoked at 30 s intervals by electrical stimulation of the Schaffer collateral synaptic pathway and recorded using extracellular electrodes positioned in the cell body layer of CA1 pyramidal neurons from adult Wistar and Sprague-Dawley rats. Most positive reference agents elicited concentration-dependent increase in PS area and/or PS number, indicating a sensitivity of 88% (15 out of 17 agents). The responses to 4 ionotropic glutamate receptor agonists differed markedly: AMPA, CX546 (Ampakine), and kainic acid (KA) enhanced PS area and/or PS number, but KA (at higher concentration) and NMDA induced a reversible reduction or abolishment of PS area and/or PS number. Interestingly, NMDA increased PS area and/or PS number only under modification of ACSF composition (i.e. Mg^{2+}, Ca^{2+}, and glycine level). Among 3 groups of metabotropic glutamate receptor (mGluR) ligands, only DHPG (group I mGluR agonist) and MSOP (group III mGluR antagonist), but not LY341495 (group II mGluR antagonist), induced seizure-like activities consistent with *in vivo* EEG data. All 10 negative reference agents tested had little

effect on PS area and PS number, showing a 100% specificity of the assay to date. Overall, our study demonstrated that the rat brain slice assay can provide a good (93%) predictivity. The main limitation (false negative) could be related to the potential low expression of specific receptors or co-factors within the neuronal pathway(s). This *in vitro* hippocampal rat brain slice model is therefore of potential value to detect seizure liability at preclinical development stage(s) with a higher throughput, lower compound and animal usage than existing *in vivo* models.

Disclosures: **J. Zhai:** A. Employment/Salary (full or part-time):; Merck & Co.. **Y. Zhou:** None. **H. Zeng:** None. **A. Lagrutta:** None. **F. Sannajust:** None.

Poster

226. Excitotoxicity and Metabolic Stress

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

Support: Academy of Finland grant 218081

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Doctoral program FGSN/Brain & Mind

Doctoral program GPBM

Title: Nmda receptor- and mitochondria-dependent neurotoxicity of berberine

Authors: *K. KYSENIUS, C. A. BRUNELLO, H. J. HUTTUNEN
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Abstract: The global incidence of metabolic and age-related diseases, including type 2 diabetes and Alzheimer's disease, is on the rise. In addition to traditional pharmacotherapy, drug candidates from complementary and alternative medicine are actively pursued for further drug development. Berberine, a nutraceutical traditionally used in Chinese and Ayurvedic medicine as an antibiotic, has recently been proposed to act as a multi-target protective agent against type 2 diabetes, dyslipidemias, ischemic brain injury and neurodegenerative diseases, such as Parkinson's and Alzheimer's disease. However, the safety profile of berberine remains

controversial, as isolated reports suggest risks with acute toxicity, bradycardia and exacerbation of neurodegeneration. We report that low micromolar concentrations of berberine cause rapid mitochondria-dependent toxicity in primary cultures of murine cerebellar granule neurons and rat hippocampal neurons, characterized by mitochondrial swelling and depletion of ATP content. Berberine induced cell death remained insensitive to pan-caspase inhibitor z-VAD-FMK treatment. However, inhibition of NMDA receptors by memantine and MK-801 were able to completely block berberine-induced neurotoxicity, indicating an excitotoxic cell death mechanism. The mitochondrial permeability transition pore inhibitor cyclosporine A could also partially protect neurons from berberine toxicity. Additionally, subtoxic nanomolar concentrations of berberine were sufficient to sensitize neurons to secondary insults by glutamate and rotenone. Our results indicate mitochondrial function and NMDA receptors as the central adverse targets of berberine in neurons. Our study highlights the need for thorough safety assessment, especially with the chronic use of berberine, due to its tendency to accumulate in the CNS and the potential risk of neurotoxicity as a consequence of increased bioavailability of berberine pro-drugs.

Disclosures: **K. Kysenius:** None. **C.A. Brunello:** None. **H.J. Huttunen:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.26/P10

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

Support: VA Merit

Title: Heterogeneous nuclear ribonucleoprotein a1 as a target disease-associated protein in progressive multiple sclerosis

Authors: ***S. LEE**[1,2], Y. SHIN[1,2], M. C. LEVIN[1,2]
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Abstract: Deficiency in repair of nuclear and mitochondrial DNA damage has been linked to several neurodegenerative disorders. Many recent experimental results indicate that the post-mitotic neurons are particularly prone to accumulation of unrepaired DNA lesions potentially leading to progressive neurodegeneration. Recently our observations in progressive multiple sclerosis (MS) patient's peripheral blood mononuclear cell (PBMC) and brain suggest that the

accumulation of point mutations (N265D, F273L, P275S, K277N, F281L, R284G and S285G) in the nuclear localization sequence (NLS) region (transportin 1 binding site) of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) may be important in progressive MS pathology. Mutations in genetic coding for stress granule-associated hnRNP A1 lead to enhanced stress granule formation, which accelerates the pathophysiology of protein aggregation in neurodegenerative diseases. Thus, dysregulated aggregation caused by a potent mutant in RNA binding proteins might initiate progressive MS. We propose that two pathological hits, namely nuclear import defects and cellular stress, are involved in the pathogenesis of hnRNP A1 mutations in neurodegenerative disease like progressive MS. Furthermore, increased proteolytic activity is a hallmark of several pathological processes, including neurodegeneration. Increased expression and activity of cathepsins and caspases, during degeneration of the central nervous system is frequently reported. We found that the addition of apoptosis-inducing agent (camptothecin) or transcription inhibition agent (actinomycin D) to cells results in the cleavage of hnRNP A1 isoforms by cathepsin S and caspase-3. In addition, proteolytic degradation of hnRNP A1 in the experimental allergic encephalomyelitis (EAE) MS model was confirmed. This cleavage of hnRNP A1 separated the N-terminal region, containing RRM1-RRM2-RGG box, from the C-terminal region, containing the NLS. Our data indicate that there are two cathepsin S target sites and one caspase-3 target site in hnRNP A1 isoforms, namely LFIG19↓GLSF, SNFG255↓GGGS and SYND261↓FGNY. The N-terminal hnRNP A1 fragments localized to the cytoplasm, as opposed to the nucleus where most C-terminal hnRNP A1 fragments were found. Moreover, these C-terminal hnRNP A1 fragments showed toxicity effects on neuronal cell lines. Taken together, these data suggest an important role of hnRNP A1 in the damage and degeneration of neurons in progressive MS.

Disclosures: **S. Lee:** None. **Y. Shin:** None. **M.C. Levin:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.27/P11

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

Support: DTRA-JSTO CBM NEURO 01 10 RC 007

Title: Caramiphen as adjunct to standard treatment suppresses GD-induced status epilepticus, attenuates neuropathology, and prevents cognitive deficits in rats

Authors: *M. K. SCHULTZ[1], L. K. M. WRIGHT[1], M. DE ARAUJO FURTADO[2], S. H. ROBERTSON[1], M. F. STONE[1], M. C. MOFFETT[1], A. R. BOURNE[1], C. R. SCHULTZ[1], J. E. SCHWARTZ[1], L. A. LUMLEY[1]
[1]USAMRICD, Aberdeen Proving Ground, MD; [2]Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: The progression of epileptiform activity following soman (GD) exposure is characterized by a period of excessive cholinergic activity followed by excessive glutamatergic activity resulting in status epilepticus (SE), which may lead to neuropathological damage and behavioral deficits. Caramiphen edisylate is an anticholinergic drug with antiglutamatergic properties, which conceptually may be a beneficial therapeutic approach to the treatment of nerve agent exposure. In the present study, rats were exposed to 1.2 LD50 GD or saline, treated with atropine sulfate (2 mg/kg, im) and HI-6 (93.6 mg/kg, im) 1 min after GD exposure, and monitored for seizure activity. Rats were treated with diazepam (10 mg/kg, sc) and caramiphen (0, 20 or 100 mg/kg, im) 30 min after seizure onset. Following GD exposure, rats received a series of behavioral tests to assess cognitive and motor capabilities. Caramiphen treatment reduced GD-induced deficits in locomotor and rearing activity in the open field, spatial memory in the Morris water maze, associative learning in the fear conditioning test, and efficiency in the differential reinforcement of low rate operant conditioning schedule, but did not prevent GD-induced increase in acoustic startle response and decrease in prepulse inhibition. In addition, caramiphen in combination with diazepam reduced seizure activity and dose-dependently reduced GD-induced neuronal fiber degeneration and neuronal loss. These findings show that physiological, behavioral, and neuropathological effects of GD exposure can be attenuated by treatment with caramiphen as an adjunct therapy, even if administration is delayed to 30 min after seizure onset. This research was supported by the Defense Threat Reduction Agency-Joint Science and Technology Office, Medical S&T Division & Physical Science Division.

Disclosures: M.K. Schultz: None. **L.K.M. Wright:** None. **M. de Araujo Furtado:** None. **S.H. Robertson:** None. **M.F. Stone:** None. **M.C. Moffett:** None. **A.R. Bourne:** None. **C.R. Schultz:** None. **J.E. Schwartz:** None. **L.A. Lumley:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 226.28/P12

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

Support: DA037673

Title: Validating protein-protein interactions and disruptions using AlphaScreen methodology

Authors: *W.-H. LEE[1], Y. LAI[2], A. HOHMANN[2,1,3]
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Abstract: **Background:** Activation of NMDA receptors and the neuronal nitric oxide (nNOS) signaling cascade requires the binding between nNOS and the scaffold protein postsynaptic density 95 kDA (PSD95), which tethers nNOS to the NMDAR signaling complex. This cascade plays a critical role in neuronal excitotoxicity and pathological disorders of the nervous system. The PDZ2 domain of PSD95 interacts with the β-finger of nNOS, coupling NO production and p38 activation to NMDAR activity. Due to the physiological importance of this interaction, *in vitro* approaches to quantify protein-protein interactions and measure their disruption need to be developed and validated. **Objective:** We developed a protein-protein interaction bead-based assay using AlphaScreen (Perkin Elmer) methodology to validate known protein-protein interactions between nNOS and PSD95. We also used this assay to characterize putative nNOS-PSD95 protein-protein interaction disruptors such as the natural product honokiol. **Methods:** AlphaScreen involves phthalocyanine-containing donor beads and thioxene derivatives-containing acceptor beads. When excited by 680nm light, the donor bead will convert ambient oxygen into singlet oxygen. The singlet oxygen will be accepted by the acceptor beads when the beads are within 200 nm range. The singlet oxygen activated beads emit light at 520-620nm that can be detected by the Enspire instrument. We used glutathione-coated donor beads and Ni-chelate coated acceptor beads to recognize GST-tagged and His-tagged protein, respectively. The derived 50% binding was used to determine the concentrations of the protein to be used in the latter disruption assay. Here, we used the PSD95 constructs containing PDZ1-3 and nNOS constructs containing PDZ and β-finger in the interaction and disruption assay. Titrations of GST-PSD95 and His-nNOS were performed to determine 50% binding. Next, we used a non-tagged protein construct in a competition assay and compared it with honokiol, a natural product recently postulated to serve as a PSD95-nNOS inhibitor based upon co-immunoprecipitation studies. **Results:** The 50% binding between GST-PSD95 and His-nNOS determined by AlphaScreen was in the10-30 nM range. Moreover, PSD95/nNOS was disrupted by a known plant derivative, honokiol. **Conclusion:** AlphaScreen is a reliable technology for assaying disruption of protein-protein interactions. **Support:** DA037673.

Disclosures: **W. Lee:** None. **Y. Lai:** None. **A. Hohmann:** None.

Poster

226. Excitotoxicity and Metabolic Stress

Location: Halls A-C

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Program#/Poster#: 226.29/Q1

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

Support: FCT SFRH/BD/72071/2010

FCT PTDC/NEU-NMC/0237/2012

COMPETE: FCOMP-01-0124-FEDER-029649

Title: Neurodevelopment, mitochondrial dynamics, autophagy and huntingtin aggregation differ in cortical vs. striatal neurons: Effects of HDAC6 inhibition

Authors: P. GUEDES-DIAS[1,2,3], M. R. DUCHEN[2], *J. M. OLIVEIRA[1,3]
[1]Univ. of Porto - Fac. of Pharm., Porto, Portugal; [2]Cell and Developmental Biol., Univ. Col. London, London, United Kingdom; [3]REQUIMTE, U.Porto, Porto, Portugal

Abstract: Differential mitochondrial-dependent calcium handling may contribute for enhanced striatal vs. cortical vulnerability in Huntington's disease (HD). We previously showed that histone deacetylase (HDAC) inhibitors accelerate mitochondrial-dependent calcium recovery following NMDA receptor activation in neurons from HD mice. Here we test whether neurite outgrowth, mitochondrial and autophagy dynamics differ in primary rat cortical and striatal neurons, together with the consequences for mutant huntingtin (mHtt) clearance, in the presence or absence of a selective HDAC6 inhibitor (tubastatin A). In live imaging experiments, striatal neurons displayed decreased neurite outgrowth and a smaller motile mitochondria fraction than cortical neurons. Also, striatal mitochondria moved slower and stopped more frequently than that in cortical neurons. Furthermore, striatal neurons presented a lower autophagic flux than cortical neurons, as suggested by LC3-II and p62 immunoblots. Live imaging of neuronal EGFP-Htt(exon1)Q74 expression revealed that the proportion of neurons exhibiting mutant huntingtin inclusion bodies is smaller in striatal vs. cortical neurons. HDAC6 deacetylase activity was previously reported as a key step in autophagosome-lysosome fusion, and affecting mutant huntingtin clearance in cell lines. In live imaging experiments with neurons expressing mCherry-EGFP-LC3B, tubastatin did not inhibit autophagosome-lysosome fusion, but increased the retrograde flux of LC3-positive vesicles. Consistently, tubastatin also increased the number of autolysosomes in neuronal somata. Nevertheless, tubastatin treatment did not alter mutant huntingtin inclusion body formation in either cortical or striatal neurons. Together, these results suggest that selective HDAC6 pharmacological inhibition does not interfere with mutant huntingtin proteostasis in neurons. Also, we report the differential neurite outgrowth, mitochondrial dynamics and autophagy in cultured cortical and striatal neurons that may assist our understanding of differential neuronal vulnerability in HD. Supported by Fundação para a

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Disclosures: **P. Guedes-Dias:** None. **M.R. Duchen:** None. **J.M. Oliveira:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.01/Q2

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

Support: CNPq

Title: Crude venom of the South American rattlesnake (Crotalus durissus terrificus) induces CA1 neuronal death in rats' organotypic hippocampal slices

Authors: ***D. CARVALHO**[1], E. GERACE[2], D. PELLEGRINI-GIAMPIETRO[2], I. S. SANO-MARTINS[3], G. F. XAVIER[1]
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Abstract: Intrahippocampal administration of the South American rattlesnake (Crotalus durissus terrificus, Cdt) venom in rats permanently disrupts performance in spatial memory tasks, thus indicating the occurrence of damage to this brain area. The present study investigated the time course of neurotoxic effects of 0.05-1 μg/mL of crude Cdt venom on organotypic hippocampal slice cultures. The intensity of propidium iodide (PI) fluorescence levels in different hippocampal subfields either 2, 4, 6 or 24 h after incubation of the slices with venom were compared to corresponding scores of both (1) slices treated only with vehicle (negative control) and (2) slices incubated with 1 mM glutamate (positive control for maximal neuronal death). While negative control slices did not exhibit any neuronal damage, positive control slices exhibited strong neuronal damage in the CA1, CA3 and dentage gyrus. The ANOVA revealed dose- ($F(5,312)=7.1229$, $p=0.00001$) and time- ($F(3, 312)=4.8483$, $p=0.00260$) dependent significant increases in neuronal damage in slices exposed to Cdt venom. This effect was particularly prominent in the CA1 sub-field as compared to the CA3 and dentate gyrus ($F(2, 624)=20.763$, $p=0.00001$); in these latter brain areas damage occurred only after exposure to 0.5 and 1 μg/mL venom concentrations. In conclusion, these data show that crude Cdt venom induces selective CA1 damage (at lower concentrations) in organotypic hippocampal slice

cultures. These findings may be helpful in the elucidation and management of rattlesnake envenomation.

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Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.02/Q3

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

Support: Mount Sinai Department of Anesthesiology Research Fellowship

Title: Long-term effects of single or multiple neonatal sevoflurane exposures on hippocampal ultrastructure

Authors: L. G. AMROCK[1], M. STARNER[2], K. L. MURPHY[3], *M. G. BAXTER[2]
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Abstract: Neonatal exposure to general anesthetics (GA) may pose significant neurocognitive risk. Human epidemiological studies demonstrate higher rates of learning disability among children with multiple, but not single, exposures to anesthesia. Here we employ a rat model to provide a histological correlate for these population-based observations. Twenty male Long-Evans rat pups (n=5/condition) were exposed to 2.5% sevoflurane under one of four conditions: a single two-hour exposure on postnatal day 7 (P7); a single six-hour exposure on P7; repeated two-hour exposures on P7, P10, and P13 for a cumulative six hours of GA; or a control exposure to 30% oxygen on P7, P10, and P13. At P91, rats underwent behavioral testing at using a spatial memory task, followed by euthanasia on P105-112. Long-term differences in hippocampal CA1 synaptic density, mitochondrial density, and dendritic spine head morphology were examined using unbiased electron microscopy. Repeated two-hour exposures to GA resulted in greater synaptic loss relative to a single two-hour exposure ($p<0.001$) and a single six-hour exposure ($p=0.022$). Repeated exposures did not alter the distribution of postsynaptic density length, indicating a uniform pattern of neurodegeneration across spine types. In contrast, mitochondrial toxicity was best predicted by the cumulative duration of exposure. Both repeated two-hour exposures and a single six-hour exposure were associated with equivalent reductions in the

fraction of presynaptic terminals containing mitochondria ($p<0.001$). These findings suggest a "threshold effect" for GA-induced neurotoxicity, whereby early mitochondrial damage sensitizes surviving synapses to subsequent exposure.

Disclosures: L.G. Amrock: None. **M.G. Baxter:** None. **M. Starner:** None. **K.L. Murphy:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

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Program#/Poster#: 227.03/Q4

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

Support: NIDA/NIH Grant R01 DA020142

Title: Leptin attenuates the methamphetamine-induced apoptosis and inflammatory response in the murine striatum

Authors: *N. H. KUTUB[1,3], J. LIANG[2], V. PAHALYANTS[2], J. MAMTORA[2], J. A. ANGULO[1]
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Abstract: Methamphetamine (METH) is an addictive psychostimulant that is neurotoxic and causes cognitive and motor deficits. METH-induced neurological damage colocalizes with deficits among neurodegenerative injuries such as Parkinson's and Huntington's disease. We seek to identify neuroprotective agents that can attenuate degeneration. Leptin is a peripheral hormone produced mainly by adipocytes, circulates in the plasma and the central nervous system (CNS). Leptin mainly acts via its long form receptor, ObRb, identified in many brain areas including the hypothalamus, hippocampus, cerebellum, brain steam and striatum. The precise molecular pathway underlying the direct effects of leptin in these regions is mostly unknown. *In vitro* and *In vivo* studies reported antiapoptotic properties of leptin upon neural injury in these areas. Little is known about the neuroprotective abilities of leptin in the striatum. To measure the role of leptin on the METH-induced apoptosis, a single toxic dose of METH (30 mg/kg) and several doses of leptin (0.25, .5, 1, 2, 3 mg/kg) were administered to 10-week old mice. METH-induced apoptosis was measured 24hr post injections by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) immunofluorescence. A reliable baseline of the total

number of neurons in the striatum was established following published laboratory procedures. Percentage of TUNEL-positive cells relative to baseline was analyzed. Our results show that different doses of leptin alone do not cause any pro-toxic effects in the striatum. Furthermore, we found attenuation of apoptosis in the leptin + METH group across all five doses compared to METH alone group. METH alone caused 20-25% of the striatal neurons to undergo apoptosis and leptin treatment significantly attenuated the apoptosis. The minimum optimal leptin dose that elicited peak attenuation is 1mg/kg leptin, this dose was used in all future experiments. Furthermore, we found leptin does not protect dopamine (DA) terminals from METH toxicity by western blot analysis measuring tyrosine hydroxylase which is the rate-limiting enzyme of DA production. We also found both short and long isoforms of the leptin receptor to be expressed in mice striatal neurons. Finally, leptin can also attenuate METH-induced inflammatory response by reducing the over activation of astrocytes and microglia by 50-60% in expression levels for both signals within 72 hour post treatment when compared to METH alone groups. We demonstrate here that leptin signaling can be neuroprotective against METH-induced toxicity in striatal neurons of mice.

Disclosures: **N.H. Kutub:** None. **J. Liang:** None. **V. Pahalyants:** None. **J. Mamtora:** None. **J.A. Angulo:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.04/Q5

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

Title: Toxicity of the antimalaria drug mefloquine and novel protection by quinolinic acid

Authors: *K. E. HOLMES, D. SMITH, G. W. GROSS
Univ. of North Texas, Denton, TX

Abstract: Lab Administrator 9 7 2014-05-02T16:45:00Z 2014-05-02T16:56:00Z 1 334 1797 University of North Texas 14 4 2127 14.00 Clean Clean false false false EN-US X-NONE X-NONE MicrosoftInternetExplorer4 /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:10.0pt; font-family:"Calibri","sans-serif";} Toxicity of the antimalaria drug mefloquine and

novel protection by quinolinic acid. Katelyn E. Holmes*, David Smith, Guenter W. Gross
Abstract Mefloquine Hydrochloride (MEF) has been used as an antimalarial agent for the past 40 years. The US Army to discontinue its use in 2013. However, MEF is still widely used because of its ability to interfere with all five species of plasmodium that cause malaria [1]. Despite serious side effects, such as depression, general anxiety disorder, psychoses, convulsions, seizures, tinnitus, and movement disorders, there are no quantitative data on MEF toxicity in the literature [1]. We have used primary cultures of mouse cortical tissue, growing on MEAs, to investigate MEF toxicity and potential blockers of such toxicity. At low concentrations, MEF is functionally toxic by suppressing spontaneous network activity with an IC_{50} of 420.7 +/- 14.3 nM (n=6). At 1 microM, MEF becomes cytotoxic within 10 min after compound application. Spontaneous activity cannot be retrieved by 2 medium changes and neurons and glia show clear signs of cell stress, apoptosis, and extensive membrane blebbing. This is followed by cell death of all neurons and glia in the network (n=3). We have found that the ubiquitous metabolite, quinolinic acid (QA), is protective by shifting the IC_{50} to higher concentrations. In the presence of 100 uM QA, the MEF IC_{50} is 1.06 +/- 0.07 uM, a 2.5-fold increase (n=5). Within the brain, QA is synthesized from L-tryptophan in the kynurenine pathway and is produced by microglia and resident macrophages [3]. However, such responses have not been seen in cultured networks until QA concentrations reach 500 microM (n=6) Furthermore, for 10 min MEF exposures, blebbing of neurons is shifted from 1 micoM to 3 microM in the presence of QA. QA and MEF do not interact directly (UNT mass spectrometry data), implying that QA may interfere with apoptosis receptors or pathways [5]. We hypothesize that the highly variable responses of mefloquine users are at least partially caused by different endogenous concentrations of quinolinic acid.

Disclosures: K.E. Holmes: None. **D. Smith:** None. **G.W. Gross:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.05/Q6

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

Title: Cranial irradiation regulates multiple brain-derived neurotrophic factor transcript variants in the mouse hippocampus

Authors: *S. KANG[1], Y. SON[1], M. YANG[1], J. KIM[1], S. LEE[1], J. KIM[1], S.-H. KIM[1], J.-S. KIM[2], U. JUNG[3], C. MOON[1]
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Abstract: The brain can be exposed to ionizing radiation in many fields, but it can develop adverse effects on brain functions, such as memory ability. However, little is known of the molecular and cellular mechanism of cognitive impairments induced by cranial irradiation. In the hippocampus, brain-derived neurotrophic factor (BDNF) plays a role in neurogenesis, neuronal survival, differentiation and synaptic plasticity, and the importance of BDNF transcript variants is emerging in numerous neurologic disorders. In this study, the object recognition memory and contextual fear conditioning task performance in adult C57BL/6 mice at 1 month after single exposure to γ-rays (10 Gy) was assessed to evaluate hippocampus-related behavioral dysfunction following cranial irradiation. The changes in common BDNF expression, phosphorylation of cAMP response element binding protein (CREB), and mRNA expression of individual BDNF transcript variants were analyzed in the hippocampus 1 month after cranial irradiation. In the object recognition memory test and contextual fear conditioning, mice at 1 month after irradiation displayed significant memory deficits compared to the sham-irradiated controls, while no apparent change was detected in locomotor activity. The levels of BDNF expression and CREB phosphorylation were significantly down-regulated in the mouse hippocampus after irradiation. Cranial irradiation significantly declined mRNA levels of BDNF exon IV, VI, VII, VIII, and IXA transcripts in the hippocampus. Therefore, the decrease of CREB-BDNF signaling and the differential regulation of BDNF exon transcripts may be associated with memory deficits after cranial irradiation.

Disclosures: S. Kang: None. **Y. Son:** None. **M. Yang:** None. **J. Kim:** None. **S. Lee:** None. **J. Kim:** None. **S. Kim:** None. **J. Kim:** None. **U. Jung:** None. **C. Moon:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.06/Q7

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

Title: The modulation of GABA-ergic system in postnatal development and trimethyltin-induced neurodegeneration in the mouse hippocampus

Authors: *J. KIM[1], J. KIM[1], M. YANG[2], S. LEE[1], S. KANG[1], S.-H. KIM[1], T. SHIN[3], H. WANG[2], C. MOON[1]
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Abstract: The organotin compound trimethyltin (TMT) induces excitotoxicity in hippocampal neurons accompanied by transient seizure/tremor behavior in mice. γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter, and GABA-mediated signaling has been considered to be implicated in brain development and neurotoxicity. Here, we examined the mRNA expressions of GABA receptors subunits and GABA-related pre- and postsynaptic signals in the hippocampus of mice using real-time RT-PCR to understand the regulation of GABA-ergic system during postnatal development and TMT neurotoxicity in the mouse hippocampus. During postnatal development, the mRNA levels of GABAA receptor subunits (α1, α2, α4, α5, β1, β2, β3, γ1, γ2 and γ3), GABAB receptors subunits (GBR1 and GBR2), and pre- (VGAT, GAD-65 and GAD-67) and post-synaptic signals (KCC2 and gephyrin) were significantly increased in the mouse hippocampus. In adult hippocampus after TMT treatment, the mRNA levels of GABAA receptor α1, α2, α4, α5, β2, β3, and γ2 subunits and GABAB receptors GBR1 and GBR2 subunits were significantly decreased in the hippocampus 1-4 days post-treatment, and then the levels were recovered. Further, the mRNA levels of VGAT and KCC2 were also significantly decreased 1-2 days post-treatment, while the levels of GAD-65, GAD-67 and gephyrin remained relatively stable in the hippocampus after TMT treatment. Therefore, we suggest that the differential regulation of GABA receptor subunits and related pre-/post-synaptic signals may be associated with maturation of excitatory/inhibitory neurotransmission during postnatal development, and dysregulation of synaptic plasticity and seizure/tremor behavior in TMT-induced neurotoxicity.

Disclosures: J. Kim: None. **J. Kim:** None. **M. Yang:** None. **S. Lee:** None. **S. Kang:** None. **S. Kim:** None. **T. Shin:** None. **H. Wang:** None. **C. Moon:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.07/Q8

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

Title: Activation of extracellular signal regulated kinase 1/2 in the mouse hippocampus following trimethyltin treatment

Authors: *S. LEE[1], J. KIM[1,2], M. YANG[1,3], S. KANG[1], J. KIM[1], H.-I. IM[2], H. WANG[3], C. MOON[1]
[1]Vet. anatomy, Chonnam Natl. Univ., Gwangju, Korea, Republic of; [2]Ctr. for Neurosci., Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [3]Dept. of Physiol. and Neurosci. Program, Michigan State Univ., East Lancing, MI

Abstract: Trimethyltin (TMT) intoxication induces both histopathological damage to the hippocampus and clinical symptoms, including seizure, in mice. The lesions and symptoms spontaneously recover over time. However, little is known about the precise mechanisms of recovery from TMT-induced neurodegeneration. In the present study, we investigated the change of ERK1/2 activation and Wnt/PI3K signaling pathways in the mouse hippocampus following TMT intoxication. Mice (7 weeks old C57BL/6) administered with TMT (2.6 mg/kg, i.p.) showed severe acute neurodegeneration with markedly increased TUNEL-positive cells in the dentate gyrus (DG) of the hippocampus. Western blot analysis revealed that phosphorylation of ERK1/2 in the mouse hippocampus was significantly increased 1-4 days after TMT treatment. Immunohistochemical analysis revealed that TMT treatment markedly increased phosphorylated ERK1/2 expression in the DG, whereas the expression was decreased in mossy fibers of CA3. As well, the upstream signaling Wnt/PI3K, including Wnt5a/b, AXIN, PI3K and Akt, were dynamically regulated 1-8 days after TMT treatment. Therefore, the dynamic changes of ERK and Wnt/PI3K signaling pathways may be involved in spontaneous recovery from TMT-induced hippocampal degeneration.

Disclosures: **S. Lee:** None. **J. Kim:** None. **M. Yang:** None. **S. Kang:** None. **J. Kim:** None. **H. Im:** None. **H. Wang:** None. **C. Moon:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.08/Q9

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

Support: NIH Grant RO1NS071263

Title: Postnatal glucocorticoids suppress glutamatergic neurogenesis

Authors: M. T. K. ZIA[1], A. Z. KIDWAI[3], G. VINUKONDA[3], L. CARSON[3], *P. BALLABH[2]
[1]Pediatrics, Cell Bio & Anat., New York Med. Coll, Valhalla, NY; [2]Pediatrics, Cell Bio & Anat., New York Med. Coll, VALHALLA, NY; [3]Cell Biol., NewYork Med. Col., Valhalla, NY

Abstract: Background: Postnatal glucocorticoids (GCs) are widely used in the prevention of chronic lung disease in premature infants. Their use is associated with reduced cortical growth and neurodevelopmental delay. However, the underlying mechanism of GC-induced impairment of cortical growth remains elusive. Cerebral cortex consists of glutamatergic (80%) and GABAergic neurons (20%). Glutamatergic neurogenesis is orchestrated in the dorsal ventricular (VZ) and subventricular zones (SVZ) of the cerebral cortex. Radial glia cells in the VZ generate intermediate progenitor cells (IPCs) that migrate to the SVZ to further divide, migrate, and form projection neurons in the cortical layers. Pax6 is a key transcription factor regulating production of glutamatergic neurons. Since glutamatergic neurogenesis continues in preterm infants until 28 gestational weeks, it is important to determine whether GCs influence generation of projection neurons. **Objective:** Evaluate the effect of postnatal dexamethasone and betamethasone on glutamatergic neurogenesis in preterm rabbit pups. **Design/Methods:** Preterm rabbit pups were delivered at E29 (term=32d) by C-section. The pups were randomized at 24 h age into three groups: IM betamethasone (0.5mg/kg/dose once daily for 5d), dexamethasone (0.25mg/kg/dose twice daily for 5d), or saline. The pups were euthanized at postnatal d3 and 7. The coronal sections at mid-septal nucleus level were immunostained with Sox2 (radial glia) or Tbr2 (IPC) antibodies along with Ki67(cell proliferation) and DAPI. The cells were counted in the dorsal SVZ using stereological protocol. Pax6 was assayed by Western blot analysis. **Results:** Stereological analysis of immunostained sections revealed that both total and cycling $Tbr2^+$ IPCs were significantly less abundant in betamethasone and dexamethasone treated pups compared with saline controls ($P<0.05$, n=5 each group) at d3 and 7. However, $Sox2^+$ radial glia were comparable between GC treated groups and controls at d3. Western blot analysis showed that Pax6 levels were reduced in betamethasone treated pups compared with controls at d3 ($P<0.05$), but not at d7. **Conclusions:** Postnatal GCs suppress glutamatergic neurogenesis by downregulating the Pax6 transcription factor. We speculate that postnatal GCs reduce cortical growth by suppressing glutamatergic neurogenesis and subsequent corticogenesis.

Disclosures: M.T.K. Zia: None. **A.Z. Kidwai:** None. **G. Vinukonda:** None. **L. Carson:** None. **P. Ballabh:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH RO1NS071263.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.09/R1

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

Title: Striatal dopaminergic neurotoxicity and neuroinflammation caused by methamphetamine are differentially affected by *in vivo* stressors versus corticosterone treatment

Authors: *D. B. MILLER, K. A. KELLY, J. P. O'CALLAGHAN
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Abstract: In the mouse methamphetamine (METH) causes striatal dopaminergic nerve terminal damage accompanied by neuroinflammation as evidenced by a loss of dopamine (DA), tyrosine hydroxylase protein (TH), an increase in the astrogliosis marker GFAP and an increase in the mRNA of a number of proinflammatory cytokines and chemokines (e.g., IL-1B, LIF, CCL-2). Previously, we found exposure to corticosterone (CORT), a known anti-inflammatory agent, for 7 days in the drinking water prior to METH dosing unexpectedly enhanced the striatal damage and neuroinflammation. As CORT is released with *in vivo* stressor exposure, here we determined how METH neurotoxicity and neuroinflammation were affected by exposure to a different daily stressor for 4 days. Male C57Bl6J mice were exposed to a single s.c. injection of SAL or METH (20 mg/kg) ~ 22 hrs after the final stressor exposure and their rectal temperatures monitored at 0, 1, 3, 5, 7, 9, and 24 hrs post dosing. Striatum and cortex samples were collected 6 & 12 hrs after METH for cytokine gene expression and at 72 hrs for neurotoxicity evaluation. On the 4 days prior to dosing, mice were weighed daily and the control group (No Stress: NS) remained in their home cage while the stressed groups (S) were exposed to the following stressors: (Day 1) damp bedding 6PM to 6AM; (Day 2) a strobe light 6PM to 6AM; (Day 3) restraint from 6AM to 6 PM in home cage; (Day 4) restraint and 1.0 mA inescapable 5 sec electric shock every 30 sec for 100 shocks. Surprisingly, stressor exposure reduced the mortality associated with METH. Stressor exposure reduced thymus weight by 39 & 82% in the S-SAL & S-METH indicating the stressor exposures caused CORT release. *In vivo* stressor exposure did not modify METH hyperthermia. In contrast to our previous work, *in vivo* stressor exposure did not exacerbate striatal neuroinflammation but rather reduced cytokine expression. Striatal astrogliosis was not exacerbated but greater striatal DA and TH reductions were observed. The differences in these neurotoxicity biomarkers may reflect an ability of the *in vivo* stressor protocol to down-regulate DA and TH rather than an increased neurotoxicity. (Supported by CDC-NIOSH intramural funds)

Disclosures: **D.B. Miller:** None. **K.A. Kelly:** None. **J.P. O'Callaghan:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.10/R2

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

Support: NIH R15 NS070774

NIH T34 GM08612

Title: Cellular zinc levels are modulated by TRPML1-TMEM163 interactions

Authors: *J. SILVA, L. BASILIO, T. HART, M. P. CUAJUNGCO
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Abstract: Loss of function mutation in the Mucolipin-1 (TRPML1) ion channel protein causes the human lysosomal storage disorder Mucolipidosis type IV (MLIV). We previously reported that the brain tissues of mice knocked out of TRPML1 protein have significantly higher zinc levels compared to wild-type mice. Tissue culture models using MLIV patient fibroblasts and RNA interference (RNAi) of TRPML1 in human embryonic kidney cells confirmed that the loss or knock down of TRPML1 protein results in intracellular zinc accumulation. However, the mechanism of zinc elevation in these paradigms remains unknown. Using a membrane-based yeast two-hybrid technique, we identified a putative zinc-binding protein called transmembrane (TMEM)-163 protein as an interacting partner for TRPML1. We confirmed the interaction by co-immunoprecipitation and mass spectrometry. Confocal microscopy of TMEM163 and TRPML1 each tagged with a unique fluorescent protein showed partial co-localization and a punctate distribution pattern in human embryonic kidney (HEK)-293 cells. We hypothesized that the interaction between TMEM163 and TRPML1 plays a role in regulating TMEM163, and thus intracellular zinc. Interestingly, we found that TMEM163 mRNA and protein levels were markedly reduced in MLIV fibroblast cells when compared to wild-type control. This result correlated with our observation of intracellular zinc accumulation in lysosomes of MLIV patient fibroblast cells. RNA interference targeting TMEM163 alone, or TMEM163 and TRPML1 in HEK-293 cells resulted in significant increase of intracellular zinc. Meanwhile, deletion of the N-terminus of TMEM163 protein disrupts its binding to TRPML1, and resulted in relatively higher intracellular zinc levels when exposed to exogenous zinc. Evidence from cell surface biotinylation showed that co-expression of TRPML1 reduced TMEM163 levels localized within the plasma membrane of HEK-293 cells. This result explains why a reduction of cellular zinc flux is evident when TRPML1 is co-expressed with TMEM163, while the loss of interaction by

TMEM163 deletion mutants with TRPML1 results in intracellular zinc elevation. Overall, our data suggest that both TRPML1 and TMEM163 proteins play a role in regulating intracellular zinc. Thus, the loss of TRPML1 protein interaction with TMEM163 and/or the reduction of TMEM163 protein could result in zinc dyshomeostasis in MLIV.

Disclosures: J. Silva: None. **L. Basilio:** None. **T. Hart:** None. **M.P. Cuajungco:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.11/R3

Topic: C.17. Drugs of Abuse and Addiction

Title: The levels of E3 ligase Parkin are decreased in rat colon after self-administration of neurotoxic doses of methamphetamine

Authors: *A. FLACK[1], A. L. PERSONS[2], S. M. KOUSIK[2], T. NAPIER[2], A. MOSZCZYNSKA[1]
[1]Wayne State Univ., Detroit, MI; [2]Rush Univ. Med. Ctr., Chicago, IL

Abstract: Methamphetamine (METH) is a highly abused psychostimulant that is associated with an increased risk for developing Parkinson's disease (PD; Callaghan et al. Drug Alcohol Depend 120:35,2012). This enhanced vulnerability may relate to the known neurotoxic effects of METH via oxidative stress, mitochondrial dysfunction and inflammation (Yamamoto et al. Ann NY Acad Sci. 1187,2010), factors also implicated in PD pathology. Both PD and high-dose METH decrease the function of E3 ligase Parkin, a neuroprotective protein, in the striatum. Self-administration of lower doses of METH decreases levels of tyrosine hydroxylase in the straitum (Kousik et al. Eur J Neurosci. In press). Evidence from the PD field suggests that peripheral factors may contribute to central degenerative processes; for example, α-synuclein aggregates have been identified in the enteric nervous system of the colon of PD patients prior to the onset of motor symptoms (Shannon et al. Mov Disord. 27:709,2012). The aim of our study was to measure the levels of Parkin in the colon at different times following withdrawal from self-administered METH in the rat. We hypothesized that self-administered METH will cause a deficit in colon Parkin at 14 days or earlier. Male Sprague-Dawley rats were implanted with an indwelling jugular catheter allowing self-administration of METH at the concentration of 0.1 mg/kg per infusion. The animals were allowed to self-administer the drug for 3 h per day for 7 days on an FR-1 schedule. At day 8, the administration schedule was increased to FR-5 for 14

days. Control rats received saline. Colon tissue was analyzed at 24 h and 56 d after cessation of METH self-administration. Coronal colon sections were examined for Parkin and Neu-N immunoreactivity, using immunofluorescence and confocal microscopy. Total levels of parkin and its levels in Neu-N positive cells were markedly decreased at 24 h post METH as compared to their saline-treated counterparts. At 56 d post METH, Parkin levels returned to the levels similar to those demonstrated in the saline treated controls. Since we observed a deficit in colon Parkin earlier than deficits in striatal tyrosine hydroxylase, colon Parkin might serve as an early sign of METH neurotoxicity in the brain of human chronic METH users.

Disclosures: **A. Flack:** None. **A.L. Persons:** None. **S.M. Kousik:** None. **T. Napier:** None. **A. Moszczynska:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.12/R4

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

Support: NIH Grant NS43997C

Swedish Medical Center

Title: Methamphetamine induces activation of eNOS in cultured mouse brain endothelial cells

Authors: *J. SEO[1], S. M. JONES[1], J. P. ELLIOTT[2], G. A. WEST[3]
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Abstract: Methamphetamine is a potent psychostimulant producing neurotoxicity and blood-brain barrier dysfunction in the brain. Also it is known to cause vessel dysfunction in human and animals. In our previous study, we reported that methamphetamine caused ET-1 mediated vasoconstriction of isolated intracerebral arterioles from mice. Prior to vasoconstriction, vessels exhibit slight vasodilation that lasts for approximately 5 min. Some studies report that methamphetamine induces nitric oxide, which may cause neurotoxicity by inducing BBB dysfunction. To understand the mechanism underlying this bi-phasic response of vessels, we investigated the involvement of NO in methamphetamine-induced responses. Western blot of total eNOS and phosphorylated eNOS on cultured bEND3 mouse brain endothelial cells showed

that activated eNOS proteins were increased significantly in cells treated with 10 μM and 100μM methamphetamine for 24hrs. However, qRT-PCR of eNOS shows that synthesis of eNOS was not induced by methamphetamine treatment. These findings demonstrate that short-term vasodilation which precedes long-lasting vasoconstriction induced by methamphetamine might be caused by NO produced from post-translational modification and activation of eNOS.

Disclosures: J. Seo: None. **S.M. Jones:** None. **J.P. Elliott:** None. **G.A. West:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.13/R5

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

Support: ARISTEIA II Grant, K.A. 3948

Title: Effect of the synthetic neurosteroidal derivatives BNN 20 and 27 in an *ex vivo* model of chemical ischemia and the *in vivo* STZ-model of diabetic retinopathy: Neuroprotection via TrkA signaling

Authors: *K. A. THERMOS, N. MASTRODIMOU, S. LISA, R. IBÁN-ARIAS, S. POULAKI, P. IORDANIDOU, P. GIANNOGONAS, D. KOKONA, M. KAMARATOU, E. VOLITAKI, I. CHARALAMPOPOULOS, A. GRAVANIS
Med., Univ. of Crete, Heraklion, Crete, Greece

Abstract: The main objective of this study was to investigate the neuroprotective actions of the novel spiro-epoxy derivatives of the neurosteroid Dehydroepiandrosterone (DHEA), BNN27 and BNN20, that were shown to have antiapoptotic and neuroprotective activities and no peripheral side effects. Earlier studies in our laboratory showed that DHEA protected the retina from AMPA excitotoxicity *in vivo* via NGF TrkA receptor activation. In a preliminary study, we showed that the BNN27 analogue (10mg/kg, i.p) afforded neuroprotection to amacrine cells but not to ganglion axons, in the streptozotocin (STZ)-model of diabetic retinopathy (DR). The focus of the present study was to investigate further the neuroprotective properties of the novel synthetic neurosteroid BNN27, but also BNN20, in an *ex vivo* model of chemical ischemia (C.I) and in the *in vivo* STZ-model of DR, and examine the involvement of the NGF TrkA receptor in the neuroprotection. Adult Sprague-Dawley rats were used. Retinal C.I was induced by the use of NaCN (25mM) and iodoacetic acid (5mM), whereas diabetes was induced by a single

injection of STZ (70mg/kg, i.p). Antibodies against retinal markers, known to be affected in the two models, ganglion cell axons (NFL), amacrine cells [nitric oxide synthase (bNOS)] and rod bipolar cells (PKC), were employed to assess retinal cell loss and neuroprotection. BNN 27 protected the bNOS and PKC expressing cells, mimicking the DHEA effect in the C.I model, reaching control levels at 100nM concentration. The neuroprotection of both neurosteroids was attenuated in the presence of an inhibitor of TrkA receptor (Calbiochem 648450). BNN20 was less efficacious than BNN27 in affording neuroprotection. In the STZ-model, BNN27 protected both the nitric oxide synthetase expressing cells and ganglion axons in a dose-dependent manner. Full protection of the amacrine cells was reached at the dose of 10mg/kg (i.p), whereas the ganglion axons at 50mg/kg (i.p). To determine if TrkA receptor and its downstream pathways were activated in retinas of control and diabetic rats, as well diabetic rats treated for one week with BNN27 (2,10,50mg/kg), western blot analysis was performed using specific antibodies against the phosphorylated and total isoforms of TrkA, as well as phosphorylated and total ERK1/2 proteins. At the higher dose of 50mg/kg, the levels of pTrkA and pERK in retinal tissue were significantly increased. The results from both the *ex vivo* and *in vivo* models suggest that the neurosteroid BNN 27 provides neuroprotection to rat retina via TrkA signaling. Ongoing studies are investigating further the mechanisms involved in the neuroprotection of BNN27 and BNN20 and the biology of DR.

Disclosures: K.A. Thermos: None. **N. Mastrodimou:** None. **S. Lisa:** None. **R. Ibán-Arias:** None. **S. Poulaki:** None. **P. Iordanidou:** None. **P. Giannogonas:** None. **D. Kokona:** None. **M. Kamaratou:** None. **E. Volitaki:** None. **I. Charalampopoulos:** None. **A. Gravanis:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

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Program#/Poster#: 227.14/R6

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

Support: NIAAA

Title: Ethanol induces chemokine MCP-1 increasing neuroinflammation and neurodegeneration in mouse brain

Authors: *L. QIN, F. T. CREWS
Bowles Ctr. Alcohol Studies, Univ. North Carolina, Sch. Med., Chapel Hill, NC

Abstract: Alcohol abuse is associated with proinflammatory gene induction, neuroinflammation and neurodegeneration. We have found the chemokine MCP-1 is elevated with both acute and chronic ethanol treatments in the brain. Binge drinking levels of ethanol treatments of C57BL/6 mice increased brain mRNA and protein levels of MCP-1, as well as many other innate immune genes as determined by real-time PCR and ELISA. To investigate the effect of ethanol on innate immune gene induction, mice were treated with acute ethanol (6 g/kg, i.g., 25% ethanol w/v, 1 dose), and brain chemokines, cytokines (mRNA and ELISA) and other innate immune genes were determined at various times and compared to blood alcohol levels. Innate immune gene induction by ethanol was greatest after ethanol clearance. Acute ethanol treated mice increased the number of activated caspase-3 (a cell death marker) +IR cells by 110% ($p<0.05$) in the prefrontal cortex (PFC) 24 hours after ethanol treatment. Confocal microscopy shows that MCP-1+IR cells are co-localized with Neu-N (a neuronal marker) and with activated caspase-3, suggesting that MCP-1 contributes to acute ethanol neurodegeneration. To investigate the effects of prolonged alcohol abuse, mice were treated with ethanol for 10 days (5 g/kg, i.g., 25% ethanol w/v, daily for 10 days). At 24 hours after ethanol treatment, the chemokine MCP-1 and other innate immune genes were measured; neurotoxicity was measured using activated caspase-3 immunohistochemistry and Fluoro-Jade B (FJB) staining and compared to the orbito-frontal cortex (OFC) of human post-mortem alcoholics. Ethanol treated mice showed increased markers of neuronal cell death: Fluoro-Jade B positive staining and activated caspase-3 immunohistochemistry in several brain regions including cortex and hippocampus. These markers of cell death were co-localized with Neu-N, consistent with ethanol-induced neurodegeneration. In the PFC mice showed a 605% increase in FJB and in the OFC human post-mortem alcoholic brain showed an 85% increase in FJB cell death. Together, these results suggest that both acute and chronic ethanol induce MCP-1 increasing neuroinflammation and neurodegeneration mimicking human alcoholic pathology. (Supported by NIAAA.)

Disclosures: L. Qin: None. **F.T. Crews:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.15/R7

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

Title: Assessment of reflex sympathetic activity after guanethidine-induced depletion of postganglionic neurons in rat

Authors: *C.-N. LIU, M. ZAHNER, C. OKERBERG, I. PARDO, C. NORTHCOTT, D. PELLETIER, J. AUBRECHT, C. SOMPS
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Abstract: Guanethidine is a peripherally restricted adrenergic-neuron-blocking agent, that when administered in toxic doses, destroys sympathetic postganglionic neurons in rat. By virtue of its ability to selectively deplete postganglionic neurons while sparing the preganglionic neurons guanethidine is a very useful tool that can be used to better understand sympathetic activity. While many aspects of neuronal depletion using guanethidine have been well characterized, the degree of neuronal loss required to produce functional deficits in sympathetic outflow is not well known. Thus, the goal of these experiments was to determine the degree of sympathetic postganglionic neuronal loss that correlates to a reduced excitability of the sympathetic nervous system. To do this we treated rats with guanethidine (100 mg/kg/day, i.p.) or vehicle (saline, n=4) for either 5 days (n=4) or 11 days (n=4). We assessed sympathetic activity by measuring the renal sympathetic nerve activity (RSNA) during basal conditions and during baroreflex and chemoreflex testing. Neurophysiological testing was performed 24-28 days after the final guanethidine dose, in order to minimize confounding effects of decreased body weight and guanethidine pharmacology during the dosing phase of the experiment. Although modest decreases in baseline blood pressure were observed in both groups of guanethidine-treated rats there was no effect on basal heart rate. Both vehicle-treated and 5-day guanethidine-treated rats displayed strong pulse-synchronous RSNA during baseline and robust baroreflex and chemoreflex responsiveness during sympathetic testing. However, rats treated with guanethidine for 11 days displayed decreased basal sympathetic tone and attenuated baroreflex and chemoreflex responsiveness. When comparing basal sympathetic activity to baseline blood pressure and heart rate, we found that guanethidine-treatment resulted in greater decreases in sympathetic activity than blood pressure. In 5-day guanethidine-treated rats a 19% decrease in ongoing basal sympathetic activity correlated to only a 7% decrease in ongoing basal blood pressure and in the 11-day guanethidine-treated rats a 61% decrease in ongoing basal sympathetic activity correlated to only a 13% decrease in ongoing basal blood pressure. Whereas the disproportionate decrease in basal blood pressure to sympathetic neuronal depletion may be attributed to long-term blood pressure compensatory mechanisms these data suggest that treatment reported in the literature to cause loss of sympathetic postganglionic neurons, and confirmed by us using stereologic techniques, may be tolerable under basal conditions.

Disclosures: C. Liu: None. **M. Zahner:** None. **C. Okerberg:** None. **I. Pardo:** None. **C. Northcott:** None. **D. Pelletier:** None. **J. Aubrecht:** None. **C. Somps:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.16/R8

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

Support: DA07427

Title: MDMA-induced reductions in hippocampal parvalbumin interneurons is associated with NMDA receptor activation

Authors: *S. A. COLLINS[1], G. A. GUDELSKY[2], B. K. YAMAMOTO[1]
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Abstract: MDMA is a widely abused synthetic psychostimulant which causes rapid and extensive release of the monoaminergic neurotransmitters dopamine and serotonin. Several studies have shown that MDMA is selectively neurotoxic to serotonergic (5HT) terminals in the brains of exposed rats. This neurotoxicity is prevalent throughout limbic and cortical regions and parallels the learning deficits in rats exposed to MDMA. Previous findings by our lab and colleagues revealed a significant increase in glutamate within the hippocampus during binge MDMA exposure suggesting that excitotoxicity is involved. This effect was dependent on increases in extracellular serotonin and the activation of 5HT2a receptors. In addition to the long term depletions of 5HT, the acute increases in extracellular glutamate coincided with a loss of parvalbumin-immunoreactive (PV-IR) interneurons of the dentate gyrus region. Given the known susceptibility of PV interneurons to excitotoxicity, we examined whether MDMA-induced increases in extracellular glutamate in the dentate gyrus are necessary for the loss of PV cells. The results indicate that extracellular glutamate is increased (52% increase, p=.003) in the dentate gyrus of the hippocampus and that the NMDA receptor antagonist, MK-801, when administered during MDMA (7.5 mg/kg x 4, ip) exposure, prevented the loss of PV-IR interneurons seen 10 days after MDMA exposure. Additionally, the administration of the 5HT2a antagonist MDL100907 during MDMA exposure, blocked the reduction of PV-IR. MDMA-induced hyperthermia was maintained during MK801 and MDL100907 treatments by elevating ambient temperature. Moreover, the increases in extracellular glutamate and the subsequent decrease in PV-IR (30% decrease; p<.001) were prevented when rats were treated with the prostaglandin EP1 receptor antagonist, SC-51089, during MDMA exposure. These findings are consistent with previous studies by others showing that 5HT2a receptor activation promotes prostaglandin production that in turn, can be excitotoxic. Therefore, increases in extracellular serotonin during MDMA exposure may promote excitotoxicity to PV interneurons through 5HT2a activation and increased production of prostaglandins. Given the known role of the

hippocampus in learning and memory, it remains to be determined if this loss of GABAergic interneurons could lead to a loss of cognitive function.

Disclosures: **S.A. Collins:** None. **G.A. Gudelsky:** None. **B.K. Yamamoto:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

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Program#/Poster#: 227.17/R9

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

Support: NIH/NICHD HD44517 (to V.J-T.), NIH/NICHD HD44517-S (to V.J-T.), Harold Carron endowment (to V.J-T), John E. Fogarty Award TW007423-128322 (to P.I. V.J-T.), the National March of Dimes Award (to V.J-T.).

V.J-T. was an Established Investigator of the American Heart Association.

Title: An early exposure to general anesthesia modulates gene expression in the developing rat subiculum

Authors: H. OSURU, L. DALLA MASARA, A. OKLOPCIC, *V. JEVTOVIC-TODOROVIC
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Abstract: Background: Clinically- used general anesthetics, alone or in combination, cause damage to the developing mammalian brain. Many cellular mechanisms have been investigated and several were found to play potentially important role in anesthesia-induced killing of young neurons. While many studies have focused on morphological outcomes, little is known about anesthesia-induced alteration in transcription and DNA methylation of genes important for neuronal development. **Aims:** To decipher the potential for anesthesia-induced modulation of gene expression during early brain development. **Experimental Design**: At PND7 (peak of synaptogenesis) experimental rats were administered midazolam (9 mg/kg, intraperitoneally), followed by 6 hours of nitrous oxide (70%), isoflurane (0.75%) anesthesia (in 30% oxygen). Sham-controls received 6 hours of mock anesthesia (vehicle+air). Rats were sacrificed at PND8; fresh subicular tissue was collected for microarry profiling and DNMT/DNdeMt enzyme activity. Differentially and significantly modulated brain development- and disease-associated genes were further confirmed by qRT-PCR. In addition, locus Specific 5-mC and 5-hmC levels were also estimated by methylation-insensitive (MspI) and sensitive (HpaII) restriction

digestion-qPCR. **Results:** Using microarray approach we found that in anesthesia-treated group compared to the control group total of 266 genes were significantly up-regulated and 158 genes were significantly down regulated ($p<0.05$) in developing subiculum. The qRT-PCR analysis of genes found to be most profoundly modulated by anesthesia; i.e. target genes (e.g. *Oxtr, Mecp2, Avpr2, Mrgprb2, Cdk5,Grik1,Wnt1and Drd2*) confirmed good correlation with microarray findings and significant modulation when compared to controls. When 5-mC and 5-hmC levels and the activity of DNMT/DNdeMt enzymes were assessed we found a strong correlation between anesthesia-induced effects on DNA methylation and target gene expression. **Conclusions:** An early exposure to general anesthesia disrupts target gene expression via altered DNA methylation. Since these genes are important for functional and morphological synapse development and behavioral defense responses we conclude that anesthesia-induced developmental neurotoxicity could be at least in part caused by early genetic modulations.

Disclosures: H. Osuru: A. Employment/Salary (full or part-time):; Research Associate,NIH/NICHD HD44517 (to V.J-T.), NIH/NICHD HD44517-S (to V.J-T.), Harold Carron endowment (to V.J-T), John E. Fogarty Award TW007423-128322 (to P.I. V.J-T.), the National March of Dim. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NICHD HD44517 (to V.J-T.), NIH/NICHD HD44517-S (to V.J-T.), Harold Carron endowment (to V.J-T), John E. Fogarty Award TW007423-128322 (to P.I. V.J-T.), the National March of Dimes Award (to V.J-T. **L. Dalla Masara:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NICHD HD44517 (to V.J-T.), NIH/NICHD HD44517-S (to V.J-T.), Harold Carron endowment (to V.J-T), John E. Fogarty Award TW007423-128322 (to P.I. V.J-T.), the National March of Dimes Award (to V.J-T. **A. Oklopcic:** A. Employment/Salary (full or part-time):; NIH/NICHD HD44517 (to V.J-T.), NIH/NICHD HD44517-S (to V.J-T.), Harold Carron endowment (to V.J-T), John E. Fogarty Award TW007423-128322 (to P.I. V.J-T.), the National March of Dimes Award (to V.J-T.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NICHD HD44517 (to V.J-T.), NIH/NICHD HD44517-S (to V.J-T.), Harold Carron endowment (to V.J-T), John E. Fogarty Award TW007423-128322 (to P.I. V.J-T.), the National March of Dimes Award (to V.J-T. **V. Jevtovic-Todorovic:** A. Employment/Salary (full or part-time):; University Of Virginia, Medical School,Anesthesiology,Research Associate. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Dr.Vesna jevtovic-todorovic.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.18/R10

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

Support: National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010-0023638) and NRF-2013R1A1A2074860

Title: Egb761 attenuates zinc-induced tau phosphorylation at Ser262 via GSK3β regulation in rat primary cortical neurons

Authors: *K. KWON[1], E. LEE[2], K. CHO[2], S. YANG[2], M. KO[2], C. SHIN[3], S.-H. HAN[4]
[1]Dept. of Neurology, Sch. of medicine, Konkuk Univ., Seoul, Korea, Republic of; [2]Dept. of Neurosci., [3]Dept. of Pharmacol., [4]Dept. of Neurol., CGNR, IBST, Konkuk Univ. Sch. of Med., Seoul, Korea, Republic of

Abstract: In brain, excess zinc promotes the deposition of amyloid β protein and intraneuronal accumulation of neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau protein. Egb761, a standardized Ginkgo biloba extract, is known to be a powerful antioxidant and exhibits neuroprotective effects. In this study, we investigated the effects of Egb761 on zinc-induced tau phosphorylation in rat primary cortical neurons. We investigated whether administration of Egb761 can modify tau phosphorylation in primary cortical neurons employing Western blotting analyses: 1) the site of zinc-induced tau phosphorylation; 2) the effects of Egb761 on zinc-induced tau hyperphosphorylation; 3) the signal pathway regulated by Egb761, 4) we also test the effects of Egb761 on cell viability and ROS generation by MTT reduction assay and ROS measurement. Zinc induced tau phosphorylation at Ser262 in time and dose-dependent manner in rat primary cortical neurons. However, other sites of tau were not phosphorylated. Tau phosphorylation at Ser262 was elevated at 30 min, peaked at 3 h after the zinc treatment (control: 100±1.2%, 30 min: 253±2.24%, 3h: 373±1.3%). Egb761 was attenuated zinc-induced tau hyperphosphorylation at Ser262 in concentration-dependent manner. NAC, an antioxidant, also inhibited zinc-induced tau phosphorylation at Ser262. Egb761 was prevented the zinc-induced MAPKs and GSK3β activation. Lithium inhibited zinc-induced tau phosphorylation. Zinc treatment increased ROS generation and neuronal cell death which was effectively prevented by Egb761. These results suggest that Egb761 inhibited zinc-induced tau phosphorylation dependently of an antioxidative action through GSK3β regulation, and support the efficacy of Egb761 for treatment of tauopathy in neurological disorder such as Alzheimer's disease. Acknowledgment: This work was supported by the National Research Foundation of

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Disclosures: **K. Kwon:** None. **E. Lee:** None. **K. Cho:** None. **S. Yang:** None. **M. Ko:** None. **C. Shin:** None. **S. Han:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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

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John E. Fogarty Award TW007423-128322 (VJT)

The National March of Dimes Award (VJT)

V.J-T. was an Established Investigator of the American Heart Association

RO1 GM 102525 (SMT)

Title: Early exposure to general anesthesia alters developmental synaptic transmission and excitability

Authors: *N. LUNARDI[1], M. R. DIGRUCCIO[2], S. M. TODOROVIC[1], V. JEVTOVIC-TODOROVIC[1]
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Abstract: Objectives: Exposure of young rats to general anesthesia (GA) during critical stages of their brain development leaves many surviving synapses with ultrastructural changes indicative of degeneration and is followed by cognitive deficits that persist in their adult life. In view of the possibility that early GA exposure may affect neurons ability to connect and establish proper synaptic circuitries, *we asked whether and how GA alters the synaptic*

transmission of neurons surviving an initial early exposure to GA. **Methods:** At post natal day (PND) 7 (peak of synaptogenesis) experimental rats were administered midazolam (9 mg/kg, intraperitoneal), followed by 6 hours of nitrous oxide (70%), isoflurane (0.75%) and oxygen (30%). Sham-controls received 6 hours of mock anesthesia. Rats were sacrificed from PND 8 to 15 for extensive electrophysiology studies of excitatory synaptic transmission (i.e., miniature post synaptic currents (mEPSCs), evoked post synaptic currents (eEPSCs) and action potential firing patterns) from freshly prepared CA1-subiculum brain slices. We focused on the subiculum since it is very sensitive to the toxic effects of GA and crucial for learning and memory. **Results:** I. Early GA alters spontaneous excitatory synaptic currents. While mEPSCs recordings from sham-controls showed evenly distributed, very low frequency, single-spike currents, traces from GA-treated rats showed irregularly distributed, high frequency, multiple-spike currents. Upon quantification, we found about a 20-fold increase in the frequency of mEPSCs (****, $p<0.0001$) and a significant decrease in the decay time constant (**, $p<0.01$) in GA-treated rats compared to sham-controls, with no changes in amplitude and net charge transfer. II. Early GA alters evoked excitatory synaptic currents. Upon analysis of eEPSCs recordings, GA-treated rats showed a significantly decreased decay time (**, $p<0.01$) and responded to increasing stimulus strengths with significantly higher amplitude depolarizations compared to sham controls ($p< 0.05$), with no changes in paired pulse ratio. III. GA causes hyperexcitability of subicular electrical networks. Our cell-attached patch clamp recordings of spontaneous action potential (AP) firing showed about a 4-fold increase in the frequency of AP firing in GA-exposed rats compared to sham-controls (**, $p<0.01$). **Conclusions:** Early exposure to GA disrupts the function of surviving synapses by altering both pre- and post- synaptic aspects of excitatory synaptic transmission in rat subiculum. Early exposure to GA also disturbs excitatory synaptic transmission by forcing surviving synapses into a state of heightened excitability.

Disclosures: **N. Lunardi:** None. **M.R. DiGruccio:** None. **S.M. Todorovic:** None. **V. Jevtovic-Todorovic:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.20/R12

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

Support: NIH Grant DA013978

Title: Selective sigma receptor ligands attenuate methamphetamine-induced neurotoxicity and genetic alterations in the striatum: Microarray study and validation of select targets

Authors: *R. R. MATSUMOTO[1], M. J. ROBSON[1], N. KAUSHAL[1], A. COOP[2]
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Abstract: Methamphetamine (METH) is a widely abused substance worldwide with neurotoxic potential after high and/or repeated dosing. AC927 is a selective sigma receptor ligand shown in preclinical studies to attenuate depletions of dopamine and 5-HT levels and transporter expression in the striatum resulting from neurotoxic exposure to METH. To identify potential mechanisms that contribute to the neuroprotective potential of AC927 and other sigma ligands, a microarray and validation study was conducted. Male, Swiss Webster mice were treated four times at 2 h intervals using effective doses from earlier studies with: Saline (Sal)/Sal, AC927/Sal, Sal/METH, or AC927/METH. Six hours after the last treatment, the striatum was dissected and later processed for microarray analysis using Mouse Exon 1.0 ST arrays. Neurotoxic METH significantly ($P<0.05$) changed the expression of 2131 genes compared to saline. Of these genes, AC927 pretreatment significantly attenuated the METH-induced changes in 22 genes (greater than 2-fold change) to 119 genes (false discovery rate correction). The changes observed in the microarray studies were independently confirmed for three genes (OSMR, GFAP, Lilrb4) using qRT-PCR, and also a time course series involving a second sigma ligand SN79. The METH-induced changes and protection by sigma ligands were also confirmed at the protein level for OSMR and GFAP using Western blots and immunohistochemistry and shown to specifically involve astrocytes. Together, the data suggest that sigma ligands can target multiple mechanisms, including glial processes, to mitigate the neurotoxic effects of METH.

Disclosures: R.R. Matsumoto: None. **M.J. Robson:** None. **N. Kaushal:** None. **A. Coop:** None.

Poster

227. Drugs, Pharmaceuticals, and Toxicity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 227.21/S1

Topic: C.10. Trauma

Title: Cerebromicrovascular human endothelium: Characterization of functional responses induced by arachidonoyl serine

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Abstract: INTRODUCTION Arachidonoyl serine (ARA-S) is one of many endogenous lipids found in the brain. This agent is chemically related to the endocannabinoid N-arachidonoylethanolamine also known as anadamide and was shown to have similar physiologic and pathophysiologic functions. Overall the reports indicate that ARA-S possesses vasoactive and neuroprotective properties resembling those of cannabinoids. In contrast to other cannabinoids, ARA-S is considered a 'cannabinoid-like' substance since it binds weakly to its known classical receptors - CB1 and CB2. The originally described ARA-S endothelial-dependent vasorelaxation induced in rat abdominal and mesenteric vessels was not abrogated by CB1, CB2 or TRPV1 receptor antagonists. Other reports suggested that G protein-coupled receptor 55 (a putative cannabinoid receptor may be involved in mediating the ARA-S effects. In addition, the reports indicate that ARA-S stimulated phosphorylation of the kinases MAPK and AKT. This report will demonstrate that ARA-S stimulated these kinases as well JNK and cJUN via CB1, CB2 and TRPV1 receptors. In particular the findings will focus on the involvement of Rho/PI3/Akt pathway in the ARA-S-induced phosphorylation of kinase and actin reorganization in human brain endothelial cells (HBEC). METHODS Cultured HBEC which were >95% Factor VIII+ were exposed to ARA-S for 15 min alone or pre-treated with selective antagonists for CB-1, CB-2 and TRPV1 receptors (SR141716A, SR141728A and capsazepine, respectively). The levels of phosphorylation of p44/42 MAPK, Akt, JNK and c-Jun were determined by Western blot analysis. RESULTS 1. ARA-S activated both CB1 and CB2 receptors in HBEC as demonstrated by increased fluorescence (3-4 fold). 2. ARA-S stimulated phosphorylation of the above mentioned stress kinases (up to 3 fold) was inhibited by CB1, CB2 and TRPV1 receptor antagonists. 3. (a) ARA-S also increased the activity of Akt as demonstrated by the phosphorylation of Glycogen synthase kinase 3. (b) The ARA-S induced phosphorylation was enhanced (2-fold) by inhibition of Rho kinase. 4. ARA-S reduced (30%) activity of Rho kinase in control and ET-1-stimulated cells. 5. ARA-S induced reorganization of cellular actin in control and ET-1-stimulated HBEC. CONCLUSION The HBEC responses induced by ARA-S are mediated by CB1, CB2 and TRPV1 receptors and involve Rho /PI3/Akt signal transduction pathway. The findings suggest that ARA-S is an inhibitor of rho kinase and may play a critical role in the regulation of rho kinase activity and its subsequent effects of cell properties including the cytoskeleton.

Disclosures: T. Kino: None. **T. Tomori:** None. **P. Castri:** None. **Y. Chen:** None. **F. Lenz:** None. **R. Abutarboush:** None. **R. McCarron:** None. **M. Spatz:** None.

Poster

228. Gene Therapy

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 228.01/S2

Topic: C.14. Gene Therapy

Support: NIH/NINDS 1R21 NS078314

NIH T32 GM8243-27

Title: Choroid plexus-directed gene therapy as a source of alpha-N-acetyl-glucosaminidase-IGF2 fusion protein in Sanfilippo B mice

Authors: *S.-H. KAN[1], S. Q. LE[1], M. HADDAD[2], E.-Y. CHOI[2], A. DONSANTE[2], S. G. KALER[2], P. I. DICKSON[1]
[1]LA Biomed At Harbour UCLA, Torrance, CA; [2]Eunice Kennedy Shriver Natl. Inst. of Child Hlth. and Develop. at the Natl. Inst. of Hlth., Bethesda, MD

Abstract: Mucopolysaccharidosis type IIIB (MPS IIIB; Sanfilippo B) is an inherited neurodegenerative disorder for which no effective treatment is currently available. The cause of MPS IIIB is deficiency of the lysosomal enzyme, α-N-acetyl-glucosaminidase (NAGLU) and resultant storage of heparan sulfate. Impediments to enzyme replacement therapy include the short half-lives, absence of mannose 6-phosphate (M6P), and poor blood-brain barrier penetration associated with recombinant human NAGLU.. A modified human NAGLU fused to the receptor binding motif of insulin-like growth factor 2 (hNAGLU-IGF2) has been shown to enhance entry to MPS IIIB fibroblasts via M6P/IGF2 receptor-mediated endocytosis. In this study, we administered a recombinant adeno-associated virus, serotype 5 (AAV5) vector expressing rhNAGLU-IGF2 that targets the choroid plexus epithelia via lateral ventricle injection to deliver the missing enzyme to the cerebrospinal fluid (CSF) of MPS IIIB mice. We cloned the hNAGLU-IGF2 cDNA into an AAV vector plasmid and generated recombinant AAV5 vector by the triple transfection method. NAGLU activity assay and western blots confirmed robust expression of the recombinant protein in (what cell type) . An *in vivo* pilot study was then performed in MPS IIIB mice by injecting 5x10E10 vector genomes (v.g.) of rAAV5-rhNAGLU-IGF2 to the lateral ventricles of adult mice, or 5x10E9 v.g. in neonatal mice. The brains were taken 2 weeks after viral injection and compared with brains of untreated MPS IIIB or control (heterozygous) mice. NAGLU activity reached twice normal levels in the AAV5-treated brains. β-hexosaminidase activity, which is abnormally elevated in MPS IIIB, was significantly reduced in the AAV5-treated brains. Histochemical evaluations confirmed

NAGLU-IGF2 expression in the choroid plexus epithelium. NAGLU-IGF2 was also detected in brain parenchyma, even contralateral to the administered site, indicating delivery from the CSF. Evaluation of the efficacy of AAV5 gene therapy to correct brain lysosomal storage is in progress. Our results suggest that the combination of M6P/IGF2 receptor-mediated endocytosis and choroid plexus-targeted viral gene therapy may overcome the major obstacles for enzyme replacement therapy for MPS IIIB and enable feasible, sustained, and efficient distribution of NAGLU throughout the brain.

Disclosures: S. Kan: None. **S.Q. Le:** None. **M. Haddad:** None. **E. Choi:** None. **A. Donsante:** None. **S.G. Kaler:** None. **P.I. Dickson:** None.

Poster

228. Gene Therapy

Location: Halls A-C

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Program#/Poster#: 228.02/S3

Topic: C.14. Gene Therapy

Support: Department of Defense/W81XWH-09-1-0381

JPB Foundation

Title: P11 gene therapy for Parkinson's disease L-Dopa induced dyskinesias

Authors: *R. MARONGIU[1], M. ARANGO-LIEVANO[1], A. FELIZ[1], A. CENCI NILSSON[2], P. GREENGARD[3], M. G. KAPLITT[1]
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Abstract: P11 (S100A10) is a scaffold protein involved in the membrane localization and activity of its interactors, including ion channels and serotonin receptors. P11 KO mice have altered dopamine responsiveness in the 6OHDA mouse model of Parkinson's disease (PD). Here we investigated the relationship between dopamine signaling and striatal p11 in 6OHDA mice. We generated an AAV vector which blocks production of murine p11 (AAV-sh.p11) and stereotactically injected it into the dorsal striatum, which is the area that receives dopamine produced in the substantia nigra and is lost in PD. We first found that inhibition of striatal p11 significantly decreased rotational behavior in response to acute treatment with D1 and D2

receptor agonists, apomorphine, and L-Dopa. This suggested a reduction in hypersensitivity to dopamine due to decreased striatal p11. We therefore explored the effects of p11 on chronic complications of L-Dopa therapy in mice. We observed that inhibition of p11 in the dorsal striatum significantly decreases all abnormal involuntary movements due to L-Dopa chronic treatment by roughly 50% when compared to control mice. This indicates that normal striatal p11 levels are necessary for full expression of L-Dopa-induced therapy dyskinesias, consistent with our rotational data. Finally, we sought to study if p11 influence on striatal motor function is mediated by either D1 or D2 receptors by using the Cre-LoxP system. We developed a novel CRE inducible AAV vector, lox.sh.p11, to selectively express the sh.p11 only in the presence of CRE recombinase. To specifically silence p11 in D1R or D2R expressing neurons, we injected either lox.sh.p11 or lox.sh.luc (control) AAV vectors into the ipsilateral dorsal striatum of previously 6OHDA lesioned D1R-Cre and D2R-Cre transgenic mice. As controls, the respective wild-type (wt) mouse littermates were lesioned and received a striatal injection of either sh.Luc or sh.p11. Inhibition of p11 in only D1R-Cre mouse line recapitulated motor behaviors previously obtained by knocking down p11 in every cell of the dorsal striatum. A comparable significant decrease was observed in the wt littermates when p11 was knocked down in all cell types, as observed earleir. D2R-Cre mice injected with AAV.sh.p11 did not show any changes compared to their Taken together our results demonstrate that normal p11 levels are necessary for striatal dopamine responsiveness and also suggest that blocking striatal p11 may be a potential therapeutic target to treat PD and dyskinesias following chronic dopaminergic drug therapy.

Disclosures: R. Marongiu: None. **M. Arango-Lievano:** None. **A. Feliz:** None. **A. Cenci Nilsson:** None. **P. Greengard:** None. **M.G. Kaplitt:** None.

Poster

228. Gene Therapy

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 228.03/S4

Topic: C.14. Gene Therapy

Support: National Research Foundation of Korea (NRF) grant (No. 2008-0061888)

National Research Foundation of Korea (NRF) grant (No. 2012R1A1A1039140)

Title: Transduction with hRheb(S16H) induces the production of ciliary neurotrophic factor and its receptor in the nigral dopaminergic neurons *in vivo*

Authors: *K. JEONG[1,2], J. NAM[3], R. E. BURKE[4,5], B. JIN[3,6], S. KIM[1,2,7,8]
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Abstract: Adeno-associated virus 1 (AAV1) transduction with an active human ras homolog enriched in brain [hRheb(S16H)] induces trophic and protective effects on the nigrostriatal dopaminergic projection in neurotoxin models of Parkinson's disease. Moreover, hRheb(S16H) transduction stimulates the production of neurotrophic factors such as glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor, contributing to the survival of dopaminergic neurons, suggesting that its expression can impart to mature dopaminergic neurons the important ability to produce multi-neurotrophic agents for anti-neurodegeneration in the adult brain. Cilliary neurotrophic factor (CNTF) is another representative agent for the survival of dopaminergic neurons, showing the remarkable reduction in dopaminergic neurons in Parkinson's disease. In the present study, we have investigated the cellular alteration on the levels of CNTF and CNTF receptor α (CNTFRα) by transduction with hRheb(S16H) in the substantia nigra of rats. AAV1 packaging hRheb(S16H) was unilaterally injected into the rat substantia nigra, and 1-methyl-4-phenylpyridinium (MPP^+) was injected into the medial forebrain bundle for a neurotoxin model of Parkinson's disease at 3 weeks after injection of hRheb(S16H). Our results show that transduction with hRheb(S16H) significantly up-regulates the expression of CNTF and CNTFRα in the nigral dopaminergic neurons at 4 weeks after injection of AAV-hRheb(S16H). In addition, the expression of CNTFRα increased by hRheb(S16H) is significantly preserved against the MPP^+-induced neurotoxicity. These results suggest that transduction of dopaminergic neurons with hRheb(S16H) may contribute to the activation of CNTF signaling pathway as a potential therapeutic mechanism against Parkinson's disease. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (No. 2008-0061888 and 2012R1A1A1039140).

Disclosures: K. Jeong: None. **J. Nam:** None. **R.E. Burke:** None. **B. Jin:** None. **S. Kim:** None.

Poster

228. Gene Therapy

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Program#/Poster#: 228.04/S5

Topic: C.14. Gene Therapy

Support: Graduate School of Michigan State University

Mercy Health Saint Mary's

Morris K. Udall Center of Excellence for Parkinson's Disease Research at Michigan State University NS058830

Title: Recombinant adeno-associated and lentiviral vector transduction efficiency in the young adult and aged rat midbrain

Authors: *N. POLINSKI, F. P. MANFREDSSON, M. BENSKEY, C. J. KEMP, N. C. KUHN, A. COLE-STRAUSS, K. STEECE-COLLIER, K. L. PAUMIER, J. W. LIPTON, C. E. SORTWELL
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Abstract: Clinical trials currently examine the efficacy of viral vector-mediated gene delivery for treating age-related neurodegenerative diseases such as Parkinson's disease. While viral vector strategies have been successful in preclinical studies, to date, human clinical trials have disappointed. This may be partially due to the fact that preclinical studies fail to account for aging as an important covariate. In fact, aging is the greatest risk factor for developing PD, and cellular processes used by viral vectors for gene transduction can also be altered with age. Previously, we found that gene transfer utilizing recombinant adeno-associated virus serotype 2/5 (rAAV2/5) results in decreased transduction efficiency in the aged rat midbrain as compared to the young adult rat. Injection of rAAV2/5 expressing green fluorescent protein (rAAV2/5 GFP) to the substantia nigra (SN) of aged rats resulted in: 1) ~60% fewer transduced cells, 2) ~50% less striatal protein, and 3) 4-fold lower mRNA expression than identical injections into the young adult rat SN. These results were generalizable over rat strain, duration of expression, and location sampled in the nigrostriatal system. In the present series of experiments, we investigate if the phenomenon of deficient transduction in the aged brain is generalizable to other vector constructs. We chose to analyze the transduction efficiency of rAAV constructs 2/2 and 2/9, which utilize different receptors for capsid endocytosis, to examine whether deficiencies in rAAV transduction in aging are receptor-dependent. Additionally, we chose to analyze the transduction efficiency of vesicular stomatitis virus envelope glycoprotein-G pseudotyped lentivirus (LV) due to its transduction processes that are distinct from rAAV. Aged (20 month) and young adult (3 month) male Fischer 344 rats were stereotaxically injected into the SN with either rAAV2/2, rAAV2/9, or LV expressing GFP. Outcome measures four weeks after surgery will include striatal GFP protein expression, nigral GFP qPCR, stereological quantification of THir SN neurons, and total number of GFPir cells as well as GFP mRNA visualization using RNAscope *in situ* hybridization. Aging-related deficits in transduction have the potential to

impact current and future gene therapy clinical trials. The identification of vector constructs that can more efficiently transduce the aged brain will provide insight into approaches to optimize future gene therapy clinical trials for age-related neurodegenerative diseases.

Disclosures: **N. Polinski:** None. **F.P. Manfredsson:** None. **M. Benskey:** None. **C.J. Kemp:** None. **N.C. Kuhn:** None. **A. Cole-Strauss:** None. **K. Steece-Collier:** None. **K.L. Paumier:** None. **J.W. Lipton:** None. **C.E. Sortwell:** None.

Poster

228. Gene Therapy

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Support: 7th European Community programme project Persisting Transgenesis (Persist)

Deutsche Forschungsgemeinschaft

LOEWE Center for Cell and Gene Therapy Frankfurt, funded by the Hessian Ministry of Higher Education, Research and the Arts

Title: Cell surface receptor targeting expanded: From oncolytic viruses to lentiviral and AAV vector

Authors: ***T. ABEL**, R. C. MÜNCH, C. J. BUCHHOLZ
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Abstract: Viral vectors are important tools for the delivery of genes in basic research and molecular medicine. Examples include but are not limited to gene marking and gene function studies or the destruction of unwanted (tumor) cells. While gene transfer with viral vectors is usually very efficient, it does not discriminate between target and non-target cells. Attachment of vector particles to a cell surface receptor is the primary step of gene transfer. Altering the receptor usage of a viral vector was first accomplished for oncolytic measles viruses (MVs) by fusing a tumor antigen specific single chain antibody fragment (scFv) to the virus attachment protein and abolishing natural receptor usage through point mutations. We have transferred this strategy to lentiviral vectors (LVs) by pseudotyping LV particles with the engineered MV glycoproteins (Anliker et al., 2010). More recently, the approach has been extended to the non-enveloped AAV vectors by mutating the native heparan-sulfate proteoglycan binding site and

simultaneously fusing a designed ankyrin repeat protein (DARPin) providing receptor specificity to the capsid protein (Münch et al., 2013). Cell surface targeted viral vectors have been described for a variety of target receptors. We have shown that oncolytic MVs targeted to CD133, a putative marker of tumor stem cells in glioblastoma, selectively eliminate CD133-positive tumor cells and substantially prolong survival in an orthotopic glioma tumor model (Bach et al., 2013). LVs have for example been targeted to subtypes of mouse neurons by addressing particular glutamate receptor subunits or to mouse endothelial cells (ECs) including brain ECs. Surface targeted AAV vectors allow the specific and exclusive genetic modification of rare target cells, both *in vivo* upon systemic injections or *ex vivo* in cell cultures. Thus three different vector types can now be manipulated in a flexible and rationally based approach to enter cells via a free-chosen surface molecule

Disclosures: T. Abel: None. **R.C. Münch:** None. **C.J. Buchholz:** None.

Poster

228. Gene Therapy

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 228.06/S7

Topic: C.14. Gene Therapy

Title: Gene therapy for spinal cord injury using hypoxia-inducible neuron-specific VEGF expression system

Authors: *Y. YUN[1], D. YOON[2], M. LEE[3], Y. HA[2]
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Abstract: Objective : To increase vascular endothelial growth factor(VEGF) gene expression in spinal cord injury lesion but avoid unwanted overexpression in normal site, we developed hypoxia-inducible tissue-specific gene expression system consisting of the erythropoietin(Epo) enhancer and neuron-specific enolase(NSE) promoter. We assessed whether NSE promoter is specific to neuron and gene expression is more inducible under the hypoxia condition. Methods : The tissue-specific luciferase or VEGF plasmid was constructed using NSE promoter with Epo enhancer. The constructed plasmid was transfected mNSC cells by Polyethylenimine(PEI), followed by 48-hr incubation in hypoxia or normoxia. Spinal cord injury was made using clip

compression. Plasmids were injected directly into the injured spinal cord immediately following injury. The gene expression was assessed by luciferase assay. Results : The gene expression system containing NSE promoter showed the higher amount of luciferase under normoxia condition than the gene expression system containing SV promoter. Also, Epo enhancer/NSE promoter combined hypoxia-inducible tissue-specific gene expression system increased the expression of the reporter luciferase under the hypoxia condition. Conclusion : These results strongly suggest the Epo enhancer and NSE promoter systems are specifically effective in neurons under the hypoxic condition. This Epo-NSE-VEGF gene expression system has therapeutic effect in the spinal cord injury.

Disclosures: Y. Yun: None. **D. Yoon:** None. **M. lee:** None. **Y. ha:** None.

Poster

228. Gene Therapy

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Program#/Poster#: 228.07/S8

Topic: C.14. Gene Therapy

Support: R01 EY007141

R01 EY012355

Title: Mito-mouse model of Leber hereditary optic neuropathy and the rescue

Authors: *H. YU, J. GUY
Bascom Plamer Eye Inst., Miami, FL

Abstract: Purpose: To describe the vertical gene transfer of the mutant human *ND4* (*hmutND4*) bred from founder mice generated by mitochondrial targeting AAV infection of mouse embryonic stem cells, and the rescue of mito-mice. **Methods:** *hmutND4* with a *FLAG* epitope and mitochondrial encoded m*Cherry (mtCherry)* were put under the control of a mitochondrial promoter and packaged into mito-targeted AAV2. The resulting rAAV was microinjected into the mouse blastocyst to generate mitochondrial transgenic founder mice. Gene expression was assessed using confocal laser scanning ophthalmoscopy (CLSO), PCR, 2D blue native polyacrylamide gel electrophoresis, complex I activity assay, histopathology and ultrastructural analysis. Visual function and retinal structure was monitored using serial pattern electroretinography (PERG) and SD-OCT. *In vitro* DNA replication and next generation

sequencing were performed using transgenic mouse mtDNA. **Results:** 60 founder mice were generated that contained varying expression of *mCherry* in the eye. Three females with the highest *mtCherry* expression were backcrossed with C57BL/6 males for over 8 generations, resulting in a total of 233 viable, fertile mutND4 mito-mice, Among them, 77% showed red fluorescent particles in retina and optic nerve head under the CLSO, and cells with fluorescence increased as mice aged. The expressed mutND4 assembled into mouse complex I and induced complex I dysfunction in various tissues. Pathological features of mito-mice could be observed in the eye as visual loss, swelling of the optic nerve head with a progressive demise of ganglion cells in the retina and their axons comprising the optic nerve, mild demyelination and mitochondrial abnormalities in the brain and fiber degeneration in the skeletal muscle but not evidence of abnormality in the heart. Mito-mice regained PERG amplitudes back to the normal level at younger age when rescued with the injection of mitochondrial targeting AAV carrying wild type human *ND4*, otherwise, they exhibited a progressive decline in PERG amplitudes from 3 months after birth to noise levels by 11 months of age. No integration of the delivered DNA was detected in the mouse mitochondrial genome and a 2kb band was generated in *in-vitro* DNA replication using transgenic mouse mitochondria after hybridized to human *ND4* probe, suggesting that the construct might replicate independently of mitochondrial genome. **Conclusions:** Vertical transmission of mutant human ND4 into subsequent generations of transgenic mito-mice results in the characteristic hallmarks of human LHON affecting predominantly the optic nerve and retina. They could be rescued in earlier stage.

Disclosures: H. Yu: None. **J. Guy:** None.

Poster

228. Gene Therapy

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Topic: C.14. Gene Therapy

Support: Merit Review Funding from the Department of Veterans Affairs

Department of Defense

New York State Spinal Cord Injury Research Board

Title: A comparison of effects of (i) AAV-mediated delivery of NG2 function-neutralizing antibody (AAV-NG2Ab), (ii) delivery of NG2-Ab via intrathecal mini-pumps and (iii)

intraspinal injections of Chondroitinase-ABC on synaptic transmission and functional recovery after SCI in adult rats. Translational potential of treatments for clinical application

Authors: *V. L. ARVANIAN[1,2], H. PETROSYAN[1,2], V. ALESSI[1], S. SANDLER[2], J. LEVINE[2]
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Abstract: An increased level of proteoglycan NG2 and insufficient neurotrophin (NT-3) support are among the major factors restricting synaptic transmission and plasticity in the damaged spinal cord. We have successfully created a new construct, i.e. cDNA for NG2 function-neutralizing antibody (Mab 69, Levine lab; packaged into AAV10 vector by PENN vector core) and examined its effects on neuro-muscular transmission and recovery of motor function following T10 contusion (150 kdyn) SCI (Arvanian lab). We found that intraspinal injections of AAV-NG2Ab induced sustained improvements of BBB scores. These improvements of function associated with strengthened transmission. AAV10-based extended treatment with NG2-Ab induced a significantly greater degree of recovery compared with effects of NG2-Ab delivered for 2 weeks via minipump (*Petrosyan et al., 2013*). Best improvements of both transmission and motor function were seen in animals that received treatment with AAV-NG2 combined with AAV-NT3. We have recently reported improvements of transmission and motor function following the same T10 contusion (150 kdyn) injury but using AAV10-NT3 combined with intraspinal injections of Chondroitinase-ABC (ChABC) (*Hunanyan et al., 2013*) as a treatment. Thus, it was important to establish whether treatment with AAV-NG2Ab/AAV-NT3 may have advantages over treatment with ChABC/AAV-NT3 following contusion SCI. Comparisons revealed that intraspinal injections of AAV-NG2Ab/AAV-NT3 after contusion SCI induced significantly better improvement of synaptic transmission ($P < 0.05$) and locomotor function ($P < 0.05$) than intraspinal injections of ChABC/AAV-NT3. In an attempt to improve the delivery method for these treatments, we examined less invasive intrathecal injections since this method is used in clinics for drug administration. We found an excellent transduction of spinal cord tissue by AAV10-gfp that was injected intrathecally 2 weeks following initial moderate (150 kdyn) and severe (250 kdyn) T10 contusions. Transduction of different cell types in the vicinity of contusion injury generated by delayed intrathecal administration of AAV-gfp was comparable with intraspinal injections of AAV-gfp immediately after contusion. These results confirm that the AAV10-based approach for transgene delivery of NG2-Ab and NT-3 can be used for extensive treatment delivery via intrathecal administration at delayed time points after moderate and severe contusion injuries and thus carries a translational potential for clinical application.

Disclosures: V.L. Arvanian: None. **H. Petrosyan:** None. **V. Alessi:** None. **S. Sandler:** None. **J. Levine:** None.

Poster

228. Gene Therapy

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 228.09/S10

Topic: C.14. Gene Therapy

Title: Non-invasive widespread gene delivery to rat striatum using MRI-guided focused ultrasound

Authors: *M. A. STAVARACHE[1], J. MARKUS[2], J. T. SCHWARZ[2], Z. ROSENFELD[2], S. A. MUSATOV[2], M. G. KAPLITT[2]
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Abstract: The past few years have seen significant progress in vector-mediated gene therapy of neurologic disorders. To date, however, this has required invasive surgery to infuse vectors into the brain, and delivery to large brain regions has been a challenge. Recently, focused ultrasound (FUS) coupled with intravenously circulating lipid-coated microbubbles, under MRI-guidance, has been successfully employed in animals to deliver therapeutic or diagnostic agents to brain regions of interest by transient disruption of blood -brain barrier (BBB) permeability. FUS has also been reported to safely generate highly focal lesions in human brain to treat tremors and pain, thus raising the potential for therapeutic BBB disruption in humans. Here we report widespread and uniform gene delivery throughout the rat striatum using non-invasive intravenous infusion combined with FUS. Sprague-Dawley rats (300-350gr) were anesthetized and the tail vein catheterized for administration of microbubbles, viral vector and Gd-DTPA contrast during sonication. An adeno-associated virus (AAV) vector encoding the marker gene green fluorescent protein (GFP) was used as the gene delivery agent. BBB disruption was performed with a 3-axis positioning system and a 1.145 MHz single-element focused ultrasound transducer in combination with Optison microbubbles. FUS (10ms pulses, 1Hz pulse-repetition frequency, 120sec total duration) was applied to four points located at the level of striatum. Contrast -enhanced T2 weighed MRI scans were performed before sonication to determine the target and T1 weighed MRI scans after the sonication to assess the spread of the Gd-DTPA contrast as an indicator of the adequacy of BBB opening. All animals recovered well and showed no clinical signs of brain injury or deficit following treatment. Four weeks post sonication, animals were sacrificed for histological analysis. GFP staining covered up to 80% of the sonicated striatum while the striatum on the opposite side was unaffected. With this method, the BBB remained open for up to 4 hrs post sonication. There appeared to be a good correlation between the extent of Gd-DTPA contrast extravasation during FUS sonication and the spread of AAV-mediated gene delivery as measured by GFP staining 4 weeks later. There was no gross histological evidence of necrosis, edema or brain injury in the area of sonication 4 weeks following treatment. These findings indicate that a high viral transduction at the level of rat

striatum can be achieved safely using FUS, suggesting that clinical applications of human CNS gene therapy may be possible using non-invasive intravenous gene delivery.

Disclosures: **M.A. Stavarache:** None. **J. Markus:** None. **J.T. Schwarz:** None. **Z. Rosenfeld:** None. **S.A. Musatov:** None. **M.G. Kaplitt:** None.

Poster

228. Gene Therapy

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Topic: C.14. Gene Therapy

Support: HW Grant A120254

KG Grant 2012-0001560

Title: Development of tissue-specific gm-csf gene expressing system and combined cell therapy in spinal cord injury model

Authors: ***Y. YOU**[1], J. OH[1], D. YOON[2], Y. HA[2]
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Abstract: Spinal cord injury (SCI) induces a great deal of cell loss, then followed secondary degenerative response. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that associates with neuronal survival and anti-apoptosis. We hypothesized that combined stem cells with tissue specific gene expression system would be enhanced cell therapeutic effects in SCI models. The aim of this study is to make modified neural stem cells(mNSCs) which are over-expressing neuron specific GM-CSF and to assess the effects of transplanted cells combined with gene in SCI model. Before we constructed the GM-CSF plasmid, we produced mNSCs expressing luciferase with SV or NSE promotor and showed that NSE-LUCI-NSCs were over-expressing luciferase *in vitro* and *in vivo*. Like a preliminary study, we manufactured mNSCs expressing GM-CSF and those were compared with SV-GM-CSF-NSCs measured by western blot. As a result, expression of NSE-GM-CSF was higher than SV-GM-CSF. Moreover, *In vivo* model, each group of mNSCs expressing GM-CSF was transplanted into mice after SCI, NSE-GM-CSF-NSCs show that significantly higher amount of surviving NSCs in SCI model compared with those of SV-CM-CSF.In this experiment, we represented that

combining NSE promotor with NSCs can increase expression of GM-CSF and enhance the cell therapeutic effects in SCI model. Furthermore, this system may be clinically useful to treat SCI.

Disclosures: **Y. You:** None. **J. Oh:** None. **Y. Ha:** None. **D. Yoon:** None.

Poster

228. Gene Therapy

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Topic: C.14. Gene Therapy

Support: National Research Foundation of Korea (NRF) Grant (No. 2008-0061888)

National Research Foundation of Korea (NRF) Grant (No. 2012R1A1A1039140)

Title: *In vivo* adeno-associated virus 1 transduction with hRheb(S16H) protects hippocampal neurons by the production of brain-derived neurotrophic factor

Authors: ***M.-T. JEON**[1,2], J. NAM[4,5], H. KIM[1,2], N. KHOLODILOV[6], R. E. BURKE[6,7], B. JIN[4,5], S. KIM[1,2,3,8]
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Abstract: Recent evidence has shown that Ras homolog enriched in brain (Rheb) is dysregulated in Alzheimer's disease (AD) brains. However, it is still unclear whether the activation of Rheb contributes to survival and protection of hippocampal neurons in the adult brain. To assess the effects of active Rheb in hippocampal neurons *in vivo*, we transfected neurons of the cornu ammonis 1 region in normal adult rats with adeno-associated virus 1 to induce expression of a constitutively active human Rheb [hRheb(S16H)], and evaluated effects on thrombin-induced neurotoxicity. Here we report that transduction with hRheb(S16H) significantly induces neurotrophic effects in hippocampal neurons through the activation of mammalian target of rapamycin complex 1, and the expression of hRheb(S16H) prevents thrombin-induced neurodegeneration *in vivo*, an effect that is diminished by treatment with specific neutralizing

antibodies against brain-derived neurotrophic factor. These results suggest that viral vector transduction with hRheb(S16H) may have therapeutic value in the treatment of neurodegenerative diseases such as AD. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (No. 2008-0061888 and 2012R1A1A1039140).

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Poster

228. Gene Therapy

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 228.12/T1

Topic: C.14. Gene Therapy

Support: Medical research council

Rett syndrome association Scotland

Title: Assessing early and late gene therapy intervention using adeno-associated virus serotype 9 in a mouse model of Rett syndrome

Authors: K. K. E. GADALLA[1,2], M. E. S. BAILEY[3], P. ROSS[1], S. J. GRAY[4,5], *S. R. COBB[1]
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Abstract: Rett syndrome (RTT) is a neurological disorder affecting girls and is characterized by impairment of motor and cognitive functions. Typical RTT is caused in > 95% of cases by de novo mutation of the X-linked gene MECP2. The protein product of this gene, MeCP2, is expressed globally, but at especially high levels in the nuclei of postnatal neurons. Studies in mice suggest that reactivation of a silenced Mecp2 allele can reverse and prevent RTT-like neurological deficits. More recently, we and others have demonstrated the effectiveness of viral-based gene therapy approaches in ameliorating aspects the RTT-like phenotype in knockout mice. There are many variables to consider for translational application including vector design,

route and age of delivery. The aim of this study was to compare perinatal versus juvenile administration of transgenic MeCP2 using a self-complementary AAV9 vector. Human MECP2 minigene was cloned as a fusion with a Myc epitope tag under the control of a Mecp2 endogenous core promoter fragment (MeP). This construct was flanked by AAV2 ITR elements and used to generate self-complementary (SC) AAV9 virus particles. After testing in primary neuronal culture, viral particles were injected intravenously into wild-type and knockout (Mecp2 -/y) mice at P0-3 days (perinatal) and 28-35 days (juvenile). Perinatal injection of scAAV9-MeP/MECP2 was able to deliver exogenous MECP2 into the brain of neonatal mice (brain transduction efficiency ~ 8 - 12 %; MeCP2 levels ~1.4-1.8x endogenous). At the organismal level, the injected mice displayed an impairment of hindlimb function in both wild-type and Mecp2-/y mice, which developed at around 3 weeks post-injection. Histological examination of the lumbar spine of affected mice revealed axonal degeneration within the posterior column. In contrast, juvenile Mecp2-/y mice treated with identical vector did not display this hindlimb dysfunction. Importantly, while the transduction efficiency was less in juvenile mice (2-4% neurons), these mice showed increased survival. The current study therefore suggests that the timing of MeCP2 delivery may be another important factor in maximizing therapeutic benefits and minimizing adverse effects

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Poster

228. Gene Therapy

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Program#/Poster#: 228.13/T2

Topic: C.14. Gene Therapy

Title: Clinical grade-MSCs for the treatment of brain tumor after retroviral transduction of a cytosine deaminase gene

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Abstract: Human mesenchymal stem cells (MSCs) have emerged as attractive cellular vehicles to deliver therapeutic genes for ex-vivo therapy of diverse diseases; this is in part because they

have the capability to migrate into tumor or lesion sites. However, concerns about the clinical application of gene modified MSCs still exist because of the risks associated with the viral vector-based methods. Previously, we showed that MSC could be utilized to deliver a bacterial cytosine deaminase suicide (CD) gene to brain tumors. Here, we report that MSCs were isolated in a GMP facility as colony forming-unit fibroblasts from bone marrows of 10 healthy donors. Colony formation ability and proliferation rates differed among divers bone marrow donors. The cells were transduced with a retroviral vector encoding CD gene and further expanded. We found that proliferation and differentiation potentials, chromosomal intergrity, and surface antigenicity of GMP-compliant MSC were not altered by retroviral transduction. The results indicate that retroviral vectors can be safely utilized for delivery of suicide genes to MSC for ex-vivo therapy. We also found that a single retroviral transduction was sufficient for sustained expression of CD up to passage 10. The results indicate that retroviral vector-transduced MSCs provide a practical approach for potential use in allogeneic therapy. This study was supported by a grant (HI10C1411/A101446) of the Korea Health technology R&D Project, Ministry of Health & Welfare and a grant (NRF-2012M3A9C6049725) of National Research Foundation of Korea.
For more information, contact Sung-Soo Kim at kimdmg@ajou.ac.kr.

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Poster

229. Psychosis: Neuropathology

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 229.01/T3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH53327

Title: Immunoaffinity isolation of COPII vesicles from human brain

Authors: *S. D. YATES[1], J. H. MEADOR-WOODRUFF[1,2]
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[2]Evelyn F. McKnight Brain Inst., Birmingham, AL

Abstract: Schizophrenia (SZ) has a complex pathophysiology which is not well understood. Currently, data from our lab suggests that patients with SZ are more vulnerable to endoplasmic reticulum (ER) stress and activation of the Unfolded Protein Response (UPR). Part of the stress

response is the impairment of properly folded proteins being trafficked by COPII vesicles from the ER to the ER-Golgi Intermediate Compartment (ERGIC) and on to the Golgi for further processing. Recent studies have shown that the components of these vesicles are degraded under ER stress. COPII vesicles are formed when a cargo protein is targeted for transport out of the ER and into the Golgi. Although the exact mechanism for the recognition of which cargo proteins are ready for transport is still not known, it is established that certain components are activated sequentially to form a budding reaction, scission, and formation of the vesicle before reaching the ERGIC. In this study, we have successfully isolated intact COPII vesicles from postmortem human brain tissue using an immunoaffinity isolation technique targeting one of the outer coat components of the vesicle, Sec31. This technique utilizes a magnetic bead-antibody complex to isolate Sec31 which is then prepared for Western Blot analysis for confirmation or embedded and fixed for Electron Microscopy (EM). We have previously used this approach to successfully isolate other subcellular compartments from human brain. Using this novel approach, we plan to assay COPII vesicle content in SZ and comparison subjects with a hypothesis that alterations in COPII vesicle content lead to a decrease in trafficking of cargo in the face of ER stress in SZ.

Disclosures: S.D. Yates: None. **J.H. Meador-Woodruff:** None.

Poster

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Program#/Poster#: 229.02/T4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Circadian rhythm abnormalities of somatostatin expression in the amygdala of subjects with bipolar disorder

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Abstract: Growing evidence supports a role for circadian rhythm abnormalities in the pathophysiology of bipolar disorder (BD). Subjects with BD exhibit shorter circadian periods, and the most effective treatments, lithium and valproic acid, modulate expression of core clock proteins and lengthen circadian period. In addition, multiple genetic polymorphisms for core

clock molecules have been associated with BD. Despite this evidence, little is known regarding how circadian rhythm abnormalities contribute to mood dysregulation in BD. Recent rodent work has reported that somatostatin (SOM), a neuropeptide with strong anxiolytic effects in the amygdala, is rhythmically expressed in the amygdala of mice and regulates anxiety-like behavior in a circadian manner. In human subjects, reports of altered levels of SOM in the cerebrospinal fluid of depressed subjects only in the early morning suggest that altered rhythm of SOM expression may be present in subjects with mood disorders. We tested the hypothesis that SOM may be rhythmically expressed in the human amygdala, and that this rhythm may be altered in subjects with BD. Serial sections including the entire rostro-caudal extent of the amygdala from 15 BD and 15 control subjects were processed for immunocytochemical detection of SOM. Total numbers (TN) and numerical densities (ND) of immunoreactive (IR) neurons were measured in the lateral (LN), basal (BN), accessory basal (AB), and cortical (CO) nuclei using computer-assisted light microscopy. Time of death for each subject was used to analyze circadian expression of SOM-IR neurons. Stepwise linear regression models were used to test for the main effect of diagnosis together with a broad range of confounding factors. In control subjects, numbers of SOM-IR neurons plotted by time of death displayed a circadian rhythm, with a peak at 9 AM, antiphase to the rhythm reported in the mouse amygdala. In subjects with BD, this rhythm was reversed in comparison to control subjects, with a low point at 9 AM. SOM-IR neurons were found to be decreased selectively in the LN of BD (TN, $p= 0.003$; ND, $p = 0.007$), with a significant effect of time of death as a co-variate ($p=0.02$). This effect was driven by marked decreases of SOM-IR neurons in BD subjects who died in the morning. Our results show, for the first time, that the expression of SOM in the human amygdala changes according to a circadian rhythm, and that this rhythm is abnormal in subjects with BD. Together, these abnormalities may contribute to a disruption of circadian rhythms in BD, accompanied by marked anxiety and vulnerability to stress.

Disclosures: H. Pantazopoulos: None. **J. Wiseman:** None. **M. Markota:** None. **L. Ehrenfeld:** None. **S. Berretta:** None.

Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH098566

NIH Grant MH066123

Title: Tyrosine hydroxylase levels in the caudate, putamen, and nucleus accumbens in postmortem schizophrenia

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Abstract: Schizophrenia is a severe mental illness affecting approximately 1% of the population. The disorder is highly debilitating, with symptoms including cognitive impairments, psychosis, and flat affect, with limited treatment options. A better understanding of the pathology of the disease could improve drug therapies for patients. While pathology of the dopamine system in the striatum is well documented in schizophrenia, it lacks solid support from postmortem tissue, which allows for detailed subregional brain analysis. Further, a region of the ventral striatum, the nucleus accumbens, is traditionally assumed to play an important role in these abnormalities; however, this has never been validated. In the present study, postmortem tissue from schizophrenia subjects and demographically matched controls was used to study the dopamine system in the nucleus accumbens and dorsal striatum. All schizophrenia subjects had been chronically medicated; thus, to verify these results were not due to medication, rats were chronically treated with antipsychotics for 6 months. Levels of tyrosine hydroxylase (TH), a synthesizing enzyme of dopamine, were analyzed using immunohistochemistry and optical densitometry. When compared to controls, schizophrenia subjects did not significantly differ in levels of TH in the nucleus accumbens core and shell, but had reduced levels in the dorsal striatum. Antipsychotic drug treatment did not significantly affect TH levels in rats, suggesting the findings in postmortem tissue are not due to chronic medication. These findings suggest that abnormalities in the dopamine system in schizophrenia may be localized within the dorsal striatum, not in the ventral striatum, offering new insight into the dopamine hypothesis of schizophrenia.

Disclosures: L.A. McCollum: None. **R.E. McCullumsmith:** None. **C.K. Walker:** None. **R.C. Roberts:** None.

Poster

229. Psychosis: Neuropathology

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Program#/Poster#: 229.04/T6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH53327

Title: Abnormal expression of adaptor protein complex subunits in the superior temporal gyrus in schizophrenia

Authors: *S. L. MOORE, JR[1], V. HAROUTUNIAN[2], J. H. MEADOR-WOODRUFF[1]
[1]Dept. of Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Dept. of Psychiatry, Mt. Sinai Sch. of Med., New York, NY

Abstract: Schizophrenia is a devastating psychiatric illness thought to involve the dysfunction of several neurotransmitter systems, including glutamatergic and GABAergic transmission, which have been hypothesized to contribute to the pathophysiology of schizophrenia. Recent work from our lab has shown that glycosylation provides a common mechanism thought to contribute to the observed synaptic dysfunction in these various neurotransmitter systems. In schizophrenia, glutamatergic and GABAergic neurons may exhibit altered cellular trafficking that we hypothesize is vital to the underlying mechanisms of the illness. Adaptor protein (AP) complexes are involved in the transport of vesicular glutamate transporters (VGLUTs) and the GluA2 subunit of the AMPA receptor, components previously shown to be altered in schizophrenia. AP complexes are heterotetramers composed of two large chains (alpha/gamma and beta), a medium chain (mu), and a small chain (sigma). Considering that the AP complexes may have a regulatory role in AMPA receptor and VGLUT cellular localization, we measured transcript expression of AP1/2 complex subunits, VGLUTs, and AMPA receptor subunits in the superior temporal gyrus (STG) of patients with schizophrenia (N=16) and matched comparison subjects (N=16). We found significant increases in the transcript expression of select AP complex subunits, including AP1B1 and AP2A1, and GluA2. In addition, AP subunits and VGLUT1/2 or GluA2 transcript levels were analyzed for correlation in comparison (N=22) and schizophrenia (N=16) samples. AP complex genes AP1M1, AP1S1, AP2B1, and AP2S1 had significantly altered associations with specific glutamatergic components in schizophrenia. Given the significant alterations in gene expression and correlation of these AP subunits with GluA2 and VGLUTs, the APs may be an underlying mechanism contributing to the altered trafficking and subsequent synaptic dysfunction observed in schizophrenia.

Disclosures: **S.L. Moore:** None. **V. Haroutunian:** None. **J.H. Meador-Woodruff:** None.

Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

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Swiss National Science Foundation (# 130090) to PH

Title: A redox dysregulation affects the integrity and function of the fornix / fimbria and anterior commissure: Relevance to schizophrenia

Authors: *P. STEULLET[1], A. CORCOBA[1,2], P. S. BAUMANN[1,4], A. GRIFFA[3], J. DUARTE[2,5], Y. VAN DE LOOIJ[2], C. FERRARI[1], J.-P. THIRAN[3,5], M. CUENOD[1], P. HAGMANN[3,5], P. CONUS[4], R. GRUETTER[2,5], K. Q. DO[1]
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Abstract: Compelling evidence indicate oxidative stress and dysregulation of antioxidant systems in schizophrenia. In this study, we investigated the impact of impaired synthesis of the antioxidant and redox regulator, glutathione, on white matter (WM) using knockout mice for the modulatory subunit of the key enzyme of glutathione synthesis (Gclm KO mice). We first analyzed in a longitudinal study WM integrity throughout the brain of Gclm KO and wild-type

(WT) mice using diffusion tensor magnetic resonance imaging (DTI). After correction for multiple testing, we found significant reduced fractional anisotropy (FA) in Gclm KO compared to WT mice only in the fornix-fimbria (FF) (-4.5%, p=0.03) and the anterior commissure (AC) (-7.5%, p=0.005). Reduced FA in these two fiber tracts was present from adolescence to adulthood. We also detected a significant reduction in myelin basic protein immunoreactivity in AC (-18.8%, p=0.046), but not in FF. Using electrophysiological methods, we found a small but significant decrease in conduction velocity along the fast-conducting fibers in the posterior limb of the AC (-14%, p=0.024) and the slow-conducting fibers of the FF (-9%, p=0.027). Non-significant decrease in conduction velocity was also observed along the fast-conducting fibers of the FF and of the anterior limb of the AC. Together, these data suggest that FA alterations in FF and AC may reflect different structural aberrations at the cellular level. In the AC, reduced myelin basic protein and conduction of the fast-conducting fibers point to anomaly at the level of myelination. In the FF, other processes than myelination may be also implicated in the WM anomalies observed in Gclm KO mice. Collectively, this study reveals that two fiber tracts affected in schizophrenia patients are particularly vulnerable to a deficit in glutathione. Interestingly, we also found that patients in early phase of psychosis (EP) have significantly reduced hippocampal volume (p=0.004) and generalized FA (p=0.005) in the FF compared to age-matched controls. Association between hippocampal volume, FF metrics and redox dysregulation in patients are under investigation.

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Poster

229. Psychosis: Neuropathology

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Program#/Poster#: 229.06/T8

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH53327

Title: Double labeled quantitative immunohistochemistry of midbrain dopamine neurons in human brain

Authors: ***M. S. SIMMONS**, J. MEADOR-WOODRUFF
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Abstract: Schizophrenia (SZ) is a severe psychiatric illness with a complex pathophysiology. To gain insight into the complexity of this illness we developed a new tool for quantitative double immunohistochemical labeling to study proteins associated with dopaminergic neurotransmission in postmortem human brain. A constant finding in patients with SZ is dysregulation of dopamine neurotransmission. Tyrosine hydroxylase (TH) and the dopamine transporter (DAT) were chosen in the development of this tool based on the key roles each has in regulating synaptic dopamine levels. Assay validation began with sections of human midbrain containing the substantia nigra individually labeled with TH and DAT antibodies. Using the Odyssey® imaging system by LI-COR, slides were scanned at 21μm after infrared conjugated secondary antibodies were incubated for 1 hr at room temperature. After confirmation of specific labeling, dual labeling was tested on dot blots with serial dilutions of human striatal homogenates spotted on nitrocellulose membrane. The mean optical densities (OD) from each blot were plotted; no signal change or cross reactivity from individual or dual labeling was observed. Next, midbrain sections were dual labeled with TH and DAT antisera, along with a dot blot. The slide and dot blot were scanned simultaneously, then the mean OD was measured from TH and DAT expression and plotted on the standard curve from each dot blot to insure linearity of both signals. We next determined the coefficient of variation for each antibody which was % for TH and % for DAT labeling. This new approach to generate quantitative dual channel immunohistochemical data will be used to investigate the status of the midbrain dopamine neurons in SZ and comparison subjects.

Disclosures: M.S. Simmons: None. **J. Meador-Woodruff:** None.

Poster

229. Psychosis: Neuropathology

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MNIRGDP-12-258900

SFARI 274443

NARSAD 21069

Title: RCAN1/CaN signaling modulates behavioral expression in schizophrenia

Authors: *P. CAIN[1], J. LEVENGA[2], H. WONG[3], A. FALKNER[3], M. ROCHE[2], B. ROTHERMEL[4], C. HOEFFER[2]
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Abstract: Schizophrenia is estimated to afflict approximately 1% of the world's population, typically appearing in early adulthood and continuing throughout life. There is strong evidence that heritable genetic contributions combine with environmental factors to trigger the disorder. Recent studies have shown that genes highly correlated with the appearance of schizophrenia control the production of calmodulin-dependent phosphatase calcineurin (CaN) isoforms. Regulator of calcineurin 1 (RCAN1) and Ca2+/CaM are major modulators of CaN and perturbation of RCAN1 and CaN function have been implicated in the pathophysiology of schizophrenia. Previously we showed that innate anxiety and anxiogenic responses, along with memory, are modulated by RCAN1. Each of these has been implicated in the constellation of behaviors displayed in schizophrenia. Our new preliminary data indicate that RCAN1 may control the expression of behaviors associated with schizophrenia. We examined the role of RCAN1/CaN signaling in the expression of schizophrenia-related behaviors in Rcan1 knockout (KO) mice and transgenic Rcan1 overexpression (Rcan1tg) mice as compared to wild-type (WT) mice. We found differences among the groups in response to contextual fear conditioning extinction, social interaction and novelty response. To test the idea that these behavioral abnormalities are related to hippocampal-prefrontal function, we recorded differences in local field potentials (LFP) from the subiculum of each of these three groups. The critical finding was associating the differences in LFPs with schizophrenia-related behaviors. These studies may link RCAN1 and its modulation of CaN with the neuronal substrates underlying schizophrenia-related behavior.

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Poster

229. Psychosis: Neuropathology

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH53327

Title: Abnormal protein levels of cytosolic chaperone subunits in the superior temporal gyrus in patients with schizophrenia

Authors: *C. E. REMEDIES[1], S. D. YATES[2], V. HAROUTUNIAN[3], J. H. MEADOR-WOODRUFF[2]
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Abstract: Schizophrenia is a devastating psychiatric illness that has a complex pathophysiology that is not well understood. Recent data suggest that a component of this illness includes endoplasmic reticulum (ER) stress and activation of the Unfolded Protein Response. An aspect of this stress response is impairment of properly folded proteins. We have recently found that cytosolic chaperones, including the Chaperonin containing TCP1 complex (CCT) and heat shock proteins (40kDa and 70kDa), involved in proper protein folding are impaired at the transcript level. CCT is a major cytosolic chaperone involved in folding dozens of substrates including actin and tubulin. These heat shock proteins are involved in protein folding and regulation of endoplasmic reticulum associated degradation (ERAD). In this study, the protein levels of these chaperones and co-chaperones, specifically three subunits of CCT: TCP-1, CCT-4, and CCT-7, and three heat shock proteins: HSPA4L, DNAJC4, and DNAJB9, were measured by Western Blot analysis in the superior temporal gyrus of patients with schizophrenia (N=13) and matched comparison subjects (N=13). Data shows a 21% decrease in TCP-1 in schizophrenia. We hypothesize protein expression of these subunits are abnormal in schizophrenia leading to an increase in misfolded proteins and ER stress in this illness.

Disclosures: C.E. Remedies: None. **S.D. Yates:** None. **J.H. Meador-Woodruff:** None. **V. Haroutunian:** None.

Poster

229. Psychosis: Neuropathology

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Program#/Poster#: 229.09/T11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH

Title: Detailed characterization of olfactory cells via nasal biopsy: Surrogate biospecimens to define brain-relevant molecular and functional changes

Authors: *Y. CHUNG, S. NARAYAN, H. YUKITAKE, T. TSUJIMURA, N. J. GAMO, T. MASEDA, Y. HORIUCHI, S.-I. KANO, K. ISHIZUKA, A. SAWA
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Abstract: Comparison of gene expression and epigenetic profiles of biospecimens from patients and controls is one of the methods to identify biomarkers and to elucidate disease mechanisms. However, difficulty of accessing cells and tissues relevant to the disease pathophysiology has been a major barrier in promoting this strategy in brain disorder research. To overcome this question, we have developed olfactory cells via nasal biopsy (Kano et al, Mol Psychiatry, 2013). Through unbiased expression analysis, we have defined that the expression profile of the olfactory cells is similar to that of mesenchymal stem cells that can be differentiated into neurons and glia, suggesting that olfactory cells might be a good surrogate system to investigate disease-associated molecular changes in the brain (Horiuchi et al, Neurosci Res, 2013). In order to further characterize and validate the utility and validity of olfactory cells, further analyses are being taken place at molecular, protein, and functional levels. References: Kano, S. et al. Genome-wide profiling of multiple histone methylations in olfactory cells: further implications for cellular susceptibility to oxidative stress in schizophrenia. Mol Psychiatry 18, 740-742 (2013). Horiuchi, Y. et al. Olfactory cells via nasal biopsy reflect the developing brain in gene expression profiles: Utility and limitation of the surrogate tissues in research for brain disorders. Neurosci Res 77 (4), 247-250 (2013).

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Poster

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Program#/Poster#: 229.10/T12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: The differential effects of Activin and TGFbeta on signaling pathway transduction in mouse primary neurons

Authors: *Q. CHEN[1], K. TAJINDA[1], C.-J. HAN[2], S. MIYAKE[1], N. M. WALTON[1], M. MATSUMOTO[1]

[1]Neuroscience, Astellas Res. Inst. of Ameri, Skokie, IL; [2]Master of Biotech. program, Northwestern Univ., Evanston, IL

Abstract: Activins are members of the Transforming growth factor beta (TGF-beta) superfamily that participate in regulation of many biological processes, including cell differentiation and proliferation, apoptosis , hormone homeostasis and brain functions. Activin/TGFbeta and their receptors are highly expressed in embryonic and adult brain. Accumulating evidence suggests that Activin / TGFbeta signaling pathways are involved in the neurological diseases, such as depression and Alzheimer's disease. Our in-house data also showed hippocampal Activin infusion could reverse the molecular and behavioral abnormalities in CaMKIIa heterozygous knockout mouse, an animal model of psychiatric disorders (data will be presented in a separate abstract). To further elucidate Activin / TGFbeta function in adult neurons, we investigated the Activin and TGFbeta signaling pathways in primary neurons. E19 mouse hippocampal neurons were cultured in serum-free neuronal culture medium. Quantitative RT-PCR results demonstrated that both Activin and TGFbeta receptors (type 1 and type 2) were expressed in day8 cultured neurons. In order to investigate the downstream Smad2/3 signaling pathways, 100ng/ml Activin A and 10ng/ml TGFbeta1 were administrated to primary neuron. There were dynamic and significant increases in phosphor-Smad2 and phosphor-Smad3 expression after Activin A stimulation. In the meantime, immunocytochemistry experiment also showed the nuclear translocation of Smad2 and Smad3 after Activin stimulation. Very surprisingly, we only detected the increase in phosphor-Smad3 after TGFbeta 1 treatment, TGFbeta 1did not alter phosphor-Smad2 expression. In addition, we performed lentivirus Smad binging element (SBE) - Luc reporter assay in primary neurons with Activin A and TGF beta1 stimulation. Consistent with our previous data, Activin A stimulation significantly enhanced SBE - Luc reporter signal. No increase in reporter signal was detected with different doses of TGFbeta stimulation. These data indicated the selective activation of Smad2/3 signaling by Activin A in primary neuron. Furthermore, gene expression profiling by microarray analysis also showed the differential expression of downstream target genes after Activin A and TGFbeta1 treatment. Together, these results suggest the plausible differential effects of Activin and TGFbeta in adult neurons.

Disclosures: **Q. Chen:** A. Employment/Salary (full or part-time):; Astellas Research Institute of America LLC, Astellas Pharma. **K. Tajinda:** A. Employment/Salary (full or part-time):; Astellas Research Institute of America LLC, Astellas Pharma. **C. Han:** A. Employment/Salary (full or part-time):; Astellas internship. **S. Miyake:** A. Employment/Salary (full or part-time):; Astellas Research Institute of America LLC, Astellas Pharma. **N.M. Walton:** A. Employment/Salary (full or part-time):; Astellas Research Institute of America LLC, Astellas Pharma. **M. Matsumoto:** A. Employment/Salary (full or part-time):; Astellas Research Institute of America LLC, Astellas Pharma.

Poster

229. Psychosis: Neuropathology

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH53327

Title: ER stress and unfolded protein response in schizophrenia

Authors: *P. KIM[1], V. HAROUTUNIAN[2], J. MEADOR-WOODRUFF[3]
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Abstract: The pathogenesis of schizophrenia (SZ) is poorly understood but recent reports have found gene and protein expression changes in SZ. The endoplasmic reticulum (ER) is an essential cellular compartment for protein synthesis and maturation. The exposure to diverse cellular stress factors can lead to dysfunction of the ER and to imbalance between protein-folding capacity and protein-folding load. Protein-misfolding events have been observed in a variety of disorders and may contribute to both the initiation and the progression of disease through endoplasmic reticulum (ER) stress. To cope with ER stress, a signaling network called the unfolded protein response (UPR) is activated. The UPR is signaled through three ER transmembrane protein sensors: inositol-requiring enzyme1 (IRE1), protein kinase RNA like ER kinase (PERK) and activating transcription factor 6 (ATF6). The UPR consists of four mechanisms:1) attenuation of protein synthesis, 2) restoration of protein folding, 3) degradation of misfolded protein (ER- associated degradation: ERAD) and 4) apoptosis. We hypothesized that there may be abnormalities of the UPR pathway by ER stress and protein misfolding in SZ. Accordingly, this study focused on measuring the expression of genes and proteins involved in multiple UPR pathways in gray matter and pyramidal neurons derived from prefrontal cortex of subjects with schizophrenia and a comparison group. A human UPR pathway PCR array was used to measure expression of 84 genes associated with ER chaperones which are responsible for the folding and response pathways following ER stress. We identified 41 genes significantly changed in prefrontal cortex in SZ and 36 genes changed specifically in pyramidal neurons. The genes clustered into 11 different functional categories associated with the UPR. These results support that there are abnormalities of UPR pathway function in SZ and suggest possible therapeutic approaches to ameliorate ER stress in this illness.

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

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Nara Medical University

Title: Altered cortical expression of immediate early gene NARP in schizophrenia: Impact on parvalbumin neurons

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Abstract: Working memory impairments in schizophrenia appear to reflect abnormalities in the generation of gamma oscillations in dorsolateral prefrontal cortex (DLPFC) neural networks. Gamma oscillations are generated by the synchronized inhibition of neighboring populations of pyramidal neurons by fast-spiking, parvalbumin (PV)-containing interneurons. Specifically, because the phasic excitation of PV interneurons plays a crucial role in gamma oscillation generation, gamma oscillations depend, in part, on the composition of synaptic glutamate receptors on PV interneurons. However, little is known about the molecular regulation underlying the alterations in glutamate receptor-mediated excitation of PV interneurons in schizophrenia. The immediate early gene (IEG) NARP, an AMPA-receptor (AMPAR) binding protein, is secreted by presynaptic pyramidal neurons in response to neuronal activation and localizes prominently to excitatory synapses on PV interneurons. NARP plays crucial roles in the formation of excitatory inputs onto, and the excitability of, PV interneurons by clustering AMPARs at the postsynaptic membrane. Since previous work has shown that fast AMPAR-mediated excitation of PV interneurons is sufficient to support gamma oscillations, altered NARP mRNA expression may lead to changes in AMPAR-mediated excitation of PV interneurons in schizophrenia. Furthermore, given that GAD67 expression is regulated by neuronal activity, alterations in NARP mRNA expression may contribute to lower GAD67 expression and GABA synthesis in PV interneurons in schizophrenia. Consequently, we first

used qPCR to evaluate the status of the representative IEGs known to regulate glutamate synaptic neurotransmission in the DLPFC from 62 matched pairs of schizophrenia and comparison subjects. *In situ* hybridization was then performed to explore the change of NARP mRNA levels at different levels of resolution. NARP mRNA levels were significantly lower in schizophrenia subjects both by qPCR and *in situ* hybridization (-26% and -40%, respectively). Of the variables frequently comorbid with schizophrenia that could be assessed, none accounted for the lower levels of NARP mRNA in schizophrenia subjects. Finally, NARP mRNA levels were positively correlated with GAD67 mRNA levels in schizophrenia subjects and in the total sample. These findings suggest that lower NARP mRNA expression in the DLPFC may contribute to lower excitatory drive onto PV interneurons, resulting in an activity-dependent down regulation of GAD67 expression and GABA synthesis, providing a potential molecular basis for altered gamma oscillations and impaired cognition in schizophrenia.

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Poster

229. Psychosis: Neuropathology

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 229.13/U3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: DA023109

Title: Altered ErbB4 splicing is associated with lower cortical parvalbumin expression in schizophrenia

Authors: *D. W. CHUNG, D. ARION, D. A. LEWIS
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Abstract: Dysfunction of the dorsolateral prefrontal cortex (DLPFC) in schizophrenia is associated with markers of lower activity in parvalbumin (PV) interneurons which might reflect impaired excitatory synapse formation in these cells. ErbB4, a receptor tyrosine kinase, plays an essential role in excitatory synapse formation in PV cells. Total ErbB4 mRNA levels are

unaltered, but levels of two minor splice variants of ErbB4, JM-a and CYT-1, are higher in DLPFC gray matter in schizophrenia, suggesting dysregulated splicing, but not altered transcription, of ErbB4 mRNA in the illness. In human DLPFC, ErbB4 is expressed predominantly in PV cells in layer 4, but predominantly in calretinin (CR) cells in layer 2. Since PV, but not CR, mRNA expression is altered in schizophrenia, these findings suggest that abnormal ErbB4 splicing is associated selectively with PV cells in layer 4. To test this hypothesis, DLPFC layers 2 or 4 were selectively laser microdissected from 39 matched pairs of schizophrenia and control subjects, RNA was isolated and qPCR was performed using primers for three house-keeping genes (beta-actin, GAPDH, cyclophilin A), two layer enriched markers (CR and PV), four ErbB4 splicing variants (JM-a, JM-b, CYT-1,CYT-2) and Pan-ErbB4. In layer 2, CR expression was ~10X fold higher than PV, whereas in layer 4 PV expression was ~10X fold greater than CR, indicating that the samples of layers 2 and 4 were enriched for CR and PV cells, respectively. In schizophrenia, CR levels were not altered in either layer, whereas PV levels were 20% lower only in layer 4. In layer 4 of schizophrenia subjects, JM-a levels were 22% higher and JM-b levels were 17% lower, suggesting that ErbB4 splicing at JM locus is dysregulated in PV cells. Finally, the JM-a:JM-b ratio was inversely correlated with PV levels in layer 4 of SZ . As PV expression depends on excitatory inputs onto PV cells, these findings support the interpretation that a higher ratio of JM-a to JM-b ErbB4 splice variants is associated with fewer excitatory synapses and lower excitatory drive onto PV cells in schizophrenia.

Disclosures: D.W. Chung: None. **D. Arion:** None. **D.A. Lewis:** None.

Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R01 MH043784

NIH Grant R01 MH096985

Nara Medical University

Title: Lower GAD65 mRNA and protein levels in the prefrontal cortex of schizoaffective but not schizophrenia subjects

Authors: *J. R. GLAUSIER, S. KIMOTO, K. N. FISH, D. A. LEWIS
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Abstract: Altered GABA signaling in the prefrontal cortex (PFC) has been associated with cognitive dysfunction in schizophrenia and schizoaffective disorder. GABAergic neurotransmission is in part regulated by activity of the GABA-synthesizing enzymes, glutamic acid decarboxylase 65kD and 67kD (GAD65 and GAD67). Lower levels of cortical GAD67 mRNA and protein have been consistently reported in postmortem studies of schizophrenia. However, fewer studies have examined GAD65 and the findings are mixed. Here, we quantified GAD65 mRNA and protein in the PFC from a large cohort of subjects. GAD65 mRNA levels were assessed by quantitative PCR in right PFC area 9 gray matter from 62 matched pairs of schizophrenia or schizoaffective disorder subjects and healthy comparison subjects. Based on these results, GAD65 protein levels were quantified in a subset of subject pairs using confocal immunofluorescence microscopy. Fluorescence intensity, which reflects relative protein levels, was measured across all cortical layers of left PFC area 9. Mean GAD65 mRNA levels were 6.2% lower in the affected subjects relative to comparison subjects ($p=0.016$, paired ANCOVA; $p=0.051$, unpaired ANCOVA). Further examination showed that GAD65 mRNA was unchanged in subjects with schizophrenia ($p=0.4$ paired; $p=0.8$, unpaired), but was 13.6% lower in subjects with schizoaffective disorder ($p=0.006$, paired; $p=0.004$, unpaired). In these subjects with schizoaffective disorder, GAD65 relative protein levels were 19.4% lower ($p=0.012$, paired; $p=0.033$, unpaired), and GAD65 mRNA and protein levels were positively correlated ($r=0.56$, $p=0.019$). Lower GAD65 mRNA and protein measures within schizoaffective disorder subjects was not attributable to factors commonly comorbid with the diagnosis. In concert with previous studies, these findings suggest that schizoaffective disorder is associated with lower levels of both GAD65 and GAD67 mRNA and protein in the PFC, whereas subjects with schizophrenia have lower mean levels of only GAD67 mRNA and protein. Because cognitive function is generally better preserved in subjects with schizoaffective disorder relative to subjects with schizophrenia, these findings may support an interpretation that GAD65 down-regulation provides a homeostatic response complementary to GAD67 down-regulation expression that serves to reduce inhibition in the face of lower PFC network activity.

Disclosures: J.R. Glausier: None. **S. Kimoto:** None. **K.N. Fish:** None. **D.A. Lewis:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Bristol-Myers Squibb, Pfizer. F. Consulting Fees (e.g., advisory boards); Autifony, Bristol-Myers Squibb, Concert Pharmaceuticals, Sunovion.

Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH084053

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Title: Differential alterations of perineuronal net components in the dorsolateral prefrontal cortex of subjects with schizophrenia

Authors: *J. F. ENWRIGHT, III[1], A. FOGLIO[2], R. BERRY[2], S. SANAPALA[2], D. ARION[1], K. FISH[1], D. LEWIS[1]
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Abstract: Alterations in GABA interneurons that express the calcium protein PARVALBUMIN (PV) have been widely reported and may underlie some of the cognitive deficits seen in subjects with schizophrenia. Interestingly, PV neurons as well as a subset of pyramidal cells are surrounded by perineuronal nets (PNNs), extracellular structures that can be histochemically identified with the plant lectin *Wisteria floribunda* agglutinin (WFA). PNNs are thought to form in an activity-dependent manner, and may be partially responsible for the fast-spiking properties of certain cell types. Furthermore, recent postmortem studies have reported PNN deficits (as determined by WFA labeling) in subjects with schizophrenia. Using spinning disc confocal microscopy, stereological approaches, and quantitative immunofluorescence, we analyzed PNNs using two different markers, WFA and an antibody raised against AGGRECAN, a major protein component of the mature PNN, in layer 3 of area 9 in 28 matched pairs of subjects (healthy comparison/schizophrenia). Along with decreased PV immunofluorescence intensity per cell in schizophrenia subjects, at low magnification we found a decrease in the percentage of PV cells with detectable PNNs. However, this deficit was significantly more robust when WFA was used as the PNN marker. Furthermore, in both healthy comparison and schizophrenia subjects the percentage of PV cells with AGGRECAN- labeled PNNs was significantly higher than the percentage of PV cells with WFA-labeled PNNs. While high magnification analyses of individual PNNs showed a decrease in the average fluorescence intensity for both WFA and AGGRECAN in schizophrenia subjects, WFA-labeling was more consistently and robustly decreased. Additionally, when observed at high magnification, the percentage of PV cells with

detectable PNNs (as determined by AGGRECAN labeling) was not altered in schizophrenia subjects. Furthermore in schizophrenia subjects, PNNs around pyramidal cells had deficits in WFA, but not AGGRECAN, labeling. These effects do not seem to result from exposure to antipsychotics as neither PV nor PNN labeling was altered in a cohort of monkeys chronically exposed to haloperidol. These data suggest that in the dorsolateral prefrontal cortex of subjects with schizophrenia various components of the PNN are differentially affected, PNNs around both PV and pyramidal cells are altered, and the detectability of PNNs is dependent both on the marker and magnification used.

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant MH051234

Title: Altered Cdc42 signaling pathway in cortical layer 3 pyramidal cells in schizophrenia

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Abstract: Disturbances in the circuitry of the dorsolateral prefrontal cortex (DLPFC) appear to contribute to the pathophysiology of the cognitive deficits in schizophrenia. In particular, pyramidal cells, the principal source of cortical glutamate neurotransmission, exhibit morphological alterations in schizophrenia. These alterations include a smaller somal size, a less complex dendritic arbor and a lower density of dendritic spines. This pattern of pathology is particularly marked in pyramidal neurons located in layer 3, and may reflect an intrinsic deficit in the expression of genes that regulate the actin cytoskeleton in these neurons. This notion is supported by limited data demonstrating that subjects with schizophrenia exhibited altered DLPFC gray matter levels of transcripts in the Rho family of GTPases (e.g., Cdc42) which regulate the organization of the actin cytoskeleton. The goal of the present study was to examine in more detail the molecular mechanisms that may contribute to the morphological alterations, especially the lower density of dendritic spines, present in layer 3 pyramidal neurons in the

DLPFC of subjects with schizophrenia. Individual pyramidal cells (400 cells/subject) in DLPFC deep layer 3 were captured using laser microdissection from 19 matched pairs of schizophrenia and healthy comparison subjects. Total RNA was isolated and levels of transcripts for the intracellular interacting partners of the Cdc42 pathway (Cdc42, Cdc42EP4, PAK1, PAK2, LIMK1, LIMK2, ACTR2, WASL and ARHGDIA) were quantified using RT-PCR. The mRNA expression of upstream regulators of the Cdc42 pathway were modestly lower (e.g., Cdc42, -11%; PAK1, -16%; WASL, -15%), whereas expression levels of downstream regulators/effectors were significantly higher (e.g., LIMK2, 59%; Cdc42EP4, 54%) in layer 3 pyramidal cells in schizophrenia. Using a cell-type specific approach, our results identify several intracellular interacting partners of Cdc42 with altered expression in DLPFC layer 3 pyramidal cells in schizophrenia. The decrease in mRNA expression of upstream regulators may directly compromise the structural stability of spines while the increase in mRNA expression of downstream regulators/effectors may serve as a compensatory mechanism to enhance the stability of remaining spines and restore spine plasticity. Our findings support the notion that altered signaling in the Cdc42 pathway represents a molecular mechanism that might contribute to the lower density of dendritic spines in layer 3 pyramidal cells in the DLPFC of subjects with schizophrenia.

Disclosures: **D. Datta:** None. **D. Arion:** None. **D.A. Lewis:** None.

Poster

229. Psychosis: Neuropathology

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH077851

Title: Whole-transcriptome analysis of hippocampal ca3 and gene verification in schizophrenia

Authors: *J. M. PEREZ, K. GLEASON, S. GHOSE, H.-C. HUANG, T. HWANG, T.-K. KIM, C. A. TAMMINGA
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Abstract: Schizophrenia (SZ) is one of the thirty most incapacitating conditions and affects over 67 million people worldwide; suicide occurs in 10% of those diagnosed with schizophrenia. Symptoms are persistent and often severe. They include hallucinations, delusions, thought

disorder, and deficits in executive function and memory. Treatments available are not always efficacious. 20-40% of people with schizophrenia are resistant to treatment and less than 20% completely recover after one episode of psychosis. Due to a lack of understanding of the molecular pathophysiology of schizophrenia, its diagnosis is based on behavioral symptomatology. Unfortunately, due to only phenomenological diagnoses, these categories are inadequate. Therefore, we are examining human tissue for molecular causes and correlates of the illness. Our lab has proposed a model of psychosis as a disorder of learning and memory that critically involves dentate gyrus (DG) and CA3. We suggest that reduced glutamatergic neurotransmission from DG to hippocampal CA3 serves to generate an increase in CA3 basal activity and function through homeostatic plasticity changes within CA3. This increase in function may lead to the generation of inappropriate or illogical memories with psychotic content. Our lab has shown an increase in perfusion in CA3 in schizophrenia, a correlate of neuronal activity level, as well as an increase in spine density and dendritic complexity and increased protein levels of synaptic plasticity markers like GluN2B and PSD-95 in CA3 of schizophrenia postmortem tissue. Using a hypothesis generating approach, we have analyzed the CA3 transcriptome from control and off-drug schizophrenia cases, in a global and unbiased manner, using whole transcriptome (WT) sequencing to identify additional molecular changes, which have not been hypothesized. These analyses have focused our interest on genes like GRPIN1, AGAP1, and BCR among others. We have conducted differential expression analysis, gene coexpression network analysis, and variant mining analysis for this data set and are currently verifying the RNA-seq results through qRT-PCR and Sanger sequencing. We expect to show a network of abnormalities in CA3, which support the increases in activity we have shown with our *in vivo* imaging analyses.

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant MH066172-10

Title: Molecular characteristics of cerebral networks for psychosis

Authors: W. LI[1], N. SAMUDRA[1], C. MEYER[1], K. GLEASON[1], E. I. IVLEVA[1], S. GHOSE[1], *C. A. TAMMINGA[2]
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Abstract: The molecular, cellular and network underpinnings of psychosis in diseases like schizophrenia are unknown but remain essential knowledge for rational treatment development. Hippocampal hyperactivity has been repeatedly demonstrated in schizophrenia, potentially generating mistakes of memory and false memories with psychotic content within relevant brain networks, a pathological alteration in learning and memory. To identify the cerebral networks associated with this hippocampal pathology, we performed regional seed connectivity analyses on N=87 resting state fMRI acquisitions from psychosis probands and found alterations in connectivity between anterior hippocampus and medial, dorsolateral and cingulate prefrontal cortex as well as with superior temporal gyrus and cerebellum. Guided by these outcomes, we are carrying out a molecular analysis of hypothesized protein alterations in the regions of these circuits. We have started by analyzing molecular markers of synaptic strength in ACC and cerebellum in a single postmortem tissue cohort (N=20 SZ; N=20 HC) and analyzing the association between these changes and changes in hippocampus. In layers 1-3 of ACC, we found trend increases of Glu2B in all schizophrenia cases (p=0.099) and the same in off medication schizophrenia cases (p=0.10). We did not observe any change in GluN1 (p=0.82), in Glun2A (P=0.49), in GAD67 (p=0.39), in ERK1 (P=0.67) or in ERK2 (p=0.93) in schizophrenia cases. In layers 5a-6 of ACC, we found a significant increase in GluN2B (p= 0.034) and in GluN2B/GluN1 (p=0.016) in off medication cases, though the increases were not significant when tested within the whole sample of schizophrenia cases (p= 0.15 for GluN2B, and p= 0.32 for GluN2B/GluN1), suggesting that these proteins may be modified by antipsychotic treatment. There was a trend increase in GluN2A (p=0.10) and a significant increase in GluN2A/GluN1 (p= 0.0089) in off medication schizophrenia cases though the increases were not observed in the on medication schizophrenia cases (p= 0.76 for GluN2A, and p=0.68 for GluN2A/GluN1). There was no significant change observed in GluN1 (p=0.19), in GAD67 (p=0.57), in PSD95 (p=0.48), in GluA1 (p=0.79), in ERK1 (p= 0.73), or in ERK2 (p=0.78) in schizophrenia cases. Data from cerebellum and other regions in this circuit will be reported and correlations between regional molecular markers and hippocampus will be developed. Ultimately, regional transcriptome analyses might be the strongest way to identify the molecular signaling systems which mediate this functional connectivity.

Disclosures: W. Li: None. **N. Samudra:** A. Employment/Salary (full or part-time):; UT Southwestern Medical Center. **S. Ghose:** A. Employment/Salary (full or part-time):; UT Southwestern Medical Center. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH grant. **C.A. Tamminga:** A. Employment/Salary (full or part-time):; UT Southwestern Medical Center. **C. Meyer:** None. **K. Gleason:** None. **E.I. Ivleva:** A. Employment/Salary (full or part-

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Poster

229. Psychosis: Neuropathology

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH071533

5T32NS007433-15

Title: Haloperidol-exposure does not affect schizophrenia-linked reductions in microtubule associated protein 2 immunoreactivity

Authors: *M. A. SHELTON[1], J. T. NEWMAN[1], H. GU[2], A. R. SAMPSON[2], K. N. FISH[1], P. P. PENZES[4], D. A. LEWIS[1], R. A. SWEET[1,3,5]
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Abstract: Background: Microtubule-associated protein 2 (MAP2) is crucial for establishing and maintaining experience-dependent plasticity of dendrite and spine structure. The neuropathology of schizophrenia (Sz) includes a drastic reduction in MAP2 immunoreactivity (IR) in a number of cortical regions; however, the contribution of anti-psychotics to this finding is unknown. Despite the loss of immunoreactivity, MAP2 mRNA expression levels are unaffected in tissue from schizophrenia subjects. Thus, we hypothesized MAP2-IR would be unaffected by anti-psychotic exposure. Methods: Using quantitative fluorescence confocal microscopy, we examined MAP2-IR intensity as well as spine density and number in human post-mortem tissue taken from BA41 of 20 individuals with Sz and matched controls. We utilized a cohort of four macaques chronically administered the anti-psychotic haloperidol and four matched sham-treated control animals in order to determine the effect of anti-psychotics on MAP2-IR. Results: MAP2-IR was reduced by 70.0% (p=0.001) in Sz subjects, with a sub-group comprising 60% of our Sz cohort that exhibited fluorescence values near background, below the lowest intensity values

observed in control subjects. In addition, spine density and number were significantly reduced exclusively within this sub-group (spine density, p=0.0018; spine number, p=0.0042). Finally, there was no effect of chronic haloperidol exposure on MAP2-IR in BA41 deep layer 3 in drug exposed macaques. Conclusions and Future Directions: MAP2-IR, spine density, and spine number are significantly reduced in BA41 deep layer 3, and our results show that these changes are not a consequence of anti-psychotic exposure. These results suggest that alterations to MAP2 represent disease pathophysiology as opposed to an effect of treatment. We previously reported no significant effect of drug treatment on spine density in these animals. Future experiments will combine quantitative microscopy, antibody-based epitope mapping, and targeted proteomics, to perform total and domain specific measures of MAP2 in order to determine how changes in MAP2's regulatory state and epitope availability contribute to the loss of MAP2-IR in Sz.

Disclosures: **M.A. Shelton:** None. **J.T. Newman:** None. **H. Gu:** None. **A.R. Sampson:** F. Consulting Fees (e.g., advisory boards); Janssen Pharmaceutical Research and Development LLC. **K.N. Fish:** None. **P.P. Penzes:** None. **D.A. Lewis:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Bristol-Myers Squibb, Pfizer. F. Consulting Fees (e.g., advisory boards); Autifony, Bristol-Myers-Squibb, Concert Pharmaceuticals, Sunovion. **R.A. Sweet:** None.

Poster

229. Psychosis: Neuropathology

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant MH083957-04

Title: Loss of pattern separation performance and fMRI BOLD activation in schizophrenia

Authors: T. DAS[1], *E. I. IVLEVA[2], C. E. L. STARK[3], A. WAGNER[4], C. A. TAMMINGA[1]
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Abstract: In healthy individuals, the hippocampus has long been implicated in forming new memories, storing them independently, retrieving memories from partial cues and flexibly

applying stored memories to partial cues. Hippocampal-mediated memory allows the discrimination of similar events as distinct and orthogonal, called *pattern separation (PS)*. Motivated by evidence that the dentate gyrus differentially mediates the *PS* component of declarative memory function and that it harbors molecular and cellular pathology in schizophrenia, we examined *PS* performance in schizophrenia (SZV) vs healthy volunteers (HV) using the Behavioral Pattern Separation (BPS) task (Stark et al., Neuropsychologia, 2013). In well-characterized SZV and HV, we contrasted behavioral performance and fMRI BOLD activation during the BPS task. Behaviorally, we have calculated two outcome measures, *PS* and Recognition Memory (RM). The BPS task includes an incidental 'encoding' phase (runs 1-2) (with 128 pictures of everyday objects) where volunteers identified each object as "indoor" or "outdoor". A subsequent 'test' phase (runs 3-4) exposes volunteers to 192 pictures/run, with 64 repetitions (old), 64 new objects (foils) and 64 similar objects (lures) to pictures shown in runs 1-2, and volunteers were instructed to identify objects as repeated, new, or similar. The SZVs showed a significant decrement in *PS* performance relative to HV (mean±SEM, SZV: 3.1±2.7%; HV: 17.1±5.8%; *p=0.039); whereas SZV and HV did not significantly differ in RM performance (SZV: 50.1±8.1%; HV: 59.3±5.5%; p=0.350) (Fig). We have acquired high resolution fMRI BOLD task-associated scans on 3T Philips Achieva. These data are being analyzed using SPM5. We hypothesize that SZV will activate the DG/CA3 hippocampal subfield to a significantly lower degree than HV for correct lure identification and that the magnitude of activation will correlate with *PS* performance. fMRI BOLD data will be presented by group and by subfield. Hence our behavioral finding of lack of pattern separation in schizophrenia leads us to expect a dysfunctional dentate gyrus in the illness, a feature that could contribute to declarative memory impairment in the disorder and possibly, schizophrenia psychosis.

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R01 MH 090067

NIH Grant F31 MH098564

Title: Prefrontal cortical interneuron transplants reverse deficits in reversal learning in a rodent model of schizophrenia

Authors: *A. M. BOLEY, S. M. PEREZ, D. J. LODGE
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Abstract: Schizophrenia is a debilitating disease that affects up to 1% of the population. Current therapies are associated with various adverse side effects or are ineffective, making identifying novel targets and treatments a necessity. A consistent pathology, observed in schizophrenia patients and rodent models of the disease, is a loss of interneuron function, specifically interneurons containing the calcium binding protein parvalbumin (PV). We have previously demonstrated a decrease in PV expression (induced by lentiviral delivered shRNA) in the ventral hippocampus was sufficient to cause hippocampal hyperactivity and downstream dysfunction of the dopamine system. Additionally, we have shown that by replacing deficient interneurons (via cell transplantation) into the hippocampus, we were able to reverse hippocampal hyperactivity, normalize aberrant dopamine neuron activity, and reverse the hyper-responsive locomotor response to amphetamine in the methylazoxymethanol acetate (MAM) model of schizophrenia. Furthermore, the loss of PV observed postmortem in schizophrenia patients is not specific to the hippocampus and involves cortical regions such as the prefrontal cortex, which may underlie symptoms that are not related to psychosis. Given the association between prefrontal cortical function and cognitive flexibility, we examined whether interneuron transplants in the medial prefrontal cortex (mPFC) could reverse cognitive deficits in the MAM rodent model of schizophrenia. Here we demonstrate that mPFC interneuron transplants are able to reverse deficits in reversal learning in an attentional set-shifting task designed to assess cognitive flexibility. These findings support the basis of targeting cortical interneuron function, in addition to the hippocampal region, to treat features of schizophrenia that encompass both psychosis and non-psychosis related symptoms.

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Poster

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Title: Cell adhesion molecules in psychiatric diseases and aging

Authors: T. BUTT[1], A. SANTIAGO[2], A. DWORK[2], G. ROSOKLIJA[1], V. ARANGO[1], R. HEN[1], J. MANN[1], *M. BOLDRINI[3]
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Abstract: Adult neurogenesis in the dentate gyrus (DG) of the hippocampus is found in rodents (Santarelli 2003), nonhuman primates (Kornack and Rakic, 1999), and humans (Eriksson 1998). Impaired neurogenesis is reported in psychiatric and neurodegenerative disorders, such as major depression (MDD, Boldrini 2009, 2012, 2013), and Alzheimer's (Winner 2011). One third of DG neurons turn over postnatally in human (Spalding 2013), three times as fast than in rodents (Imayoshi 2008). Neural progenitor cells (NPCs) decrease with age in MDD even when treated with antidepressants (Boldrini 2009), as in rodents (Couillard-Despres 2009). Adult mammalian neurogenesis involves the birth of NPCs expressing nestin (Kalman and Ajtai, 2001); later, polysialylated-neural cell adhesion molecule (PSA-NCAM) is expressed by transiently amplifying progenitors, (Kempermann 2004, Kempermann, 2011) and neuroblasts (Kempermann, 2011). PSA-NCAM mediates structural remodeling, synapses growth (Seki and Rutishauser, 1998), cell migration and axonal growth (Keynes and Cook, 1995), learning and memory (Venero 2006). Expression of PSA-NCAM molecule in relationship with aging, MDD and antidepressant treatment by quantifying immunoreactive (IR) was assessed in autopsy DG from non-psychiatric sudden deaths (controls, n=12), untreated MDD (n=12) and antidepressant-treated MDD (n=12). We performed psychological autopsy, neuropathology and toxicology. Frozen hippocampi were fixed and processed for immunohistochemistry and stereology. PSA-NCAM-IR cells with multipolar (r= -.797, *p*= .010) and immature morphology (r= -.717, *p*=

.030) in anterior DG decreased with age in controls. PSA-NCAM-IR cell number did not correlate with age in MDD, which did not differ from controls in any DG region. MDD treated with tricyclic antidepressants (TCA) have more PSA-NCAM-IR immature cells *vs*. untreated MDD (p=.027) and controls (p=.007). PSA-NCAM-IR multipolar cells are more in MDD treated with TCA *vs*. controls (p=.030), untreated MDD (p=.018) and MDD treated with selective serotonin reuptake inhibitors (SSRI, p=.030). Fewer PSA-NCAM-IR cells and NPCs in anterior DG with aging in non-psychiatric controls may contribute to age-related memory and pattern recognition decline. Cellular changes with age in anterior DG were not present in MDD, perhaps because MDD neuropathology may obscure or over-ride the age effect. The relationship between TCA and PSA-NCAM-IR cells could be explained by TCA noradrenergic effect because it is not seen with SSRIs, which have a serotonergic effect.

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Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 230.01/U13

Topic: C.15. Schizophrenia and Bi-polar Disorder

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Title: Alterations in the structure, plasticity and connectivity of inhibitory networks in a double hit model of schizophrenia

Authors: *C. GARCÍA MOMPÓ, E. CASTILLO-GÓMEZ, B. RIPOLL-MARTÍNEZ, Y. CURTO-SASTRE, J. GILABERT-JUAN, J. NÁCHER
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Abstract: Schizophrenia is a complex psychiatric disorder, which results in dramatic changes in behavior, perception and cognition, as well as alterations in the structure and function of cortico-limbic regions, including the prefrontal cortex and the amygdala. Current pathophysiological theories of schizophrenia point to abnormalities in the development, organization and physiology of inhibitory circuits as responsible for some of these alterations. However, the cellular and molecular bases of these alterations are still unclear. In this line, the generation of animal models reproducing some of the core features of schizophrenia, constitute valuable tools to investigate these alterations. By generating a double developmental/environmental mice model of schizophrenia in a transgenic strain displaying fluorescent interneurons, we sought to mimic a wide range of features of this disorder and find alterations in the structural plasticity of inhibitory circuits. Mice were subjected to a perinatal injection of a N-methyl-D-aspartate receptor (NMDA-R) antagonist, MK-801, and were socially isolated from postweaning to adulthood. We found that these mice reproduce some of the behavioral and structural alterations previously seen in other models. Consequently, abnormalities in the structural plasticity of cortico-limbic interneurons may have a key role in the pathophysiology of schizophrenia.

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Poster

230. Systems-Oriented Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R01 MH091130

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Title: Ventral pallidum is key to the endocannabinoid regulation of dopamine system function in a rodent model of schizophrenia

Authors: *D. D. AGUILAR[1], L. CHEN[2], D. J. LODGE[1]
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Abstract: Schizophrenia is a debilitating disorder that affects over 3 million Americans. Existing pharmacological therapies have pronounced side effects which lead many patients to discontinue their medication. Therefore, alternative therapies such as endocannabinoid upregulation are being studied. Clinical observations reveal an inverse relationship between levels of the endocannabinoid anandamide and psychotic symptoms in patients with schizophrenia. Furthermore anandamide upregulation alleviates social withdrawal, a negative symptom of schizophrenia, in a rodent model of schizophrenia. The psychotic symptoms of schizophrenia are correlated with augmented dopamine system activity. Here we show anandamide upregulation alleviates augmented dopamine neuron population activity in the phencyclidine model of schizophrenia. Although hippocampal activity drives this phenotype, we have demonstrated that the site of action of anandamide is the ventral pallidum. Specifically, URB597 administered directly to the ventral pallidum is sufficient to restore aberrant dopamine neuron population activity. The endocannabinoid system and/or ventral pallidum may prove to be attractive targets for reducing psychotic episodes in schizophrenia patients.

Disclosures: D.D. Aguilar: None. **L. Chen:** None. **D.J. Lodge:** F. Consulting Fees (e.g., advisory boards); Dey Pharmaceuticals.

Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

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Program#/Poster#: 230.03/U15

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Impact of age and chronic ketamine treatment on parvalbumin expression in rats: Evidence for variation in protein expression

Authors: *J. A. CORRIVEAU, C. MCMAHON, J. J. CHROBAK
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Abstract: A selective decrease in parvalbumin (PV) immunoreactivity is a stable finding in post-mortem schizophrenic hippocampus and prefrontal cortex. Animal models of schizophreniform dysfunction following acute and/or chronic ketamine treatment show comparable decreases in PV expression in rodents, and it is often considered a marker of pathology. However, there are some groups reporting conflicting findings indicating no change in PV following sub-chronic treatment, which calls into question the efficacy of using PV as a pathological marker. Upon close examination of methodology across studies, it is clear that there exist differences in

protocols, especially with regard to the age of the rodents during treatment and/or sacrifice. Furthermore, the literature lacks a sufficient understanding of baseline/normative PV expression in drug- and behaviorally-naïve tissue. In order to understand the putative role of PV in pathology, systematic characterization of normative distribution across ages is imperative. The present study examined PV expression across the septotemporal axis of the rat hippocampus and surrounding somatosensory cortex (barrel fields) in 1-, 6-, and 12-month old rats. Our findings suggest variation in expression of PV in untreated naïve rats as a function of age, with decreased PV in older rats compared to younger rats, along with changes across the septotemporal (long) axis dependant on age. Based on our data, we propose that PV expression is a more dynamic marker than previously thought, and that changes in expression based on age should be considered when modeling pathology. Ongoing research in our lab is investigating the impact of chronic ketamine administration on PV expression and behavior to better characterize the nuances and translatability of this model of schizophreniform dysfunction.

Disclosures: J.A. Corriveau: None. **C. McMahon:** None. **J.J. Chrobak:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

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Program#/Poster#: 230.04/U16

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: NMDA receptor antagonist-induced changes in rat EEG power spectra as a model of schizophrenia

Authors: *N. UPTON, G. WADSWORTH, K. WATERS, D. VIRLEY
TRANSPHARMATION LTD, LONDON, United Kingdom

Abstract: Schizophrenia is a debilitating neuropsychiatric disorder characterised by an array of symptoms, with negative symptoms (e.g. lack of emotion) and cognitive dysfunction proving resistant to current therapies. Recent EEG studies have provided evidence that changes in synchronicity in the Gamma range (30-80Hz) are associated with this treatment resistance in schizophrenic patients. A large body of evidence now points to the disruption of N-methyl-D-aspartate (NMDA) mediated signalling as a core pathophysiological deficit in schizophrenia, including the observation that pre-clinical species administered NMDA antagonists display a phenotype consistent with schizophrenia. In this study we investigated the effects of two NMDA antagonists, ketamine (Ket) and phencyclidine (PCP) on EEG spectral power and sleep-wake

stages in freely moving rats. EEG signals were recorded from electrodes over the frontal-parietal cortex using intracranial electrodes; nuchal EMG was recorded to enable sleep stage scoring. Signals were recorded from the onset of the dark period for 24hrs. The animals were dosed with either vehicle (saline s.c.), Ket (3, 10, 30mg/kg, s.c.) or PCP (0.3, 1, 3mg/kg, s.c.) 30min following the onset of the dark period. Data were analysed over the period of peak effect for each agent.

Significant effects on activity and sleep-wake (% change from vehicle)

		Wake		Sleep Stage		
Agent	Activity	Active	Quiet	1	2	PS
Ket 3mg/kg	NS	NS	NS	NS	NS	-65.0
Ket 10mg/kg	438.8	364.4	232.8	-52.78	-60.4	-97.1
Ket 30mg/kg	704.0	587.0	272.8	-66.29	-91.3	-96.1
PCP 0.3mg/kg	NS	NS	NS	NS	NS	-28.8
PCP 1mg/kg	NS	192.1	27.8	-49.3	-21.4	-73.0
PCP 3mg/kg	789.2	600.4	27.1	-92.7	-87.7	-100.0

Effect on EEG Power (% change from vehicle)

Treatment	Delta	Theta	Alpha	Beta	Gamma
Ket 3mg/kg	NS	NS	NS	NS	NS
Ket 10mg/kg	NS	NS	NS	NS	182.3
Ket 30mg/kg	NS	NS	NS	NS	349.7
PCP 0.3mg/kg	NS	NS	NS	NS	NS
PCP 1mg/kg	NS	NS	NS	-18.2	80.0
PCP 3mg/kg	-60.3	-49.2	-48.3	-36.2	265.6

Both PCP and Ket caused profound changes in the behavioural and EEG profiles of the animals. Activity was increased and sleep-wake was shifted to an arousal profile with an increase in wake phases and decrease in all sleep phases. In terms of EEG power, PCP and Ket dramatically increased gamma frequencies. These data add further weight to the use of NMDA receptor antagonists to provide a valid translational model of schizophrenia and suggest that EEG biomarkers may have utility in identifying novel treatment strategies for restoring more normal gamma synchronicity.

Disclosures: **N. Upton:** None. **G. Wadsworth:** None. **K. Waters:** None. **D. Virley:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

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Program#/Poster#: 230.05/U17

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NEWMEDS

The Danish National Advanced Technology Foundation

Title: Characterization of a mouse model of the human 22q11.2 microdeletion syndrome

Authors: ***J. B. LAURIDSEN**[1], K. FEJGIN[1], F. GASTAMBIDE[2], S. NILSSON[3], V. NIELSEN[1], D. CLAUSEN[1], P. H. LARSEN[1], M. TRICKLEBANK[2], T. BUSSEY[3], L. M. SAKSIDA[3], M. DIDRIKSEN[1], J. NIELSEN[1]
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Abstract: Certain recurrent human copy number variants have been associated with a highly increased risk of schizophrenia. A hemizygous deletion of the 22q11.2 region constitutes the strongest discovered genetic risk factor for this disease (odds ratio≈30). The region is well conserved between human and mouse which allows modelling the human 22q11.2 deletion syndrome in mice. We have generated a new mouse model of the 22q11.2 microdeletion syndrome (Df(h22q11)/+). Transcriptional analysis showed an expected 50% reduction in the expression levels of genes within the deleted region of the Df(h22q11)/+ compared to wildtype and confirmed an earlier reported increase in ER membrane protein complex subunit 10 (Emc10) as well as other genes. Df(h22q11)/+ mice were born at a non-Mendelian ratio with about 40%

hemizygous mice. Histological analysis identified no gross abnormalities in brain morphology. Likewise, Df(h22q11)/+ mice had normal basic behavior such as reflexes, thermal pain sensitivity and motor performance. Baseline exploratory behavior, motility and anxiety parameters were also unaltered. However, Df(h22q11)/+ mice displayed increased hyperactivity in response to both ketamine and phencyclidine challenge and, importantly, this hypersensitivity first occurred post puberty. Furthermore, Df(h22q11)/+ mice showed a significantly increased acoustic startle response with shorter response latencies, indicating putative changes in auditory processing. Prepulse inhibition (PPI), a measure of sensorimotor gating commonly disrupted in patients with schizophrenia, was robustly decreased across all ages tested. Finally, Df(h22q11)/+ mice showed a complex cognition phenotype including both trends towards impairments in watermaze and t-maze tests, paralleled with improvements in multiple touch screen based tests. In summary, we have successfully created a mouse model of the human 22q11.2 microdeletion syndrome that recapitulates some of the findings in patients with schizophrenia such as an increased sensitivity to NMDA antagonists and a strong PPI deficit. Further studies on the impact of the deletion on social interaction are currently ongoing.

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Poster

230. Systems-Oriented Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

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Instituto de Salud Carlos III (Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM)

SAF2012-35183 (Ministerio de Economía y Competitividad, cofinanced by ERDF)

PI12/00156 (Instituto de Salud Carlos III, cofinanced by ERDF)

Title: Brain oscillatory patterns in 22q11.2 transgenic mice model of schizophrenia. Interaction with non-competitive NMDA-R antagonists

Authors: L. LLADÓ-PELFORT, *P. CELADA, F. ARTIGAS
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Abstract: Brain oscillations are one of the pillars of neural processing allowing for the coordinated activity of brain regions and the emergence of higher brain functions. Abnormal functional connectivity between brain areas has been hypothesized to explain schizophrenia pathophysiology, which would result in alterations of brain oscillatory patterns [1]. Copy number variants (CNV) provide valuable information about the relationship between genes, brain and behavior. Individuals carrying the 22q11.2 microdeletion display a high incidence of psychiatric symptoms, including schizophrenia [2]. 22q11.2 mice models of the human microdeletion may help to understand the etiology and pathophysiology of schizophrenia, also providing a valuable tool to study new treatment strategies. Non-competitive NMDA receptor (NMDAR) antagonists like phencyclidine (PCP) and ketamine are able to induce psychotic symptoms in healthy individuals and aggravate them in schizophrenic patients. Indeed, NMDAR hypofunction has been proposed as a schizophrenia hypothesis and is routinely used as a pharmacological model of the disease [3]. Here we examined the effects of a 22q11.2 model microdeletion in basal and NMDAR antagonist-induced patterns of oscillatory activity in cortical and subcortical brain networks. We performed local field potential multi-recordings of cortical (prefrontal cortex, PFC) and subcortical brain regions (hippocampal areas and thalamic nuclei) in freely moving wild type (WT) and Df(h22q11)/+ mice (provided by H. Lundbeck A/S) receiving acute saline, PCP or ketamine. Compared to WT mice, Df(h22q11)/+ mice showed an abnormal pattern of brain oscillations, including differences at low and high frequencies in PFC and subcortical areas. PCP and ketamine differentially altered brain oscillations, with PCP showing more marked and persistent effects than ketamine in WT and Df(h22q11)/+ mice. Moreover, the effects of both drugs were significantly different in WT and Df(h22q11)/+ mice, especially in the fast oscillation bands (beta, gamma and high frequency oscillations). The present results indicate the presence of altered baseline and NMDAR antagonist-induced oscillatory patterns in cortical and subcortical areas of Df(h22q11)/+ mice. These abnormalities may underlie the cognitive and behavioral alterations of 22q11.2 deletion carriers and illustrate the usefulness of CNV as intermediate phenotypes to gain insight into the pathophysiology and treatment of schizophrenia.
[1] Uhlhaas et al., 2010. Nat Rev Neurosci. 11:100-13. [2] Bassett et al., 2008. Curr Psychiatry Rep. 10:148-157. [3] Gilmour et al., 2012. Neuropharmacology 62:1401-12.

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Poster

230. Systems-Oriented Models of Schizophrenia

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NIH Office of the Director

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Title: Gamma rhythms link interneuron dysfunction with cognitive inflexibility in Dlx5/6+/- mice

Authors: *K. K. CHO, R. HOCH, A. T. LEE, T. PATEL, J. L. R. RUBENSTEIN, V. S. SOHAL
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Abstract: Dysfunction of the prefrontal cortex (PFC) contributes to cognitive deficits that represent the core of schizophrenia. Abnormalities in prefrontal GABAergic interneurons are found in post-mortem brain tissue from individuals with schizophrenia (Lewis et al., 2005), and are particularly prominent within fast-spiking interneurons (FSINs), which can be identified by either their electrophysiological properties or their expression of the calcium-binding protein parvalbumin (PV). FSINs generate synchronized gamma frequency (~30-120 Hz) oscillations that are hypothesized to enhance cortical information processing (Cardin et al,. 2009; Sohal et al., 2009), and deficient gamma oscillations may link interneuron dysfunction to cognitive deficits in schizophrenia (Uhlhaas and Singer 2010). Indeed, task-evoked cortical gamma oscillations are deficient in patients with schizophrenia (Cho et al., 2006). However, studies that disrupt FSIN function in mice have only found increases in baseline gamma oscillations, not deficient cognitive task-evoked gamma oscillations. Thus, the hypothesized link between deficits in interneurons, gamma oscillations, and cognition remains unclear. Specific developmental abnormalities that may contribute to the post-adolescent onset of schizophrenia are also unknown. Here we show that in Dlx5/6+/- mice, which are deficient in transcription factors necessary for FSIN development (Wang et al., 2010), the post-adolescent maturation of FSINs is

impaired. This coincides with the post-adolescent appearance of behavioral, cognitive, and electrophysiological endophenotypes of schizophrenia. Notably, adult Dlx5/6+/- mice exhibit both deficient gamma oscillations during PFC-dependent cognitive tasks and elevated gamma oscillations at baseline, matching the complex pattern of changes observed in schizophrenia, whereas the behavioral, cognitive and electrophysiological properties in adolescent Dlx5/6+/- mice were normal compared to age-matched wild-type controls. These results illustrate how the post-adolescent arrest of FSIN maturation could drive key cognitive and electrophysiologic endophenotypes of schizophrenia.

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Poster

230. Systems-Oriented Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: EDF(AL)

Title: Dopaminergic responses in shell part of the nucleus accumbens to MK801 administration are disrupted in adult rats after neonatal prefrontal cortex transient inactivation

Authors: T. POUVREAU[1], E. TAGLIABUE[1], S. EYBRARD[1], F. MEYER[2], *A. E. LOUILOT[1]
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Abstract: Schizophrenia would result from a defective connectivity, between several integrative regions, stemming from developmental anomalies (Weinberger and Lipska, 1995; Bullmore et al., 1997; Lewis and Levitt, 2002). Various abnormalities reminiscent of early brain development disturbances have been observed, in particular in patients' left prefrontal cortex (PFC) (Akbarian et al., 1993; Kalus et al., 2000). A striatal dopaminergic (DA) disturbance in schizophrenia is generally well accepted (e.g. Harrison, 1999; Carlsson et al., 2001). Non-competitive NMDA/glutamate receptors antagonists, can induce psychotic symptoms in healthy humans and exacerbate these symptoms in patients with schizophrenia (Malhotra et al., 1997; Lahti et al., 1995; 2001). The striatal DAergic dysregulation in schizophrenia may be dependent of prefronto-striatal disconnection involving glutamatergic NMDA receptors (Jentsch et Roth, 1999

; Carlsson et al., 2000 ; Laruelle et al., 2005).Thus, the present study was designed to investigate the effects of the non-competitive NMDA receptor antagonist, MK801, in adult rats on ventral striatal DA responses in the dorsomedial shell, following a postnatal inactivation of the left PFC (infralimbic/prelimbic region).During the neurodevelopmental period, impulse electrical activity appears to be crucial for shaping connections once developing axons reach the target structure (Katz and Shatz, 1996 ; Frostscher et al., 2000). Tetrodotoxin (TTX), is a potent and specific Na+ channel blocker (Mosher, 1986). Therefore, transient functional inactivation of the left PFC was carried out by local TTX microinjection in 8-day-old rats, i.e a critical time of the neurodevelopmental period (Clancy et al., 2001). DA variations were recorded in the dorsomedial shell using *in vivo* voltammetry in freely moving adult rats (11 weeks). Control animals received a s.c. injection of NaCl (0.9%); MK801 was administred s.c. at 0.1 mg/kg or 0.2 mg/kg. The obtained results were the following : 1) A clear dose-dependent decrease in dorsomedial shell DA levels was observed in PBS-microinjected animals whereas dose-dependent DA increase was observed with the two MK801 doses in TTX-microinjected animals; 2) DA changes were significantly different in PBS and TTX groups in adult animals after the administration of the highest MK801 dose. These data suggest that NMDA regulation of DA release in the dorsomedial shell is disrupted in animals microinjected with TTX in the left PFC at PND8. In conclusion, these findings may provide new insights in the involvement of NMDA glutamatergic receptors in the pathophysiology of schizophrenia.

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Poster

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Title: Quantitative EEG and hippocampal-prefrontal cortex synchrony in Df(h22q11)/+ mice, a model of high risk schizophrenia

Authors: S. TAKILLAH[1,2], J. NAUDÉ[3], P. FAURE[3], C. SEBBAN[2], B. DECROS[2], M. SPEDDING[4], E. SCHENKER[5], *J. J. MARIANI[6,2,1]
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Abstract: The human brain is a complex, dynamic system with computations occurring at several levels of organization, from individual synapses to networks that span multiple brain regions. These large-scale neural systems underlie behavior and cognition. Psychotropic drug administration or neuropsychiatric diseases such as schizophrenia affect behavior by acting on such large-scale neuronal networks. Abnormal synchronization of neural activity between distal brain regions has been proposed as the underlying core symptomatology of schizophrenia. The hippocampal-prefrontal cortex (HPC-PFC) network has been studied extensively in recent years to better understand disconnectivity in a wide range of neurodevelopmental as well as neurodegenerative diseases. Multi-electrode electroencephalographic recordings (EEG) allow monitoring of electrical oscillations generated in distal brain structures involved in the same functional networks. These oscillations and their relationships (i.e. synchrony) are associated with physiological and pathological states of consciousness. The present study analyzes differences in EEG recordings of the PFC and HPC, and synchrony changes in control versus Df(h22q11)/+ mice, carrying a microdeletion on chromosome 16 synteny of del (22q11.2) in humans. To restore this functional connectivity in these mice, we measured the synchronization of neuronal activity between the HPC and the PFC in the presence and absence of clozapine, an atypical antipsychotic with known efficacy in treating symptoms of schizophrenia. We have also administered ketamine non-competitive NMDA receptor antagonist, known to mimic schizophrenia-like symptoms in human studies including memory deficit, to examine the differential response between wild type and Df(h22q11)/+ mice.

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Conacyt-Mexico CB166241

Title: Synaptic plasticity dysfunction in a model of psychosis-like behavior

Authors: *M. G. HERNANDEZ, I. RUÍZ-PÉREZ, C. LÓPEZ-RUBALCAVA, E. J. GALVAN
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Abstract: The sub-chronic, neonatal administration of MK-801 is a model that mimics the psychosis-like behavior symptoms of schizophrenia. However, the relationship between synaptic plasticity and the emergence of the behavioral alterations associated to schizophrenia remains unclear. Rats administered with saline or MK-801 (0.2 mg/kg) were behaviorally examined at 30 and 90 postnatal days (PNDs) using the novel object recognition (NOR) test and pre-pulse inhibition (PPI) of the acoustic startle response test. Consistently, MK-801 reduce the NOR and PPI indexes at 90 but not at 30 PNDs (see table). Next, hippocampal slices were prepared from these animals and extracellular recordings were performed on CA1 area to assess for possible changes in paired-pulse facilitation (PPF), long-term depression (LTD) and long-term potentiation (LTP). Pretreated animals had a reduced PPF (ISI 60 ms; PPF control, 1.54 ± 0.08; MK-801 animals, 1.01 ± 0.08; at 90 PND control, 1.71 ± 0.11; MK-801 1.28 ± 0.06). High frequency stimulation (2 trains 100 Hz, 10 sec interval), failed to induce LTP in the pretreated animals (control LTP at 60 min post HFS, 150 ± 4 n=8. In MK-801 animals, 76 ± 21 n=9; at 90 PND control LTP, 169±4, n=9; for MK-801 animals, 71±1 n=9). Conversely, induction of LTD (900 pulses at 1 Hz) was not affected by the MK-801 pretreatment (synaptic depression compared to control at 60 min post LFS, 20.98 ± 5.4 % n= 8. MK-801 animals, 30.6 ± 12.2 n= 9; at 90 PND Control LTD, 36.46 ± 3.48% n=3; MK-801, 32.3 ± 1.9% n=3). These behavioral impairments shows that neonatal administration of MK-801 leads to the onset of memory and sensorimotor deficits at 90 PNDs (young adulthood) but not at 30 PNDs. However, the extracellular recordings revealed alterations in the mechanisms that underlie neurotransmitter release and induction of LTP as early as 30 PNDs. Nevertheless, our data indicate that the cellular mechanisms necessary for induction of LTD are not affected by the neonatal pretreatment with MK-801.

Effects of the sub-chronic administration of MK-801

	30 PNDs	90 PNDs
Behavioral tests(values compared to control):	MK-801	MK-801
NOR	Normal	Decreased

PPI	Normal	Decreased
Electrophysiology(values compared to control)		
PPF ratio	Decreased	Decreased
Long-term Potentiation	Impaired	Impaired
Long-term Depression	OK	OK

Disclosures: **M.G. Hernandez:** None. **I. Ruíz-Pérez:** None. **C. López-Rubalcava:** None. **E.J. Galvan:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 230.11/U23

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant MH048404-23

Title: Comparing the response of ventral tegmental area and substantia nigra neurons during acquisition and maintenance of a reward-mediated instrumental task

Authors: ***M. A. WEGENER**, B. MOGHADDAM
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Abstract: Dopamine neurons have been the focus of many influential theories and proposed mechanisms related to motivated behavior. These mechanisms and theories focus primarily, if not exclusively, on limbic circuitry involving dopamine neurons in the ventral tegmental area (VTA) that project to the nucleus accumbens. However, much of the electrophysiological research into motivated behavior has been performed in the substantia nigra pars compacta (SNc) of primates. While both VTA and SNc contain dopamine neurons, each region has distinct afferent and efferent projections and is implicated in different aspects of motivated behavior and cognition. Here we aimed to directly compare the response pattern of VTA and SNc neurons

during reward-related behavior. Adult rats were bilaterally implanted with microelectrode arrays to conduct single-unit recordings in the VTA and SNc of adult rats during acquisition and maintenance of a reward-mediated instrumental learning task. Rats learned to execute a naturalistic nose poke during presentation of a cue to receive a single sugar pellet. During task learning, we observed phasic responses to unexpected reward in both the VTA and SNc, and cue-evoked activity developed gradually over multiple sessions. Reward-related activity persisted strongly in the SNc throughout task performance, and still was prominent in the final session. In ongoing analyses, we will further examine the comparison of VTA and SNc single-unit responses to task-related events, isolate and compare putative dopamine and non-dopamine neurons and investigate how these regions respond to the extinction of this reward-mediated behavior.

Disclosures: M.A. Wegener: None. **B. Moghaddam:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 230.12/U24

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH096539

Title: Rescue of the trafficking deficient schizophrenia associated KCNH2 splice variant hERG 3.1 for the purpose of drug discovery

Authors: N. E. CALCATERRA[1], *J. C. BARROW[2]
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Abstract: Cultivated from advances in GWAS studies, numerous exciting schizophrenia drug targets have recently been discovered, including a novel KCNH2 splice variant, KCNH2 3.1. This gene translates to an N-truncated hERG channel with fast deactivation kinetics, Kv11.1-3.1. However, preliminary efforts to develop a cell line amenable to automated patch clamp platform for drug discovery have been difficult due to low assay signal and expression inconsistency. A robust system is needed for comparison to the full-length cardiac IKr channel, Kv11.1-1A, which will be an important counterscreen to avoid off target effects on the heart. It has been suggested that the truncation of 102 amino acids seen in Kv11.1-3.1 leaves the resulting protein product

without an intact Per-Ant-Sim (PAS) domain, found to be critical in Kv11.1-1A stability, trafficking, and regulation of deactivation rate. Mutations and deletions in this domain have been well characterized; similar characterizations of poor trafficking, stability, and fast deactivation rates have been suggested of KCNH2-3.1. Here we examine the trafficking characteristics by biochemical, electrophysiological, and imaging methods compared to the full transcript. Kv11.1-3.1 shows increased colocalization to the endoplasmic reticulum (ER), decreased colocalization to the plasma membrane, and diminished current. Trafficking of the protein responds differentially to a variety of literature rescue methods. Optimization of these rescue methods has resulted in transfection/expression protocol amenable to testing inhibitors for potency and selectivity. Kv11.1-3.1 is a promising drug target, now with tools in place for drug screening.

Disclosures: **N.E. Calcaterra:** None. **J.C. Barrow:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 230.13/U25

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: CIHR

Title: Correlating ventral hippocampal lesion volume with working memory performance in an animal model of schizophrenia

Authors: *T. BADIUDEEN, J. M. HYMAN, J. K. SEAMANS
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Abstract: The neonatal ventral hippocampal lesion (NVHL) model is the most well-characterized neurodevelopmental animal model of the cognitive deficits in schizophrenia. Reminiscent of the cognitive deficits seen in human patients, NVHL animals display marked impairments in cognitive flexibility and working memory. However, inherent to any lesion model, there is notable variability in the size and extent of lesions across individual animals. To the best of our knowledge, no study has analyzed the impact of variability in NVHL lesion size on adult performance of cognitive paradigms assessing working memory. One possibility is that cognitive deficits are produced by lesions of a given size and larger lesions have little additional impact. Alternatively, the cognitive deficit could be a linear or non-linear function of lesion size. To test these possibilities, NVHL and sham operated rats were trained on a delayed non-match to

sample (DNMS) task in a T-maze as adults. Following training, magnetic resonance imaging (MRI), was performed on all animals. Consistent with the current literature, NVHL animals showed a significant deficit in task performance when compared to sham operated animals, at a 10 second delay. We also found an impressive inverse linear correlation between the size (measured in cubic mm) of the lesion and working memory performance (percentage of correct trials), as performance on the DNMS task decreased linearly with increased lesion size. Lesion size was able to account for 49% of the total variability in working memory performance across animals.

Disclosures: T. Badiudeen: None. **J.M. Hyman:** None. **J.K. Seamans:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NHMRC Project Grant 1026070

University of Newcastle Near Miss grant

SRI seed funding

Title: Impaired generation of high frequency oscillations in a rat model of schizophrenia

Authors: L. R. HARMS[1,5], D. M. HODGSON[1], W. R. FULHAM[2], A. S. W. WONG[3], M. PENTTONEN[6], U. SCHALL[2,5], J. TODD[4,5], *P. T. MICHIE[3,5]
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Abstract: Persons with schizophrenia exhibit an impaired capacity to generate high-frequency gamma (>30Hz) oscillations. Gamma oscillations play an integral role in cognition and their reduction in schizophrenia is a candidate neurophysiological mechanism underlying cognitive impairments. The aim of this study was to establish an animal model with which to explore the neurobiological and behavioural implications of disrupted gamma oscillations. Here we used a well-accepted developmental animal model of schizophrenia, the maternal immune activation

(MIA) model. MIA was induced in Wistar rats by administering pregnant rats with Poly (I:C), a viral mimic, at gestational day 19. The offspring grew to adulthood, when they underwent surgery to implant EEG electrodes. A wireless telemetric headstage was fixed to the rat's head and the EEG recorded from multiple sites while rats were exposed to auditory steady-state white noise clicks, presented at a range of frequencies (10-50Hz). Recordings were made from three separate drug-free sessions, then rats were administered MK-801, an NMDAR antagonist, over three separate sessions at escalating doses (0.1, 0.3, 0.5mg/kg). Oscillatory power and inter-trial coherence were extracted for each stimulus frequency. Auditory steady-state responses (aSSRs) were elicited to all frequencies of auditory stimulation, but the strongest responses were observed to 50Hz stimulation over frontal sites. MK-801 dose-dependently reduced aSSR oscillatory power of high-frequency (>30Hz) aSSRs. Rats exposed to MIA exhibited a reduction in frontal 50Hz aSSRs, similar to that seen in control rats exposed to a low dose (0.1mg/kg) of MK-801. MIA in rats altered the trajectory of brain development, affecting systems responsible for the generation of high-frequency gamma oscillations. MK-801, a NMDAR antagonist that also indirectly impairs inhibitory neurotransmission also was found to reduce 50Hz oscillatory activity, perhaps indicating that both MK-801 and MIA are affecting similar neurobiological pathways. Further experiments will focus on the cognitive and neurobiological correlates of MIA-associated reductions in gamma oscillations, with the ultimate goal of furthering our understanding of the neurobiology of cognitive impairments in schizophrenia.

Disclosures: L.R. Harms: None. **D.M. Hodgson:** None. **W.R. Fulham:** None. **A.S.W. Wong:** None. **M. Penttonen:** None. **U. Schall:** None. **J. Todd:** None. **P.T. Michie:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NHMRC project grant APP1026070

Title: Investigation of schizophrenia-related behavioural phenotypes following early versus late gestation maternal immune activation in a rat model

Authors: *C. L. MEEHAN[1], J. FROST[1], L. R. HARMS[1], D. M. HODGSON[1], U. SCHALL[2], J. TODD[1], K. ZAVITSANOU[3], C. SHANNON-WEICKERT[3], P. MICHIE[1]

[1]Univ. of Newcastle, Sch. of Psychology, [2]Sch. of Med. and Publ. Hlth., Univ. of Newcastle, Callaghan, Australia; [3]Sch. of Psychiatry, Univ. of New South Wales, Sydney, Australia

Abstract: Maternal immune activation (MIA) during gestation has been identified as a risk factor for schizophrenia. Evidence from a mouse model suggested that MIA in late gestation promoted schizophrenia-related cognitive dysfunction and altered NMDA receptor expression, whereas activation during early gestation was associated with changes in behaviours related to dopamine neurotransmission. The current study aimed to determine whether MIA in rats results in a reliable model of schizophrenia, and if MIA during different stages of gestation preferentially alters either dopaminergic or NMDA-related neurotransmission as observed in the mouse model. Wistar rats were administered with either 4.0mg/kg of PolyI:C or saline on either gestational day 10 (early) or 19 (late). Prepulse inhibition (PPI) of the acoustic startle response, working memory on an operant delayed non-match to position task and locomotion in response to MK-801 and amphetamine were examined in male and female adult offspring. Female rats exposed to MIA during late gestation had impaired working memory, exhibiting a reduction in the proportion of correct responses in comparison to controls in a one second delayed non-match to position task ($p = .01$). A similar trend was also seen in late gestation males, however this difference was not significant. No differences were seen at longer delays indicating that deficits in working memory are not delay-dependent. MIA during early or late gestation did not produce observable effects on MK-801- and amphetamine-induced locomotion, nor did it affect PPI of the acoustic startle response. The lack of any significant changes in the dopamine related behaviours of PPI and amphetamine-induced locomotion indicate that this rat model of MIA does not replicate the perturbed dopaminergic phenotype previously seen in the mouse model, and other rat models in which MIA was induced at different developmental time-points. Therefore, a later gestational time-point may be needed to induce changes in dopamine-related behaviours. The presence of non-delay dependent working memory deficits suggests that MIA in late gestation, in females more so than males, produces a model of schizophrenia-related cognitive impairments.

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Poster

230. Systems-Oriented Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Intramural Research Program

Title: Altered cortical neuronal avalanches in a rat model of schizophrenia

Authors: *S. SESHADRI, D. PLENZ
NIMH, Bethesda, MD

Abstract: Schizophrenia (SZ) is a psychiatric disorder whose pathophysiology is thought to involve impaired cortical balance of excitation and inhibition (E/I), as well as altered dopaminergic signaling, which may underlie cognitive symptoms such as working memory deficits. The resting state activity of cortex in humans, non-human primates, and rodents has been shown to be composed of neuronal avalanches, i.e. spontaneous spatiotemporal cascades of synchronized activity that exhibit a precise organization, described by power laws. This suggests that resting state dynamics of cortex are critical, which theory and experiment indicate is beneficial for information processing. Avalanche dynamics are highly sensitive to the E/I balance and exhibit an inverted U-shaped profile of dopaminergic dependency similar to that identified for working memory function. We therefore hypothesized that network-level disturbances in SZ may correlate with deviations from neuronal avalanche dynamics. To address this, we used the neonatal phencyclidine (PCP) model of SZ. Rat pups were injected with PCP (s.c., 10 mg/kg) at postnatal day 7, 9, and 11, and subjected to behavioral testing at 4-6 weeks. In confirmatory behavioral tests, PCP-treated rats showed hyperlocomotion in response to a PCP challenge and impairment in novel object recognition. Avalanches were first studied at the population level by recording the local field potential in layer 2/3 (L2/3) of frontal cortex of adult rats under anesthesia (1.5% isoflurane) using microelectrode arrays (MEAs, 8x4 electrodes, NeuroNexus). Ongoing LFP activity deviated from criticality, exhibiting dynamics consistent with a supercritical regime of propagation in neonatal PCP treated rats (kappa, PCP: 1.150, control: 0.934, $p=0.030$; sigma, PCP: 1.769, control: 0.825, $p=0.038$; N=7 rats). We plan to extend these results in awake, behaving rats by chronically implanting MEAs into L2/3 of cortex, and recording LFP at rest and during visual and spatial working memory tasks. Deviations in avalanche dynamics will be assessed in different phases of the task (e.g. habituation, delay, test) and correlated with performance. We found that neonatal PCP-treated rats display behavioral deficits and abnormal neuronal avalanche dynamics. These results suggest that studying disruption of neuronal avalanches in disease states may be a translatable tool for psychiatric disorders.

Disclosures: S. Seshadri: None. **D. Plenz:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

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Program#/Poster#: 230.17/U29

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NHMRC Project Grant APP1026070

Title: The effects of the maternal immune activation and MK-801 models of schizophrenia on mismatch negativity-like responses in awake, freely-moving rats

Authors: *L. R. HARMS[1], D. M. HODGSON[1], W. R. FULHAM[1], M. PENTTONEN[2], U. SCHALL[1], J. TODD[1], P. T. MICHIE[1]
[1]The Univ. of Newcastle, Callaghan, Australia; [2]Univ. of Jyväskylä, Jyväskylä, Finland

Abstract: Reductions in the amplitude of mismatch negativity (MMN) to rare, unexpected (deviant) auditory stimuli are one of the most robust neurophysiological changes observed in patients with schizophrenia and are thought to be critically dependent on glutamate n-methyl-d-aspartate receptor (NMDAR) function. In this study, we aim to establish a rat model of schizophrenia-related MMN impairments by using a novel rodent EEG recording system to assess MMN in rats exposed to pharmacological (MK-801, an NMDAR antagonist) and developmental (Maternal Immune Activation, MIA) interventions known to model schizophrenia. Pregnant rats were administered the viral mimic Poly (I:C) (4mg/kg) or saline at gestational day (GD) 19. In adulthood, stainless steel screw electrodes were surgically implanted over five cortical locations in MIA and control offspring (n=7-10). After recovery, a wireless transmitter was attached to the recording electrodes and EEG was recorded while the rats were exposed to two oddball sequences (either a high- or low-frequency deviant), and a control sequence of randomly-intermixed frequencies that controlled for adaptation. Rats were tested on each sequence during three 'baseline' (drug-free) sessions, then tested on three successive sessions after progressively escalating doses of the NMDAR antagonist MK-801 (0.1, 0.3 and 0.5mg/kg), each dose separated by at least 4 days. Five components of the auditory event-related potential (ERP) were identified: two early positive components, (P13, P30), an early negative component (N18), and two later broad negative peaks (N-shift1, N-shift2). Adaptation-independent deviance-detection was observed in control rats for high frequency, but not low frequency deviants on both early and late ERP components. MK-801 dose-dependently increased adaptation-independent deviance detection on early components, but reduced deviance detection for N-shift1. MIA increased deviance detection on early components as well, but did not reduce deviance detection on N-shift1. These findings indicate we have established a reliable rat model

of MMN, evident in the late negative peak, N-shift1, which exhibits sensitivity to reduction by NMDAR antagonists as human MMN does. However, we have also identified an unexpected increase in deviance detection in early ERP components in response to both MIA and the NMDAR antagonist. These findings suggest that the neurobiological underpinnings of deviance detection may not be conserved from early to late stages of auditory processing.

Disclosures: L.R. Harms: None. **D.M. Hodgson:** None. **W.R. Fulham:** None. **M. Penttonen:** None. **U. Schall:** None. **J. Todd:** None. **P.T. Michie:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

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Program#/Poster#: 230.18/U30

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH077235

MH97879

Title: Contribution of a novel neuronal inhibitor to the cellular and behavioral deficits observed in a mouse model of 22q11.2 microdeletion-related schizophrenia

Authors: *A. DIAMANTOPOULOU[1], B. XU[1], Z. SUN[1], K. FÉNELON[2], M. KARAYIORGOU[1], J. A. GOGOS[1]
[1]Columbia Univ., New York, NY; [2]Univ. of Texas at El Paso, El Paso, TX

Abstract: Adult Df(16)A+/− mice, a model of 22q11.2 microdeletion associated SCZ, show significant behavioral and cognitive deficits, especially in prepulse inhibition (PPI), working memory (WM) and fear conditioning tasks, accompanied with abnormalities in dendritic morphogenesis and formation of dendritic spines of hippocampal and cortical pyramidal neurons. Such abnormalities can be linked to aberrant processing of brain microRNAs (miRNAs), a result of the 22q11.2 microdeletion. Recently, Mirta22, a previously uncharacterized gene, was identified as a major miRNA target mediating the effects of the 22q11.2 microdeletion on neuronal maturation and connectivity. Elevated Mirta22 levels found in brains of Df(16)A+/− mice have been shown to inhibit dendritic and spine development in Df(16)A+/− neurons, both *in vitro* and *in vivo*, whereas reduction of abnormally increased Mirta22 levels restores structural abnormalities in the HPC of Df(16)A+/− mice. We hypothesized that such restoration would

impact behavior, leading, at least, to partial rescue of behavioral abnormalities. For that reason, we generated a Df(16)A+/-; Mirta22 +/- compound heterozygote (HET) strain. Reduction of the dosage of the Mirta22 gene by half, to almost WT levels, in the compound HET strain will be used to evaluate the contribution of this miRNA target to the behavioral phenotypes induced by the 22q11.2 deletion. Indeed, Df(16)A+/-;Mirta22+/- mice show significantly increased PPI levels, when compared to their Df(16)A+/- littermates, which reach the level of WTs, while the Df(16)A+/- PPI deficit was consistently reproduced. Normalizing Mirta22 levels in Df(16)A+/-;Mirta22+/- was sufficient to significantly increase working memory performance in a T-maze task, in which Df(16)A+/- mice have a prominent impairment. This result suggests that at least some of behavioral deficits observed in Df(16)A+/- mice can be attributed to the inhibitory influence of Mirta22 upregulation and that Mirta22 gene is a key regulator of the brain circuitry that controls PPI and WM. Also it points out that restoring normal Mirta22 levels in the brains of Df(16)A+/- mice can prevent structural changes governed by Mirta22 overexpression which would result in local and long-distance disruptions of neuronal communication. In fact, behavioral phenotyping of Mirta22-/- mice, revealed significant deficits in various domains, ranging from abnormal novelty exploration, startle reactivity and diminished anxiety to substantial increase in fear memory, compared to WT, advocating for a significant biological role of the Mirta22 protein in regulating appropriate behavioral responses.

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Poster

230. Systems-Oriented Models of Schizophrenia

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 230.19/U31

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Striatonigral contribution to individual variability in susceptibility to chronic haloperidol-induced vacuous chewing movements

Authors: *S. E. BACHUS[1], C. BLANCHARD[2]
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Abstract: We have previously found that the elevation of striatal enkephalin mRNA by chronic haloperidol, in rats, is not significantly correlated, across individuals, with variability in emergence of vacuous chewing movements (VCM) (Blanchard & Bachus, SfN Abstr. 2011), a

model for tardive dyskinesia. Here, the possibility that the "direct" striatonigral pathway might be involved in the induction of vacuous chewing movements was evaluated by exploration of striatal D1 receptor and dynorphin (DYN) mRNA levels in this same cohort. Group housed male Long-Evans rats, with starting body weights of 90-165g, were treated for 24 weeks with HAL (28.5mg/kg/ml, i.m.: n=43) or vehicle (sesame oil: n=21) injections every 3 weeks. VCM were counted for each rat for 2 minutes weekly, for 7 weeks prior to HAL treatment and then throughout HAL treatment, by observers blinded to treatment group. Over the final 2 weeks, 4 samples were rated under both quiet (only ambient air-conditioning) and noisy (constant loud music and key-rattling) conditions. Cryostat-cut sections from fresh-frozen brains, containing neostriatum and nucleus accumbens, were then assayed by *in situ* hybridization histochemistry with oligonucleotide probes complementary to D1 or preprodynorphin mRNA, or a mis-sense control probe. Neither D1 nor DYN mRNA in any striatal region was significantly altered by chronic haloperidol. Nor were D1 and DYN mRNA levels significantly correlated with each other, in either the controls or the haloperidol-treated rats. D1 mRNA was not significantly correlated with VCM under either quiet or noisy conditions. In contrast, DYN mRNA was significantly positively correlated with VCM under noisy conditions, though not under quiet conditions, in all neostriatal regions and nucleus accumbens, in the haloperidol-treated rats. DYN mRNA was also significantly positively correlated with previously measured nigral GAD67 mRNA in this cohort, in the haloperidol-treated rats, but not in vehicle controls. Conversely, significant positive correlations between striatal DYN mRNA and previously measured paraventricular hypothalamic CRF mRNA and nigral D2 dopamine receptor mRNA, in controls, were abolished in haloperidol-treated rats. These results add to evidence suggesting that differential effects on striatonigral neurons, secondary to striatal D2 receptor upregulation by chronic haloperidol, may play a role in the determination of individual vulnerability to the development of vacuous chewing movements, and, by analogy, tardive dyskinesia.

Disclosures: S.E. Bachus: None. **C. Blanchard:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Danish Agency for Science, Technology and Innovation

Title: 15q13 microdeletion syndrome - characterization of homozygous knockout mice

Authors: *A. FORSINGDAL[1,2], J. NIELSEN[1]
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Abstract: Genome wide association studies have revealed that certain copy number variants (CNVs) strongly increase the risk of schizophrenia and other psychiatric diseases. One such CNV is a 1.5 MB long hemizygous deletion located in the 15q13.3 region. Hemizygous 15q13 microdeletion increases the risk of schizophrenia, epilepsy and autism by several fold (Ben-Shachar et al., 2009). A mouse model of the human 15q13 hemizygous microdeletion has recently been generated by deleting the homologous genes on the mouse chromosome 7. Characterization of the model identified disease-related phenotypes (Fejgin et al., 2013). However, the phenotypes observed in the hemizygous mouse model are relatively subtle. Subtle alterations are not unexpected in the hemizygous model as the penetrance is also variable in human hemizygous deletion carriers (Ben-Shachar et al., 2009). Human cases of homozygous microdeletion carriers have also been reported, all with severe impairments (Hoppman-Chaney et al., 2013). These patients suffer from seizures, severe mental retardation, major motor symptoms, hypotonia, developmental delay and other deficits. The present study is a basic characterization of 15q13 homozygous knockout mice. The first tests show that these mice recapitulate some of the phenotypes seen in the human homozygous deletion carriers, namely hypotonia and decreased size, but they do not appear to be as strongly impaired as the human cases. Further characterization of the 15q13 homozygous knockout mice is ongoing. **References** Ben-Shachar S et al. (2009) Microdeletion 15q13.3: a locus with incomplete penetrance for autism, mental retardation, and psychiatric disorders. J Med Genet 46:382-388 Fejgin K, Nielsen J, Birknow MR, Bastlund JF, Nielsen V, Lauridsen JB, Stefansson H, Steinberg S, Sorensen HBD, Mortensen TE, Larsen PH, Klewe I V, Rasmussen S V, Stefansson K, Werge TM, Kallunki P, Christensen K V, Didriksen M (2013) A Mouse Model that Recapitulates Cardinal Features of the 15q13.3 Microdeletion Syndrome Including Schizophrenia- and Epilepsy-Related Alterations. Biol Psychiatry:1-10 Hoppman-Chaney N, Wain K, Seger P, Superneau D, Hodge J (2013) Identification of single gene deletions at 15q13.3: further evidence that CHRNA7 causes the 15q13.3 microdeletion syndrome phenotype. Clin Genet 83:345-351

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Poster

230. Systems-Oriented Models of Schizophrenia

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the National MS Society

Title: Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia

Authors: J. MUNIR[1], J. BATES[1], S. SCHANZ[1], S. WANG[1], *M. S. WINDREM[1], R. FINDLING[2], P. TESAR[3], S. A. GOLDMAN[1]
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Abstract: Recent genetic and neuroradiological studies have suggested a role for glia, and oligodendrocytes in particular, in the pathogenesis of schizophrenia. To assess the potential role of glia in the ontogeny of schizophrenia, we established human glial chimeric mice using glial progenitor cells produced from iPSC cells derived from patients with juvenile onset schizophrenia, or from their age- and gender-matched controls. We did so by neonatally implanting donor iPSC GPCs, derived from schizophrenic patients or their normal controls, into either normally-myelinated immunodeficient rag1-/- hosts, in which implanted GPCs remain as progenitors or become astrocytes, or into hypomyelinated and immune deficient shiverer mutant MBPshi/shi x rag1-/- mice, in which the donor GPCs also develop as myelinating oligodendrocytes. We found that by 18 weeks of age, the schizophrenic patient-derived iPSC GPCs exhibited less white matter engraftment than their matched controls, migrating prematurely and aberrantly into the overlying cortical gray matter. These schizophrenia-derived iPSC GPCs were also more proliferative, as measured by both Ki67 and BrdU. Although individual schizophrenia-derived hiPSC GPCs differentiated as normal myelinogenic oligodendroglia within the callosum and capsules, the excessive cortical influx and hence lower density of GPCs in the white matter of schizophrenic hiPSC GPC-engrafted mice, resulted in the latter's overt hypomyelination, relative to mice engrafted with control-derived hiPSC GPCs. Remarkably, we also noted that when schizophrenia-derived hiPSC GPCs were neonatally transplanted into normally-myelinated mice, yielding GPC and astrocytic chimerization of the recipient brains, that the resultant glial chimeric mice manifested overtly abnormal behavioral phenotypes relative to control-engrafted mice. The mice engrafted with schizophrenic-derived hiPSC GPCs exhibited diminished prepulse inhibition, and when evaluated at 8 months, higher anxiety in the elevated plus maze, less interest in stranger mice in the 3-chamber social test, and

socially avoidant behavior in free interaction with a stranger mouse, all as analyzed by ICY software. These data suggest a significant contribution of cell-autonomous glial pathology to the genesis and development of juvenile-onset schizophrenia.

Disclosures: J. Munir: None. **J. Bates:** None. **S. Schanz:** None. **S. Wang:** None. **M.S. Windrem:** None. **R. Findling:** None. **P. Tesar:** None. **S.A. Goldman:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 230.22/U34

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Singapore MOE2012-T2-1-021

Duke-NUS Signature Research Program (SRP) Phase 2 Research Block Grant

Title: Regulation of BDNF exocytosis and GABAergic interneuron synapse by the schizophrenia susceptibility gene, dysbindin-1

Authors: *H. S. JE[1], Y. QIANG[2], N. HUSAIN[2], Y. XIAO[2], W. HAN[3]
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Abstract: Genetic variations in the dystrobrevin binding protein 1 (DTNBP1, dysbindin-1) have been implicated as risk factors in the pathogenesis of schizophrenia. The encoded protein, dysbindin-1, functions in the regulation of synaptic activity and synapse development. Intriguingly, a loss of functional mutation in dysbindin-1 in mice disrupted both glutamatergic and GABAergic transmission in cerebral cortex- cortical pyramidal neurons showed enhanced excitability due to reduction of inhibitory synaptic inputs and inhibitory neurons. However, the underlying mechanism of inhibitory synaptic deficits due to reduced dysbindin-1 remains unknown. In this study, we identified the unexpected role of dysbindin-1 in the exocytosis of brain-derived neurotrophic factor (BDNF) from excitatory pyramidal neurons. And this reduction of BDNF exocytosis in excitatory neurons trans-synaptically reduced the number of inhibitory synapses formed on excitatory neurons. Furthermore, exogenous BDNF rescued inhibitory synaptic deficits due to reduced dysbindin-1 in cultured neurons and *ex vivo* cortical slices. Taken together, our result demonstrates that the two, well-known schizophrenia

susceptibility gene products (BDNF and dysbindin-1) function together at the protein level to regulate interneuron development and cortical network activity. Furthermore, our results provide considerable insight into the cellular and molecular mechanisms that regulate the development of the neural circuitry in the brain, and to link abnormalities in BDNF secretion to cognitive disease.

Disclosures: H.S. Je: None. **Y. Qiang:** None. **N. Husain:** None. **Y. Xiao:** None. **W. Han:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

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Program#/Poster#: 230.23/U35

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant

Title: Pathologically accumulated GAPDH in the lysosomes is associated with augmented autofluorescence in cells from patients with schizophrenia

Authors: *A. RAMOS[1], T. TSUJIMURA[1], T. SAITOH[2], F. EMILIANI[1], C.-Y. LIN[1], N. J. GAMO[1], M. KOGA[1], T. MASEDA[1], T. G. SEDLAK[1], C. KORTH[3], Y. HORIGUCHI[4], K. TAGUCHI[4], K. ISHIZUKA[1], A. SAWA[1]
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Abstract: We find increased cellular autofluorescence (AF) in lymphoblasts from patients with schizophrenia (SZ), compared to those from matched controls. In addition, two animal models relevant for mental disease, EAAC1 knockout and dominant-negative DISC1 transgenic mice, reveal elevated AF (see the abstract by Tsuyoshi Tsujimura et al, SFN 2014). In the present study, we explore a possible mechanism that underlies this cellular phenotype. To address this question, we tested compounds that may correct AF in SZ cells. Among several tested, a novel compound that selectively interfered with the GAPDH-Siah1 binding was effective in attenuating increased AF in SZ. Based on the emission wavelength of AF elevated in SZ cells, we hypothesized that lipofuscin mediates the AF. To address this question, we examined lymphoblasts from SZ patients and those from normal controls in a comparative manner with

electron microscopy (EM), and observed lipofuscin-like accumulation in lysosomes of SZ cells but not control cells. Given that GAPDH is reportedly a protein component of lipofuscin, we are currently testing immuno-EM with an anti-GAPDH antibody to validate a specific accumulation of GAPDH in lysosomes of SZ cells. The final question we are now addressing is the biological function of lysosomal GAPDH in the pathophysiology of SZ.

Disclosures: **A. Ramos:** None. **T. Tsujimura:** A. Employment/Salary (full or part-time):; Tsuyoshi Tsujimura is a employee of Dainippon Sumitomo Pharma Co., Ltd.. **T. Saitoh:** None. **F. Emiliani:** None. **C. Lin:** None. **N.J. Gamo:** None. **M. Koga:** None. **T. Maseda:** None. **T.G. Sedlak:** None. **C. Korth:** None. **Y. Horiguchi:** None. **K. Taguchi:** None. **K. Ishizuka:** None. **A. Sawa:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 230.24/U36

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Age-dependent decrease in Parvalbumin interneuron density and in inhibitory transmission in hippocampal area CA2 in the 22q11.2 mouse model of schizophrenia

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[1]Univ. Paris Descartes, CNRS8118, Paris, France; [2]Neurosci., Columbia Univ., New York, NY

Abstract: An imbalance between excitation and inhibition is observed in several pathologies. More specifically, a reduction in interneuron number has been observed in area CA2 in the hippocampi of schizophrenic patients in post-mortem studies. Our previous work has demonstrated that inhibition in area CA2 of the hippocampus can act as a gate to control the ability of CA3 to excite CA2 neurons, and thus alter information flow. Motivated by findings in human tissue, we asked whether inhibition in area CA2 might also be affected in a mouse model of schizophrenia. The highest known factor for developing schizophrenia is the 22q11 deletion syndrome. The mouse model of the 22q11 deletion syndrome, the Df(16)A+/- mice, recapitulate many of the behavioral deficits and neuroanatomical changes observed in 22q11 deletion carriers. We found that the Df(16)A+/- mice have fewer Parvalbumin (PV) expressing interneurons in CA2, with no differences found in areas CA1 and CA3. In addition, inhibitory transmission in CA2 recruited by CA3 inputs was decreased while excitatory transmission from

cortical inputs and from CA3 was not altered. Interestingly, similar to disease onset in humans, these differences did not manifest until the mice were between 8 and 12 weeks old. Furthermore, CA2 pyramidal cells of Df(16)A+/- were more hyperpolarized and had a lower membrane resistance. As a consequence, CA2 pyramidal cells in Df(16)A+/- mice displayed fewer action potentials in response to CA3 or cortical input stimulation. Finally, the decrease in inhibition in Df(16)A+/- mice impaired the activity-dependent increase in the excitatory drive between CA3 and CA2 resulting from a long-term depression of PV inhibitory synapses in CA2. These results indicate that both information transfer and synaptic plasticity are altered in Df(16)A+/- mice. Thus, this mouse line is a promising model for examining the cellular mechanisms underlying the changes occurring in the hippocampus during the onset of schizophrenia in humans.

Disclosures: V. Chevaleyre: None. **R.A. Piskorowski:** None. **J. Mukai:** None. **S.A. Siegelbaum:** None. **J.A. Gogos:** None.

Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

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Program#/Poster#: 230.25/V1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: A novel function for matrix metalloproteinases in animal models of mood disorders and schizophrenia

Authors: *K. ARDHANAREESWARAN[1,2,3,4], N. WELTY[2], H. DUYTSCHAEVER[2], B. LORD[2], J. KANERVA[2], A. BITTNER[2], L. VER DONC[2], M. LETAVIC[2], P. BONAVENTURE[2], G. CHEN[2], T. LOVENBERG[2], J. R. SHOBLOCK[2]
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Abstract: Major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia together affect over twenty million American adults in a given year. Matrix metalloproteinases (MMP's) are a family of zinc-dependent protease enzymes that degrade extracellular matrix proteins and process bioactive molecules into mature forms. Of these MMP's, MMP-9 is one of the most abundant in the brain and is involved in synaptic plasticity and long term potentiation, modulation of network connectivity, neurogenesis, and neurite growth. Furthermore MMP-9

transcription is induced by growth factors and cytokines during events that require plasticity and remodeling. The aim of the present study was to determine the effects of a highly selective and potent inhibitor of MMP-9, Compound A, in rodent models of various psychiatric illnesses. Compound A attenuated urine sniffing and escape deficits in the learned helplessness model in rats, decreased immobility time in the tail suspension test in a lipopolysaccharide (LPS)-induced model of depression, partially reversed amphetamine-disruption of prepulse inhibition, attenuated morphine-induced hyperactivity, and attenuated naloxone-induced dysphoria as measured by conditioned place aversion and escape jumping. Furthermore, preliminary data from an angiogenesis protein array and microarray showed that Compound A blunted the increases in Il-10, GM-CSF, PDGF-AA, Fractalkine, MIP-1a, and Il-1a in plasma caused by LPS as well as attenuated several transcriptional responses to LPS in frontal cortex. Hence, the data suggest that Compound A exerts its antidepressant-like, antipsychotic-like, and mood-stabilizing effects in animal models through its anti-inflammatory actions or effects on plasticity in the CNS. These data highlight MMP-9 inhibitors as a class of possible therapeutic targets.

Disclosures: **K. Ardhanareeswaran:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **N. Welty:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **H. Duytschaever:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **B. Lord:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **J. Kanerva:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **A. Bittner:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **L. Ver Donc:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **M. Letavic:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **P. Bonaventure:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **G. Chen:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **T. Lovenberg:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D. **J.R. Shoblock:** A. Employment/Salary (full or part-time):; Employee of Janssen R&D.

Poster

230. Systems-Oriented Models of Schizophrenia

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 230.26/V2

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH084906

Title: Prefrontal cortex and dopamine single neuron spike rates and LFP phase represent time over distinct scales

Authors: *B. MOGHADDAM[1], N. K. B. TOTAH[2]
[1]Univ. Pittsburgh, Pittsburgh, PA; [2]Physiol. of Cognitive Processes, Max Planck Inst. for Biol. Cybernetics, Tübingen, Germany

Abstract: Expectations are generated on multiple timescales. During behavioral planning and working memory utilization, expectations are constructed on the multi-second level (sec), whereas expectations can be externally guided at the sub-second level by rhythmic stimuli (msec). Estimation of multi-second intervals has been associated with monotonic, "climbing" changes in neuron spike rate. On the other hand, sub-second expectations have been associated with rhythmic fluctuations of neuronal excitability in relation to phase of mesoscopic signals. It is not known if changes in gradual spike rate and phase-modulation of neuronal excitability co-occur during multi-second expectations. We studied this question by measuring spike rate, across-trial phase consistency, and phase locked modulations of excitation during a multi-second stimulus expectation task. We recorded single unit spiking and local field potentials from 3 rodent brain regions that have been implicated in time interval estimation, as well as in expectancy and behavioral planning: the prefrontal cortex (PFC), the anterior cingulate cortex (ACC), and the dopamine-producing ventral tegmental area (VTA). A stimulus-evoked phase reset in the PFC, ACC and VTA was observed, which may allow adaptive coding by these neurons and underlie their necessity for behavioral flexibility. In contrast, intrinsic across-trial phase consistency was not observed and phase modulations of excitation did not change in a climbing pattern. These data suggest that expectation over multi-second time intervals is better represented by climbing activity of single dopamine and PFC neurons as compared to mesoscopic signal phase.

Disclosures: B. Moghaddam: None. **N.K.B. Totah:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.01/V3

Topic: C.17. Drugs of Abuse and Addiction

Support: U54DA031659

Title: Nicotine, even at low doses, suppresses body weight gain independent of food intake

Authors: *L. RUPPRECHT, T. T. SMITH, R. L. SCHASSBURGER, D. M. BUFFALARI, E. C. DONNY, A. F. SVED
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Abstract: The ability of nicotine (NIC) to suppress food intake and body weight (BW) is cited as a factor impacting smoking initiation and the failure to quit. Smokers have reduced food intake, but it is unknown whether NIC reduces BW when food intake is constant and restricted. Furthermore, the FDA is considering a policy of markedly reducing the allowable NIC levels in cigarettes; such a reduction could have a detrimental effect on BW. To model new smokers of reduced NIC content cigarettes, we assessed the effects of a range of self-administered (SA) doses of NIC on BW gain in food-restricted rats. Adult rats restricted to 20 g chow/day SA intravenous infusions of NIC (0, 3.75, 7.5, 15, or 60 μg/kg) in daily 1-h sessions. After 20 days, NIC dose-dependently suppressed BW. A low dose of NIC (3.75 μg/kg/infusion) that results in relatively little daily intake of nicotine suppressed BW compared to saline. We modeled NIC reduction in current smokers in a separate group of food-restricted rats that SA 60 μg/kg/infusion NIC before immediate reduction (1.875 or 3.75 μg/kg/infusion) or a gradual stepped dose reduction (dose halved every 10 days to 1.875 μg/kg/infusion). Both immediate and gradual reductions resulted in BW significantly greater than constant 60 μg/kg NIC. Reduction of NIC in cigarettes to a dose that will not maintain smoking will likely cause significant weight gain in current smokers. However, in new smokers low NIC levels may still restrict BW, possibly motivating continued use and maintaining exposure to other harmful chemicals in cigarettes.

Disclosures: L. Rupprecht: None. **T.T. Smith:** None. **R.L. Schassburger:** None. **D.M. Buffalari:** None. **E.C. Donny:** None. **A.F. Sved:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.02/V4

Topic: C.17. Drugs of Abuse and Addiction

Title: Self-administration of cigarette smoke extract vs. nicotine alone in adolescent and adult male rats

Authors: *C. GELLNER, H. R. SMITH, S. L. DO, P. KAUR, S. SALSABILIAN, J. M. L. DEGUZMAN, J. D. BELLUZZI, F. M. LESLIE
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Abstract: To challenge the current animal model of using nicotine alone to study tobacco dependence, we have established a model of intravenous self-administration of cigarette smoke extract (CSE). Previous research in our lab has shown that adult male rats will not only self-administer CSE but that they find it more reinforcing than nicotine alone at the 7.5µg/kg per infusion dose. We now test the hypothesis that CSE will be even more reinforcing in adolescence, the age at which most humans initiate tobacco use. Adolescent male rats (aged postnatal day 25, P25) and adults (P85) were trained to work for food pellets on an FR1TO20 schedule (1 pellet/ lever press, time out 20 seconds). After rats reached the reinforced lever press threshold (R=35 and 50 for adolescents and adults, respectively), they underwent surgery in which a catheter was implanted into the right jugular vein. After three days recovery, rats (P37 and P97) underwent three progressively harder schedules of lever pressing: FR1TO20, FR2TO20, and FR5TO20 for one of 5 doses of CSE or nicotine (Nic) (0, 3.75, 7.5, 15, or 30 µg/kg per infusion). The results show that both adolescent and adult male rats will self-administer CSE and Nic at all test doses. Preliminary results also suggest that there are no differences in responding for CSE and Nic at either age, but that adolescents show a dose-related increase in drug intake as well as a dose-related increase in non-reinforced responding. . These findings suggest that age and dose are important factors in tobacco dependence models.
Keywords: adolescence, tobacco dependence, nicotine, cigarette smoke extract

Disclosures: C. Gellner: None. **H.R. Smith:** None. **S.L. Do:** None. **P. Kaur:** None. **S. Salsabilian:** None. **J.M.L. Deguzman:** None. **J.D. Belluzzi:** None. **F.M. Leslie:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

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Program#/Poster#: 231.03/V5

Topic: C.17. Drugs of Abuse and Addiction

Title: Rats that self-administer cigarette smoke extract are more sensitive to drug-induced relapse

Authors: *D. REYNAGA, J. BELLUZZI, F. LESLIE
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Abstract: Tobacco dependence is extremely difficult to treat and the vast majority of those who try to quit will relapse within the first year. The most effective pharmacological treatment for smoking cessation is varenicline, an α4β2 nicotinic receptor antagonist. However, only 23% of subjects treated with varenicline maintain abstinence for over a year. Although improvements in experimental models may result in better smoking cessation treatments, current preclinical tests largely use nicotine alone, ignoring the other ~8000 constituents also found in tobacco smoke. We have recently shown that rats which self-administer aqueous cigarette smoke extract (CSE), a solution of saline containing nicotine and other non-nicotine constituents, are more sensitive to stress-induced relapse to drug-seeking behavior than those that worked for nicotine alone (Costello et al., 2014). We have now examined whether rats that self-administered CSE are also more sensitive to drug-induced relapse. Adult male Sprague-Dawley rats were allowed to self-administer nicotine (15 µg/kg/inf) or CSE (15µg/kg/inf of nicotine content) under a fixed FR5 ratio for a minimum of 10 days or until self-administration was stabilized (reinforced responses (R) within 20% of the mean over 3 days; $R \geq 2 \times$ non-reinforced (NR) responses; $R \geq 6$). Animals then began extinction training, where drug and cues were removed, for a minimum of 5 days or until reinforced responding was reduced to 20% or less of that of the last day of drug self-administration. Animals were then tested for reinstatement of responding using the following conditions in a counterbalanced design: cues alone, a priming dose of nicotine or CSE alone (0.15mg/kg; i.p), or a priming dose of drug and cues combined. We found that animals that self-administered CSE reinstated more robustly than animals that self-administered nicotine alone ($F_{1,12}= 6.03$; $p = 0.03$), when given a priming dose of CSE alone ($p = 0.046$) and paired with the introduction of drug cues ($p = 0.028$). The results suggest that the non-nicotine constituents in cigarette smoke contribute to smoking craving and relapse. Keywords: cigarette smoke extract (CSE), drug-primed reinstatement, nicotine

Disclosures: **D. Reynaga:** None. **J. Belluzzi:** None. **F. Leslie:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.04/V6

Topic: C.17. Drugs of Abuse and Addiction

Title: Differential effects of tobacco smoke containing either high or low levels of nicotine during adolescence on novelty seeking and anxiety-like behaviors

Authors: *D. P. BRANCO, M. CORREA-SANTOS, C. C. FILGUEIRAS, C. C. CAVINA, V. F. NAIFF, A. C. MANHÃES, A. RIBEIRO-CARVALHO, Y. ABREU-VILLAÇA
Dept. de Ciências Fisiológicas, Univ. do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: There is a lack of experimental studies that investigate the effects of tobacco smoke exposure during adolescence. Here, we investigated the short- and long-term effects of tobacco smoke generated from cigarettes containing either high or low levels of nicotine on novelty seeking and anxiety-like behavior of adolescent mice. From postnatal day 30 to 45 (PN30-45), male and female Swiss mice were exposed to tobacco smoke (whole body exposure for 8 h/day, 7 days/week) generated from one of two reference research cigarettes: type 2R1F (HighNIC group - 1.74mg nicotine/cigt) or type 4A1 (LowNIC group - 0.14mg nicotine/cigt). Control mice were exposed to ambient air. By the end of the exposure period (PN45) we assessed the levels of cotinine (nicotine metabolite) in serum. During exposure (PN44-45), short- (PN49-50) and long-term deprivation (PN74-75), the elevated plus maze was used to assess anxiety-like behavior and the hole board to assess novelty-seeking. Only HighNIC mice presented detectable cotinine serum levels (109.1±24.0ng/ml). There were no differences in anxiety-like behavior among groups. Effects on novelty-seeking were largely dependent on the type of cigarette. HighNic mice presented increased novelty-seeking during exposure and a late-emergent females-only decrease in exploration during deprivation. Distinctively, LowNic mice presented reduced novelty-seeking both during exposure and short-term deprivation. Differential behavioral effects of tobacco smoke containing high or low nicotine levels are associated with differences in the compounds inhaled during exposure: In addition to relevant behavioral effects of nicotine, we suggest that smoke components other than nicotine play a role.

Disclosures: D.P. Branco: None. **M. Correa-Santos:** None. **C. C. Filgueiras:** None. **C. C. Cavina:** None. **V. F. Naiff:** None. **A. C. Manhães:** None. **A. Ribeiro-Carvalho:** None. **Y. Abreu-Villaça:** None.

Poster

231. Nicotine: Reward and Seeking

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Topic: C.17. Drugs of Abuse and Addiction

Support: Supported by the National Institute for Food and Drug Safety Evaluation Grant (S14042TBC9770E)(ESC), Korea.

Title: Phosphorylation of mGluR1/5 by protein kinases after nicotine challenge

Authors: *I. RYU, J. KIM, E. CHOE
Dept. of Biol. Sci., Pusan Natioanl Univ., Pusan / Keumjeong-Gu, Korea, Republic of

Abstract: Exposure to nicotine, a psychoactive component in tobacco, alters mesolimbic dopaminergic and glutamatergic pathways innervated to the nucleus accumbens (NAc). Our preliminary study shows that nicotine challenge (0.2, 0.4, or 1.0 mg/kg/day) after six days of withdrawal, followed by fourteen daily systemic injections of nicotine (0.2, 0.4, or 1.0 mg/kg/day), significantly increased both locomotor activity and stereotypy in a dose-dependent manner. In this study, therefore, we will unravel mechanisms involving nicotine challenge-induced relapse by investigating the hypothesis that nicotine challenge phosphorylates metabotropic glutamate receptor subtype 1 and 5 (mGluR1/5) through the activation of protein kinases, which we believe it contributes to behavioral changes.

Disclosures: I. Ryu: None. **J. Kim:** None. **E. Choe:** None.

Poster

231. Nicotine: Reward and Seeking

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA017949

Title: Acute nicotine delays extinction of contextual fear in mice

Authors: M. G. KUTLU, *T. J. GOULD
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Abstract: Smoking is linked to Post-Traumatic Stress Disorder (PTSD) which suggests smoking is either a risk factor or an attempt at self-medication. The ability to reduce or extinguish fear-related memories may be altered in patients with PTSD and it is possible that nicotine modulates this. Although there are numerous studies examining the effects of nicotine on acquisition of fear learning, the effects of nicotine on extinction of contextual fear are not well understood. In the present study, we examined the effects of acute nicotine (0.18 mg/kg) on extinction of contextual fear in C57BL/6J mice. Animals were first trained in a background contextual fear conditioning paradigm using a white noise as a conditioned stimulus (CS), which co-terminated with a 2 s

0.57 mA unconditioned foot-shock stimulus (US). Animals were then administered either nicotine or saline and exposed to either the training context or a novel context in order to measure freezing to the context during extinction. Our results demonstrate that nicotine administration during extinction delays extinction of contextual freezing while nicotine did not affect cued freezing or freezing to the novel context. These results suggest that initiation of smoking after a traumatic event may interfere with the extinction of fear response to a trauma-associated context by enhancing the recall of the contextual fear memory.

Disclosures: M.G. Kutlu: None. **T.J. Gould:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

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Program#/Poster#: 231.07/V9

Topic: C.17. Drugs of Abuse and Addiction

Title: The effect of appetitive interoceptive conditioning on nicotine reinforcement: A novel nicotine self-administration model

Authors: *S. CHARNTIKOV, S. PITTENGER, N. SWALVE, R. BEVINS
Dept of Psychology, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Nicotine is the primary addictive component of tobacco and is one of the active ingredients included in e-cigarettes. Notably, nicotine's reinforcing effects appear weak at best in animal models. Although understanding nicotine's reinforcing effects is of great importance, learning processes involving nicotine are likely to be more complex. There is a need to study this complexity in order to design more relevant and efficacious treatment strategies. The goal of this study was to test whether appetitive interoceptive conditioning with the nicotine stimulus in the initial self-administration stage would enhance nicotine's rewarding properties as revealed through later responding on a progressive ratio schedule of reinforcement. To this end, rats were first trained to self-administer nicotine using a variable ratio 3 (VR3) schedule of reinforcement. There were 24 nicotine self-administration sessions using right and left levers as a manipulanda. Thirty seconds after each earned nicotine infusion, one group of rats (n=13) received 4-sec access to liquid sucrose. The comparison group (n=15) was reinforced with nicotine on a VR3, but had no access to sucrose in the initial self-administration phase. The number of infusions was limited to 10 for all rats; this provided some control over nicotine intake between the two groups. In the second phase, the response manipulanda were switched to nose-pokes and relocated on the

opposite side of the self-administration chamber. The levers were removed, but the sucrose receptacle now inactive, remained on that opposite side wall from the nose-poke holes. Also, the schedule of reinforcement for each nicotine infusion was changed to a progressive ratio (PR). A PR schedule, with its increasing response requirement after each nicotine infusion, provides a good index of reinforcer value. Rats in both groups rapidly acquired nicotine self-administration, had comparable self-administration pattern of responding on the VR3, and comparable drug intake throughout the initial phase using levers as manipulanda. In the second phase, rats with a history of nicotine-sucrose pairings had significantly higher PR responding on the active nose-poke than rats in comparison condition that only received nicotine in the initial phase. Thus, for the first time, we show that appetitive interoceptive conditioning with the nicotine stimulus can enhance later nicotine self-administration suggesting that the early establishment of an appetitive nicotine - sucrose association enhances the reinforcing value of nicotine; an understudied factor that may play a role in the progression to nicotine dependence.

Disclosures: **S. Charntikov:** None. **S. Pittenger:** None. **N. Swalve:** None. **R. Bevins:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.08/V10

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA034389

Title: Individual differences in forced and free-choice novelty differentially predict nicotine sensitization behaviors

Authors: *A. M. FALCO, R. A. BEVINS
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Abstract: Work with traditional psychostimulants has reported a relation between response to novel environments and drug-induced behaviors. This relation is less clear when reactivity to a novel environment is correlated to nicotine-induced behaviors. The current research asked whether a set of tasks that included novelty in different forms predicted later locomotor sensitization to nicotine. Male Sprague-Dawley rats were first assessed in the individual differences screens. The screens were reactivity to an inescapable novel environment, consumption of a novel tastant (sucrose), consumption of a novel food in an unfamiliar

environment (L-maze), and approach and interaction with a novel object. Following screening, rats underwent a 10-day sensitization regimen. Rats were injected SC with either 0.4 mg base/kg nicotine or saline and placed in the locomotor chamber (dia. 30.5 cm) for 30 min (n=16/group). After sensitization, a drug-free test was conducted to determine whether the chamber evoked enhanced locomotion (conditioned hyperactivity). Subsequently, two nicotine (0.4 mg/kg) challenge tests were conducted to assess the lasting effects of nicotine sensitization. Locomotor sensitization developed in nicotine-treated rats. Context-evoked conditioned hyperactivity was seen in the drug-free test. Later challenge tests found that sensitization lasted up to 7 days without nicotine. For each individual difference screen, a median split was performed. Rats with performance above the median were defined as high responders (HR); rats below the median were low responders (LR). There were no locomotor differences between saline HR and LR rats. This was not so for nicotine-sensitized rats. HRs on in the inescapable novel environment significantly higher than LR nicotine-sensitized rats on day 10, conditioned locomotor test, and nicotine challenge I and II. Interestingly, the only other novelty-related behavior that consistently predicted the effects of nicotine was average duration of sniffing the novel object. For this screen, HRs were significantly lower than LRs for day 10, conditioned locomotor test, and nicotine challenges I and II. This experiment suggests that two types of novelty, forced and free-choice, predict nicotine sensitization differentially. This likely occurs via different mechanisms that have yet to be elucidated.

Disclosures: A.M. Falco: None. **R.A. Bevins:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

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Program#/Poster#: 231.09/V11

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA grant DA027840

Title: Neuroanatomic mapping of dopamine D1 receptor involvement in nicotine self-administration in rats

Authors: *B. J. HALL, S. SLADE, C. ALLENBY, E. D. LEVIN
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Abstract: The dopamine system is known to be critically involved in addiction to nicotine, as well other drugs of abuse. The dopaminergic projections from the VTA to the nucleus accumbens and prefrontal cortex have been well established to be critical to the reinforcing effects of these drugs. However, other projections of dopamine neurons are likely have significant roles in this process. In addition, the relative contributions of dopamine D1 and D2 receptors in drug addiction and its treatment remains to be fully understood. In a series of studies we have investigated a variety of different dopaminergic projections with regard to their involvement in nicotine self-administration in rats. The relative involvement of dopamine D1 receptors were studied in specific cortical and subcortical neuroanatomic brain regions including: nucleus accumbens shell (AcS), anterior cingulate cortex (ACC), and parietal association cortex (PAC). Young adult female Sprague-Dawley rats were fitted with jugular catheters and given access to self-administer nicotine (0.03 mg/kg) on an FR1 schedule of reinforcement. A bilateral infusion cannula was implanted in each respective brain region in separate cohorts of rats to allow local infusion of the DA D1 receptor antagonist SCH23390. Infusions of SCH23390 occurred 5 min prior to the start of self-administration sessions, and each session lasted 45 min. Doses of SCH23390 (1.0, 2.0, and 4.0 μg/side) were infused in a repeated measures, counterbalanced design two times. Bilateral infusions of SCH23390 into the AcS caused significant reductions in nicotine self-administration (measured as number of nicotine infusions per session) at all doses, compared to infusions of ACSF vehicle, while infusions into the PAC resulted in significant reductions at the 2.0 and 4.0 μg doses. Infusions of 4.0 μg/side SCH23390 into the ACC resulted in a slight reduction in nicotine self-administration but did not reach the level of significance. The results of this series of experiments demonstrates the importance of D1 dopamine receptors in the aquisition and maintenance of nicotine addiction, and also may show significant contributions to this process from previously overlooked neuroanatomical brain regions. This research was supported by P50 grant DA027840 from NIDA.

Disclosures: **B.J. Hall:** None. **S. Slade:** None. **C. Allenby:** None. **E.D. Levin:** None.

Poster

231. Nicotine: Reward and Seeking

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Program#/Poster#: 231.10/V12

Topic: C.17. Drugs of Abuse and Addiction

Support: DePauw University Asher Fund

Title: Serotonin receptor type1 function in nicotine-induced locomotor behavior

Authors: *H. SCHNEIDER, B. F. KOPECKY, N. J. SNYDER, S. OWIREDU, E. E. CLOR, S. INDIA-ALDANA, K. Y. CHEN
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Abstract: The serotonin system has been linked to the modulation of psychostimulant-induced behavior and could be targeted for the treatment of nicotine dependence, the number one cause of preventable diseases in the U.S. Neurobehavioral responses of zebrafish larvae to nicotine following treatment with agonists for various serotonin receptors could lead to the identification of new chemicals for smoking cessation therapy in patients. The goal of this study was to determine the action of the htr1a agonist (R)-(+)-8-hydroxy-DAPT, the partial htr1a agonist busprione and the htr1b agonist eletriptan on nicotine-induced motor behavior in zebrafish (Danio rerio) larvae at 5-7 days post fertilization (pdf) and in comparison to the acteylcholine receptor agonist varenicline, which is used successfully in smoking cessation therapy. Treatment with (R)-(+)-8-hydroxy-DAPT, busprione or eletriptan reduces spontaneous swimming activity and the acute nicotine-induced motor response. In control experiments, a reduced locomotor response to an irritant chemical is also observed in treated zebrafish larvae. In contrast, varenicline treatment reduces the nicotine-induced motor behavior but not the response to the irritant chemical. Together, these results suggest that treatment with (R)-(+)-8-hydroxy-DAPT, busprione or ETT reduces motor behavior of 5-7dpf larval zebrafish more broadly unlike the more specific action of varenicline. Genes of htr1a and htr1b are expressed by 5dpf in zebrafish larvae. The action of additional serotonin receptor agonists and antagonists will be explored in future studies.

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Poster

231. Nicotine: Reward and Seeking

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA027840

Title: Dextromethorphan interactions with the histaminergic H1 antagonist pyrilamine and the serotonergic 5HT2c agonist lorcaserin to reduce nicotine self-administration in rats

Authors: S. A. BRIGGS, C. WELLS, S. SLADE, M. MORRISON, P. JASKOWSKI, *J. E. ROSE, E. D. LEVIN
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Abstract: Tobacco addiction is a complex syndrome involving multiple interacting neural systems. A variety of drug treatments affecting different neurotransmitter receptors have been found to reduce nicotine self-administration. Combining effective treatments for smoking cessation may provide more effective treatments by affecting multiple points of control in the neural circuits underlying addiction. Previous studies in our laboratory have shown that the H1 histamine antagonist pyrilamine and the serotonin 5HT2c agonist lorcaserin significantly reduce nicotine self-administration in the rat model. In addition, dextromethorphan a drug with alpha3beta4 nicotinic and NMDA glutamate antagonist actions has been shown to decrease nicotine self-administration in the rat model. To explore ways to enhance success for reducing nicotine self-administration and provide new, more effective treatments to help smoking cessation, the current studies were conducted to determine the combined effects of dextromethorphan with pyrilamine or lorcaserin. Young-adult female rats were fitted with jugular IV catheters and trained to self-administer with a nicotine infusion dose of 0.03 mg/kg/infusion. In an initial dose-effect function study of dextromethorphan we found a monotonic decrease in nicotine self-administration over a dose range of 1 to 30 mg/kg with lowest effective doses seen at 3 mg/kg. In the combination studies rats were given varying doses of both dextromethorphan (3.3, and 10 mg/kg) and pyrilamine (4.43, and 13.3 mg/kg) or lorcaserin (0.3125 and 0.625 mg/kg) to test an acute dose-effect function and drug interactions. The saline vehicle was used as control. As seen before, pyrilamine significantly reduced nicotine self-administration. Dextromethorphan significantly augmented this effect. Also as seen previously, lorcaserin significantly reduced nicotine self-administration. This effect was also significantly augmented by dextromethorphan. Combined treatments affecting multiple components of the neural circuits underlying nicotine dependence may be useful to enhance success at smoking cessation.

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Poster

231. Nicotine: Reward and Seeking

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA020811

Title: Nicotine-suppressive properties of the high affinity α3β4 nAChR ligand AT-1001 are different than those of varenicline

Authors: *A. CIPPITELLI[1], J. WU[1], K. GAIOLINI[1], J. SCHOCH[1], G. BRUNORI[1], R. CICCOCIOPPO[2], N. ZAVERI[3], L. TOLL[1]
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Abstract: The α3β4 subtype of nicotinic acetylcholine receptors (nAChR) has been shown to play an important role in mediating nicotine reinforcement processes. A recently reported series of compounds was found to possess high affinity and selectivity for α3β4 nAChR when measuring binding affinity *in vitro*. One such compound, AT-1001, was also found to decrease nicotine self-administration in rats. Here we present *in vitro* studies that demonstrate that AT-1001 and AT-1012 are functionally selective as antagonists for α3β4 over α4β2 nAChR, but not to the same extent as the binding selectivity. Furthermore, these compounds have partial agonist activity at α3β4 nAChR. Additional experiments demonstrate that AT-1001 and varenicline, a partial agonist at α4β2 nAChR with binding but not functional selectivity *in vitro*, have very different *in vivo* properties, indicating distinct mechanisms of action. First, AT-1001 made available intravenously, did not exhibit reinforcing properties per se in fixed and progressive ratio reinforcement schedules, while varenicline (20 μg/kg) was self-administered. Secondly, systemic treatment with AT-1001 did not induce reinstatement of extinguished nicotine seeking but in fact attenuated activation of nicotine seeking induced by varenicline (0.15 mg/kg), as well as nicotine (0.15 mg/kg). Finally, AT-1001 selectively blocked nicotine self-administration without altering alcohol lever pressing as assessed in an operant co-administration paradigm. These findings describe a more complex AT-1001 *in vitro* profile than previously appreciated and provide further support for the potential of AT-1001 and congeners as clinically useful compounds for smoking cessation, with a mechanism of action distinct from currently available medications.

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Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

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Support: DFG SM80/2-2

DFG SM80/5-1

DFG SM80/5-2

DFG SM80/7-1

SFB 940/1

Title: Hippocampus goes depression: Structural and functional correlates of negative mood states after smoking cessation

Authors: *F. WUTTIG, N. B. KROEMER, C. BURRASCH, M. N. SMOLKA
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Abstract: Individuals with a history of major depressive disorder require more attempts to successfully quit smoking, and after quitting the risk for recurrence of depressive symptoms is increased. So far little is known about the neurobiological substrates of affective symptoms which often emerge within the first days after smoking cessation and last for several weeks. Using MRI, we examined structural and functional changes in emotional circuits subsequent to smoking cessation. We hypothesized that smoking cessation should preferentially affect structure and function of the hippocampus. Thus, we investigated 68 nicotine-dependent smokers and 70 non-dependent controls that completed two MRI sessions within approximately one month. Abstinent smokers took part both before and after quitting successfully; relapsing smokers were not invited for a second scan. Images were processed with cross-sectional and, if both sessions were completed, longitudinal FreeSurfer streams. Hierarchical linear modeling was used to investigate the effects of smoking cessation and baseline depression scores (measured with BDI) on volumetric changes in subcortical areas. In addition, we measured fMRI BOLD signaling during passive viewing of emotional (pleasant, unpleasant, neutral) pictures in a subsample (23 smokers, 37 controls). Relating to structural changes, we observed greater volume loss in the right hippocampus, left amygdala and bilateral nucleus caudatus in smokers compared to controls. Moreover, volume loss in the left hippocampus occurred in more dependent and more

depressed smokers. Functional ROI analysis revealed increased bilateral hippocampal response to unpleasant, but not to pleasant stimuli in smokers after smoking cessation. Increases of BDI scores covaried with elevated BOLD signaling to unpleasant stimuli in the left hippocampus. Critically, increases in brain responses to unpleasant stimuli in bilateral hippocampus predicted relapse within the first 6 month after smoking cessation. Taken together, we were able to identify structural and functional neuroadaptations induced by smoking cessation in regions which are involved in emotion and motivational processing. Our results suggest that the hippocampal formation in a particular has a substantial association with the occurrence of negative mood states after quitting. Changes in neural reactivity might be caused by cessation-induced elevated cholinergic levels and result in increased susceptibility to unpleasant events which increases the risk for relapse.

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Poster

231. Nicotine: Reward and Seeking

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Program#/Poster#: 231.14/V16

Topic: C.17. Drugs of Abuse and Addiction

Title: Attenuating combined cue and prime-induced reinstatement of nicotine-seeking following chronic dosing of the novel D3-receptor antagonist PF-04363467

Authors: *E. R. DUNN-SIMS[1], A. ROSADO[1], A. FOOTE[1], C. TYSZKIEWICZ[1], N. C. STRATMAN[2], C. J. SCHMIDT[2], A. SAWANT-BASAK[2], A. N. MEAD[1], T. T. WAGER[2], T. A. CHAPPIE[2]
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Abstract: Learned associations between the rewarding properties of drugs of abuse and environmental cues contribute to craving and relapse in humans. Dopamine D3-receptors are preferentially expressed in mesocorticolimbic DA projection areas: areas of the brain highly associated with reward-related learning induced by drugs of abuse. Nicotine dependence is a chronic relapsing disorder and increasing evidence supports a model where D3-receptor activity is implicated in the relapse-related cellular and behavioral effects underlying nicotine-seeking behavior. We have previously reported that the novel D3-receptor antagonist PF-04363467 attenuates reinstatement of nicotine-seeking in rats under different test conditions when

administered acutely. The purpose of the present studies was to understand whether these same effects of PF-04363467 were observed when dosed chronically for 28 days. Male Sprague-Dawley rats were initially trained to self-administer nicotine, I.V., under a fixed ratio schedule of reinforcement using a standard 2-lever choice design, with infusion-paired cues. Training sessions were conducted during either short-access (90-minute) or long-access (15-hour) conditions. Following acquisition, an extinction period commenced to dissociate the act of lever pressing from delivery of nicotine. Reinstatement tests were then conducted, with reinstatement induced by a combination of nicotine prime and cues. PF-04363467 was administered once daily for a total of 28 days, and reinstatement tests were conducted once per week. Congruent with prior findings, PF-04363467 attenuated reinstatement of nicotine seeking when given acutely to rats trained under short and long-access conditions (30 mins prior to session start). However, chronic treatment of PF-04363467 resulted in tolerance to this effect after 3 weeks in rats trained under both short and long access conditions. The results are discussed in relation to potential reasons for the loss of efficacy with repeated administration of PF-04363467, as well as published examples relating to the lack of translation of chronic dosing regimens from rodents to humans. These data raise important questions regarding gaps in knowledge when pharmacological agents are chronically dosed to rats.

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Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.15/V17

Topic: C.17. Drugs of Abuse and Addiction

Title: Maternal care deprivation and chronic alcohol consumption prevents conditioned place preference to nicotine in rats

Authors: *R. GARCÍA
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Abstract: Perinatal maternal care is a condition that may influence many aspects of de brain development and behavior including the response to drugs of abuse. The deprivation of maternal care (MCD) in rats for few hours a day may predispose litters for alcohol consumption in the

adulthood. In this study we use the maternal deprivation model for three hours for 16 days in male Wistar rats to determine whether the maternal deprivation affect the quantity of chronic voluntary consumption of Ethanol solution (at 10%) across two months (during puberty). At the end of this period we applied a conditioned place paradigm in order to analyze the possible interaction with the preference to 0.8 mg /kg of nicotine. Our results show a marked influence of maternal separation on the development of aversive nicotine in CPP (66% in rats not separated vs 44% of separated rats, both groups without exposure to ethanol). By contrast, non-deprived rats exposed to voluntary ethanol consumption showed strong preference for the CPP nicotine compared to non-deprived without exposure to ethanol (54% vs 33% respectively). We found a negative correlation aversion (r=-0.77) in nicotine CPP associated with increased ethanol consumption (as much as 5.22mg/kg of ethanol) during the period of conditioning in maternal deprived rats exposed to ethanol. This study suggests that MCD plus alcohol consumption enhances nicotine intake Supported by Grants 129103 and IN224314 from CONACyT and DGAPA-UNAM respectively and Fundación Miguel Aleman to OPG, and CONACyT 240173 to Ricardo García-Ruiz

Disclosures: R. García: None.

Poster

231. Nicotine: Reward and Seeking

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Program#/Poster#: 231.16/V18

Topic: C.17. Drugs of Abuse and Addiction

Title: Choice between nicotine and sucrose in rats as a model of the development, maintenance, and treatment of tobacco use

Authors: *L. V. PANLILIO[1], L. HOGARTH[2], M. SHOAIB[3]
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Abstract: Animal models of drug abuse that allow subjects to choose between drug and nondrug reinforcers might provide unique insights into how drug use is developed and sustained, and how it might be decreased through therapeutic treatments. Choice procedures have been used extensively in rats with cocaine, but not with nicotine. Here, male hooded Lister rats were trained with a concurrent-choice schedule in which pressing one lever delivered intravenous nicotine (0.03 mg/kg) and pressing another lever delivered sucrose pellets. When rats were initially

trained with only the sucrose lever available on some days and only the nicotine lever available on other days, they immediately showed a preference for sucrose when they were given a choice. In contrast, when rats were initially trained with nicotine for three days but given no sucrose training prior to being allowed to choose, about half developed a preference for nicotine. Subcutaneous treatment with varenicline (1.5 mg/kg), a partial agonist of nicotinic receptors, selectively decreased nicotine self-administration in the choice procedure. When food-related manipulations were performed, nicotine responding increased when the sucrose lever was removed, but decreased gradually when food restriction was discontinued. The finding that rats can prefer nicotine over sucrose is surprising in light of studies showing that rats tend to prefer sweet tastes over intravenous cocaine, even when trained exclusively with cocaine prior to being given a choice. Testing with varenicline, which is known to be effective in promoting smoking cessation, indicates that the choice procedure could be useful for testing therapeutic treatments. The results obtained with food-related manipulations are consistent with previous studies showing that food restriction enhances drug self-administration, and that availability of alternative reinforcers can decrease drug use. Overall, these findings demonstrate the choice procedure's ability to reveal and account for individual differences that were not apparent with a simple schedule of nicotine self-administration.

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Poster

231. Nicotine: Reward and Seeking

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Topic: C.17. Drugs of Abuse and Addiction

Support: KFDA 13182 담배안 759

Title: The rewarding and reinforcing effects of nicotine in drug-naïve, nicotine pretreated, or tobacco smoke pre-exposed adolescent and adult rats

Authors: *A. H. MUHAMMAD, J. DE LA PENA, C. BOTANAS, R. TAMPUS, N. JEONG, J. KIM, H. KIM, J. CHEONG
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Abstract: Cigarette (tobacco) smoking is one of the most prevalent addictions worldwide. Millions are affected by this addiction, and thousands more light their first cigarette every day.

More alarming is that most of these first time smokers are adolescents. Similar to other abused substances, cigarettes are primarily taken because it makes the user “feel good”. The main substance implicated in the addictive effect of cigarettes/tobacco is nicotine. However, when tested in animal models of addiction, the rewarding and reinforcing effects of nicotine is hard to establish. Furthermore, studies delving into the effects of chronic or repeated nicotine pre-exposure have reported conflicting results. Thus, in the present study we sought to characterize the rewarding and reinforcing effects of nicotine in adolescent (4-6 weeks) and adult rats (8-10 weeks) under three conditions: (1) drug-naïve, (2) nicotine-pretreated, or (3) cigarette smoke pre-exposed. The rewarding and reinforcing effects of nicotine were evaluated in two of the most widely used and accepted animal models of addiction, the conditioned place preference (CPP) and the self-administration (SA) tests. In drug-naïve rats, adolescents demonstrated enhanced nicotine CPP (0.2 mg/kg) and SA (0.03 mg/kg/infusion) as compared to the adult group. 7 days pre-exposed rats exhibited greater CPP for the initially unrewarding high dose (0.06 mg/kg) of nicotine, especially appreciable in cigarette smoke pre-exposed adolescent rats. On the other hand, in the SA test, pre-exposed adolescent and adult rats demonstrated reduced/diminished nicotine (0.03 mg/kg) SA. These results suggest that adolescents are more vulnerable to the addictive effects of nicotine, and repeated nicotine or cigarette smoke exposure changes subsequent response towards this drug.

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Poster

231. Nicotine: Reward and Seeking

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH K Award K01 DA030445

Title: Paternal nicotine self-administration is associated with increased acquisition and maintenance of nicotine taking in offspring

Authors: *A. C. ARREOLA, B. A. KIMMEY, H. D. SCHMIDT
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Recent evidence indicates that paternal smoking is associated with nicotine dependence and increased incidence of childhood cancer in offspring. These findings indicate that tobacco smoke is capable of influencing behavioral phenotypes in future generations. Epigenetics is a key mechanism by which the environment can influence and interact with genetics to influence behavior. Epigenetic mechanisms have been shown to underlie drug-induced behavioral plasticity by coordinating expression of gene networks in the brain. Thus, one direct mechanism by which nicotine may influence genetic events involved in the development of nicotine addiction as well as its heritability in future generations is epigenetics. However, the epigenetic mechanisms by which paternal nicotine exposure influences smoking behavior in subsequent generations are not clear. The goal of this study was to establish a preclinical rodent model of inter-generational susceptibility to nicotine dependence. Male rats were allowed to self-administer nicotine (0.03 mg/kg/infusion) on a fixed-ratio 1 schedule of reinforcement for 60 consecutive days. Nicotine-experienced rats and yoked saline controls were then allowed to mate with drug-naïve dams. When the offspring reached 60 days of age, male and female progeny were implanted with jugular catheters and the acquisition of nicotine self-administration was assessed. Offspring of nicotine-experienced sires self-administered more nicotine when compared to the offspring of yoked saline controls. These data are consistent with human epidemiological studies and indicate that paternal nicotine exposure increases susceptibility to nicotine taking in offspring. Identifying novel epigenetic mechanisms underlying the transmission of enhanced vulnerability to nicotine dependence will aid in the development of novel smoking cessation medication in generations at high risk for chronic smoking behavior. This work is supported by K01 DA030445 (H.D.S)

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Poster

231. Nicotine: Reward and Seeking

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Support: Intramural Research Program of the National Institute on Drug Abuse

NIH Grant DP1 DA031387

Title: Fatty acid amide hydrolase (FAAH) inhibition blocks abuse-related effects of nicotine in squirrel monkeys

Authors: *S. R. GOLDBERG[1], L. V. PANLILIO[1], G. H. REDHI[1], G. MORENO-SANZ[2], S. I. CHEFER[3], S. YASAR[4], D. PIOMELLI[2], Z. JUSTINOVA[1]
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Abstract: Endogenous cannabinoid signaling is known to be involved in nicotine addiction, but the functions of specific endocannabinoid transmitters in the abuse-associated effects of nicotine have long remained unclear. Inhibition of fatty acid amide hydrolase (FAAH) counteracts certain aspects of nicotine reward in rats (Scherma et al., 2008), but its effects have not been studied with addiction-related procedures in non-human primates. Here we studied the effects of first and second generation FAAH inhibitors, URB597 and URB694, on reinforcing effects of nicotine in squirrel monkeys using a fixed-ratio intravenous nicotine self-administration procedure. Systemic treatment with either FAAH inhibitor: 1) almost completely blocked FAAH activity in monkey brain and liver, increasing levels of endogenous ligands (anandamide, PEA and OEA) for cannabinoid and alpha-type peroxisome proliferator-activated receptors (PPAR-α); 2) shifted nicotine self-administration dose-response functions to the right in a manner consistent with reduced nicotine reward; and 3) blocked reinstatement of nicotine seeking induced by re-exposure to either nicotine or cues that had been associated with nicotine. The behavioral effects of FAAH inhibition on nicotine self-administration and nicotine-induced reinstatement were reversed by treatment with a PPAR-α antagonist MK886. Moreover, FAAH inhibition had no effect on cocaine or food self-administration under the same schedule of reinforcement. In these non-human primate models, both URB597 and URB694 show promise for the initialization and maintenance of smoking cessation, due to their ability to block the rewarding effects of nicotine and to block nicotine-induced and cue-induced reinstatement.

Disclosures: S.R. Goldberg: None. **L.V. Panlilio:** None. **G.H. Redhi:** None. **G. Moreno-Sanz:** None. **S.I. Chefer:** None. **S. Yasar:** None. **D. Piomelli:** None. **Z. Justinova:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.20/V22

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/NIH and FDA Center for Tobacco Products U54DA031659

Title: Monoamine oxidase (MAO) inhibition decreases the threshold for nicotine self-administration in rats

Authors: T. T. SMITH[1], R. L. SCHASSBURGER[1], L. E. RUPPRECHT[1], S. N. CWALINA[1], D. M. BUFFALARI[1], S. ALI[2], E. C. DONNY[1], *A. F. SVED[1]
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Abstract: In chronic cigarette smokers brain monoamine oxidase (MAO) activity is partially inhibited. Previous studies have suggested that drugs that inhibit MAO increase nicotine self-administration in rats. The present studies sought to address three issues. First, does the MAO inhibitor tranylcypromine (TCP) lower the threshold dose of nicotine required to support self-administration in rats? Second, do other drugs that inhibit MAO have a similar effect? Third, does partial inhibition of MAO, to the extent observed in chronic smokers, similarly impact nicotine self-administration? To assess the effect of a large dose of TCP on the dose-response curve for nicotine self-administration, adult male rats responded (nose poke, fixed ratio 2 schedule) for an i.v. infusion of a nicotine solution paired with a 15-s stimulus light and received a pre-session injection of TCP (1 mg/kg i.p.) or saline (n=12-14). Self-administration sessions were one hour each day during the dark portion of the light-dark cycle. After every 7 sessions the nicotine dose was increased (0→1.875→3.75→7.5→15→30→60→90 µg/kg/infusion). TCP, at this dose of 1 mg/kg that produced near complete inhibition of MAO-A and MAO-B, shifted the dose-response curve to the left, with the threshold dose supporting nicotine self-administration being 7.5 ug/kg/inf in the TCP group versus 15 ug/kg/inf in the group of rats not receiving TCP. Similarly, in a separate group of rats (n=8) a combination of clorgyline (1 mg/kg i.p.; MAO-A inhibitor) plus pargyline (10 mg/kg i.p.; MAO-B inhibitor), which produced near complete inhibition of MAO-A and MAO-B, increased responding for a low dose of nicotine. A dose of TCP (0.1 mg/kg) that inhibited MAO activity by ~35%, comparable to what is observed in chronic smokers, also increased responding for a low dose of nicotine. Together, these studies show that MAO inhibition increases self-administration of low doses of nicotine. Furthermore the studies suggest that MAO inhibition produced by cigarette smoke may contribute to the reinforcing properties of cigarettes and may be even more important if nicotine levels in tobacco products is reduced as part of a tobacco regulatory policy.

Disclosures: **T.T. Smith:** None. **R.L. Schassburger:** None. **L.E. Rupprecht:** None. **S.N. Cwalina:** None. **D.M. Buffalari:** None. **S. Ali:** None. **E.C. Donny:** None. **A.F. Sved:** None.

Poster

231. Nicotine: Reward and Seeking

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Topic: C.17. Drugs of Abuse and Addiction

Support: ANR BLANC 2012

FRM, équipe 2013 PF

Bettencourt Schueller Foundation

Title: Nicotine and alcohol induce inhibition of medial vta dopaminergic neurons

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Abstract: Midbrain dopamine (DA) neurons are key players in motivation and reward processing. Increased DA release is thought to be central in the initiation of drug addiction. Whereas dopamine neurons are generally considered to be activated by drugs such as nicotine and alcohol, we report here that nicotine not only induces excitation of Ventral Tegmental Area (VTA) DA cells but also induces inhibition of a subset of VTA DA neurons that are anatomically segregated in the medial part of the VTA. These opposite responses are also elicited by ethanol, but do not correlate with the inhibition and excitation induced by noxious stimuli. We show that this inhibition requires D2 receptor (D2-R) activation, suggesting that an intra-VTA dopaminergic release is involved in the mechanism.. Our findings provide a novel organizing principle of concurrent excitation and inhibition of VTA DA cells in response to reinforcing stimuli. It promotes unexplored roles for DA release in addiction contrasting with the classical views of reinforcement and motivation, and give rise to a new comprehension of the mode of operation of the reward system.

Disclosures: S. Valverde: None. **R. Eddine:** None. **R. Hepp:** None. **F. Marti:** None. **S. Tolu:** None. **A. Hay:** None. **C. Morel:** None. **B. Lambolez:** None. **P. Faure:** None. **D. Dautan:** None. **L. Venance:** None. **Y. Cui:** None.

Poster

231. Nicotine: Reward and Seeking

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Program#/Poster#: 231.22/V24

Topic: C.17. Drugs of Abuse and Addiction

Support: NRF-2011-0013573

NRF-2013S1A5A8024560

Title: Repeated nicotine exposure in rats: Alteration of medial habenula and blockade effect of oxytocin

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Abstract: Nicotine can not only stimulate the reward circuitries, but also can produce aversive reaction. Although the neurobiological mechanism of nicotine reward has been heavily studied, relatively little is known about the mechanism of nicotine aversion. Because growing evidences suggest that medial habenula to interpeduncular nucleus circuitry plays a pivotal role in nicotine aversion, we investigated the neural activity of medial habenula between the repeated saline and nicotine injected rats using an extracellular recording. We found that the basal spontaneous spike frequency in medial habenula was minimized and nicotine-evoked response was also reduced in repeated nicotine injected rats compared to saline injected rats. Moreover, in repeated nicotine injected rats, increased nicotine preference was observed. Because oxytocin is implicated in drug addiction to reduce the tolerance, self-administration and even withdrawal symptoms, we determined the effect of oxytocin on nicotine-induced behavior alteration. Oxytocin completely blocked an augmentation of nicotine intake preference and an increase of nicotine-mediated anxiety-like behavior by repeated nicotine exposure. These results demonstrate a medial habenular activity is one of candidates for determining an inhibitory motivation to avoid nicotine consumption. Additionally oxytocin has fascinating potential to reverse nicotine-mediated addictive behavior and to inoculate against vulnerability to nicotine-mediated addictive disorders.

Disclosures: **H. Lee:** None. **J. Noh:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

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Topic: C.17. Drugs of Abuse and Addiction

Support: CIHR

Title: The effects of varenicline, bupropion, lorcaserin, and naltrexone on nicotine-enhanced responding for a conditioned reinforcer

Authors: *E. G. GUY[1], D. C. FISHER[5], G. A. HIGGINS[2,6], P. J. FLETCHER[5,3,4]
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Abstract: Stimuli that have been repeatedly associated with natural or drug reinforcement can become motivationally significant and elicit reward-seeking behaviors, making them conditioned reinforcers. Nicotine, the primary psychoactive ingredient in tobacco, enhances responding for conditioned reinforcement. This type of interaction between nicotine and cues associated with rewards, or conditioned stimuli (CSs), may contribute to nicotine addiction. This work examined how pharmacological interventions that are used for smoking cessation alter the ability of nicotine to enhance responding for conditioned reinforcement. Thus we looked at interactions between nicotine and the nicotinic receptor partial agonist varenicline, and between nicotine and the DAT inhibitor bupropion. Preclinical studies have shown interactions between nicotine and lorcaserin (a 5-HT_{2C} receptor agonist), and between nicotine and naltrexone (μ-opioid receptor antagonist) on several behavioral measures. Since both drugs are FDA-approved, albeit for other indications, we also examined their effects on nicotine-induced responding for conditioned reinforcement. Thirsty rats were exposed to 13 Pavlovian conditioning sessions where a CS was paired with water delivery. Nicotine (0.4 mg/kg) injections were administered prior to each of these sessions. Then, in separate groups of animals (n=10 each), a repeated-measures design was used to test the effects of varenicline (1 mg/kg), bupropion (10 and 30 mg/kg), lorcaserin (0.6 mg/kg), and naltrexone (2 mg/kg) and their interaction with nicotine (0.4 mg/kg) on responding for conditioned reinforcement. Varenicline alone modestly increased responding for conditioned reinforcement compared to saline, but fully antagonized the response-potentiating effects of nicotine. Lorcaserin and naltrexone both reduced the effect of nicotine to enhance responding for conditioned reinforcement. In contrast, the 30 mg/kg dose of bupropion enhanced responding for conditioned reinforcement, and further increased the response-potentiating effects of nicotine. These studies inform one possible mechanism by which these four, FDA approved medications affect smoking behavior in humans; by interacting with the effect of nicotine to enhance the motivating properties of nicotine-associated CSs.

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Poster

231. Nicotine: Reward and Seeking

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Topic: C.17. Drugs of Abuse and Addiction

Support: DFG grant HE 2597/4-3

DFG grant HE 2597/7-3

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DFG grant SM 80/2-2

DFG grant SM 80/5-1

DFG grant SM 80/5-2

DFG grant SM 80/7-1

Title: Smoking moderates association of 5-HTTLPR and *in vivo* availability of serotonin transporters

Authors: *M. N. SMOLKA[1], M. REIMOLD[2], A. KOBIELLA[1], G. REISCHL[2], M. RIETSCHEL[3], A. HEINZ[4]
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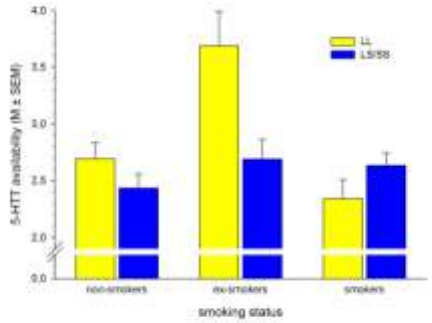
Abstract: Although preclinical studies clearly indicate an effect of 5-HTTLPR genotype on 5-HT transporter (5-HTT) expression, studies in humans provided inconclusive results, hypothetically due to environmental factors and differences in individual behavior. For example, nicotine and other constituents of tobacco smoke elevate serotonin (5-HT) levels in the brain and may cause homeostatic adaptations in 5-HTT availability that moderate effects of 5-HTTLPR genotype. To test whether 5-HTT availability in the midbrain is affected by smoking status and 5-HTTLPR genotype, we pooled data from prior studies on *in vivo* 5-HTT availability (BPND) measured with positron emission tomography (PET) and [11C]DASB. In total, we reanalyzed 5-HTT availability in 116 subjects using ANCOVA statistics. ROI analysis revealed that midbrain BPND is higher in former smokers than in smokers and non-smokers. Interestingly, smoking

status significantly interacted with 5-HTTLPR genotype, i.e. the association of active smoking with reduced 5-HTT availability was only seen in LL subjects but not in carriers of the S-allele. Or from the perspective of genotype effects, former smokers and non-smokers showed the expected association with 5-HTTLPR, i.e. higher 5-HTT availability in LL subjects compared to carriers of the S-allele, whereas this pattern was actually reversed for active smokers. Our study indicates that smoking status moderates the association of 5-HTTLPR genotype and 5-HTT expression. This may help to explain inconsistent findings in previous studies. Regarding the mechanism, we suggest that smoking induces epigenetic processes such as methylation of SLC6A4 which might differ depending on its genetic constitution.



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Poster

231. Nicotine: Reward and Seeking

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.25/V27

Topic: C.17. Drugs of Abuse and Addiction

Support: Supported by a grant from Philip Morris USA

Title: Neuroanatomical location of orexin projecting nerve fibers in mesocorticolimbic pathways of GAD67-GFP knock-in mice

Authors: *O. DEHKORDI[1], J. E. ROSE[2], S. ASADI[1], R. M. MILLIS[1], A. J. TROUTH[1]
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Abstract: Orexin-and GABA-containing neurons are implicated in the neurobiology of nicotine addiction. However, the neuroanatomical and neurochemical relationships between such neurons in the neurocircutry of addiction is not known. Therefore, In the present study in glutamic acid decarboxylase-green fluorescence (GAD67-GFP) knock-in mice, we used immunohistochemistry to identify the neuroanatomical location of orexin-containing neurons and nerve fibers with respect to GABAergic neurons in hypothalamic regions and in areas overlapping the mesocorticolimbic pathways.Consistent with previous studies, orexin inmmunoreactive (IR) cells were present exclusively in the lateral hypothalamus. Orexin IR nerve fibers were observed at multiple sites throughout the CNS. In the hypothalamic region,orexin IR nerve fibers were found to be intermingled with GAD67-GFP positive cells. The GAD67-GFP positive cells at these sites were seen medial and ventral to the orexin IR cells and at sites which correspond to the dorsomedial hypothalamic nucleus and the arcuate hypothalamic nucleus. In the ventral tegmental area (VTA), orexin IR nerve fibers were seen at areas rostral to the GAD67-GFP positive cells of interpeduncular nucleus (IPN) and at sites overlapping paranigral nucleus, parainterfascicular nucleus and parabrachial pigmented nucleus. More rostrally, orexin IR nerve fibers were seen in areas overlapping retromamillary nucleus, interfascicular nucleus and rostral VTA, at sites which were lateral to the GAD67-GFP positive cells of medial terminal nucleus and substantia nigra compact part. In nucleus accumbens (Acb) and prefrontal cortex (PFC), orexin IR nerve fibers were found to be intermingled with GAD67-GFP positive cells. In addition, orexin IR nerve fibers were detected in many other brain regions subserving the reward pathways. The present anatomical data demonstrate that orexin-containing neurons of the lateral hypothalamus have direct projections to structures of the mesocorticolimbic reward centers. The close proximity of orexin IR nerve fibers to GAD67-GFP positive cells of the prefrontal cortex and nucleus accumbens suggests that orexin-containing nerve fibers may modulate activity of GABAergic neurons at these sites.

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Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.26/V28

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NR012065

Title: Menthol speeds the desensitization of human α3β4 nicotinic acetylcholine Receptors

Authors: *H. TON, A. SMART, T. XIE, K. J. KELLAR, G. P. AHERN
Georgetown Univ., Washington, DC

Abstract: The α3β4 receptor is the most prevalent nicotinic acetylcholine receptor expressed in the peripheral nervous system. Notably, α3β4 receptors in airway sensory nerves may transduce the irritant effects of nicotine in tobacco smoke. In our study, we sought to examine potential effects of menthol, a counterirritant, at these ion channels by performing Ca^{2+} imaging, whole-cell voltage-clamp recording and [^{3}H]-epibatidine binding in HEK cells stably expressing human α3β4 nAChRs. Co-application, but not pre-treatment, of menthol with acetylcholine and nicotine inhibits the function of α3β4 nAChRs by increasing desensitization. This is demonstrated by a reduction of the current integral as well as an increase in the rate and magnitude of the current decay. Menthol does not act at the orthosteric nAChR binding site as it does not compete for [^{3}H]-epibatidine binding. Menthol-induced inhibition of α3β4 nAChRs may contribute to the counterirritant action of menthol in nicotine-induced irritation.

Disclosures: H. Ton: A. Employment/Salary (full or part-time):; Department of Pharmacology and Physiology, Georgetown University, Washington DC 20007, USA. **A. Smart:** A. Employment/Salary (full or part-time):; Department of Pharmacology and Physiology, Georgetown University, Washington DC 20007, USA. **T. Xie:** A. Employment/Salary (full or part-time):; Department of Pharmacology and Physiology, Georgetown University, Washington DC 20007, USA. **K.J. Kellar:** A. Employment/Salary (full or part-time):; Department of Pharmacology and Physiology, Georgetown University, Washington DC 20007, USA. **G.P. Ahern:** A. Employment/Salary (full or part-time):; Department of Pharmacology and Physiology, Georgetown University, Washington DC 20007, USA.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.27/V29

Topic: F.02. Animal Cognition and Behavior

Title: Menthol induces anesthetic effects mediated by GABAA-receptors in fishes

Authors: *M. KASAI
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Abstract: Menthol is well known to be able to induced cold and pain sensations in subjects. To determine whether fishes respond to menthol, Japanese medaka, goldfish and zebrafish were exposed to various types of menthol receptors agonists and the behavioral responses to these drugs were observed. Waterbone application of dl-menthol (0.5 mM) induced surgical anesthesia in 100% of medaka, 90% of goldfish, and 100% of zebrafish. The percentage of response increased dose-dependently from 0.2 mM to 0.5 mM. Sedation (motion loss) was observed more than 0.2 mM in goldfish by both d-, and l- types of menthol in goldfish. There were no differences in either percentage or the response in the anesthesia among dl-, d-, and l- types of menthol. A high (3.0 mM) concentration of any of the three types of menthol induced rapid movement followed by the surgical anesthetic response. Rapid movement was observed with allyl isothiocyanate, a cold nociceptor agonist, but not with icilin, a cold receptor agonist, in medaka and goldfish. Both allyl isothiocyanate and icilin failed to induce surgical anesthesia. To determine the involvement of γ-aminobutyric acid (GABA) system in menthol-induced anesthesia, the effect of the receptors antagonist was tested. Pretreatment with a specific GABAA receptor antagonist prolonged the latency of the surgical anesthetic responses and completely attenuated the sedation to menthol in goldfish. These results demonstrate that menthol may play a role in the induction of anesthesia in fishes, related at least in part to the activation of GABAA receptors, and of rapid movement possibly via cold nociceptors.

Disclosures: M. Kasai: None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Title: A menthol conditioned reinforcer promotes acquisition of nicotine self-administration in rats

Authors: M. R. KELLICUT[1], A. M. BROWN[1], A. B. SHEPPARD[1], E. M. ODINEAL[1], *M. I. PALMATIER[2]
[1]Psychology, [2]East Tennessee State Univ., Johnson City, TN

Abstract: Nicotine (NIC) by itself is a weak to moderate reinforcer but it potently increases responding for non-drug conditioned reinforcers (CRs). Twenty-five percent of all cigarettes sold in the United States are menthol flavored and menthol is a common flavor additive in candy, ice-cream, and other foods. Thus, menthol may serve as a CR and interact with nicotine to promote tobacco use. These experiments investigated the effects of a menthol CR on NIC self-administration in rats. To do so, menthol was established as a CR (Menthol-CR groups) or neutral stimulus (Menthol-Neutral groups). Each group received access to two gustatory stimuli (0.0005% menthol or 0.05% grape Kool-Aid). For the Menthol-CR groups, sucrose (20% w/v) was added to the menthol solution. For the Menthol-Neutral groups, sucrose was added to the grape solution. Rats were then instrumented for intravenous (IV) NIC self-administration. In Experiment 1 responses on a nose-key resulted in presentation of 0.0005% menthol solution (unsweetened) in a liquid dipper and IV NIC (1.5-60 ug/kg/infusion, dose calculated as base). The Menthol-CR robustly increased responding at low NIC doses, peak responding was observed at 3.25 ug/kg/infusion. For the Menthol-Neutral group peak responding was observed at a dose almost 5 times higher, 15 ug/kg/infusion. In Experiment 2, the experimental group (Menthol-CR+NIC) was compared to two controls - a group that provided a baseline for the primary reinforcing effects of NIC (Menthol-Neutral+NIC) and a group that provided a baseline for the conditioned reinforcing effect of menthol (Menthol-CR+SAL infusions). The peak unit dose from Menthol-CR groups in Experiment 1, 3.25 ug/kg/infusion, was available during acquisition. For all groups the reinforcers (0.1 ml menthol and IV NIC or SAL infusion) were contingent upon lick responses at a sipper tube. The Menthol-CR+NIC group made more lick responses than both control groups. The Menthol-Neutral+NIC group did not acquire reliable NIC self-administration at this dose. Subsequent tests under a progressive ratio schedule indicated that the motivation to obtain the reinforcers in the Menthol-CR+NIC group was inversely related to unit NIC-dose, with the lowest doses (3.25-6.5 ug/kg/infusion) supporting the highest responding. These experiments indicate that a menthol CR increases the reinforcing effects of low unit NIC doses, promotes acquisition of NIC self-administration, and may alter the relationship between NIC dose and reinforcer efficacy.

Disclosures: M.R. Kellicut: None. **A.M. Brown:** None. **A.B. Sheppard:** None. **E.M. Odineal:** None. **M.I. Palmatier:** None.

Poster

231. Nicotine: Reward and Seeking

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 231.29/V31

Topic: B.02. Ligand-Gated Ion Channels

Support: Virginia Youth Tobacco Program Grant Award

Title: A chilling connection; menthol's effect on nicotinic acetylcholine receptor expression and synaptic structure in the rodent brain

Authors: *S. P. BRIDGES[1], J. R. KING[1], J. C. NORDMAN[1], P. MULDOON[2], M. LIZARRAGA[1], I. DAMAJ[2], N. KABBANI[1]
[1]Dept. of Mol. Neurosci., George Mason Univ., Fairfax, VA; [2]Dept. of Pharmacol., Virginia Commonwealth Univ., Richmond, VA

Abstract: Although menthol, a common flavoring additive to cigarettes, has been found to impact the addictive properties of nicotine cigarettes in smokers, little is known about its molecular actions in the brain. To test the involvement of nicotinic acetylcholine receptors (nAChR) in menthol associated nicotine addiction, we examined proteomic and structural changes in the brain of rodents exposed to nicotine with and without menthol. Western blot analysis of α7, α4, and β2 nAChR subunit expression suggests that menthol impacts the levels and distribution of several nAChRs in various brain regions. In particular, co-administration of menthol and nicotine appears to promote significant alterations in β2 and α7 nAChR subunit expression in the hippocampus, amygdala, and striatum of mice. Immunohistochemical analysis of α7 nAChR and synaptic marker protein expression reveals dynamic alterations in post-synaptic structures within hippocampal neurons in response to menthol administration. Because the addition of menthol to tobacco products has been suggested to augment their addictive potential, the current findings reveal several new molecular adaptations that may contribute to its unique addictive profile.

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Poster

231. Nicotine: Reward and Seeking

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Topic: B.02. Ligand-Gated Ion Channels

Support: DA017279

DA019375

DA033721

Title: Menthol alone alters the number and assembly of α4β2 and α6β2* nAChRs

Authors: *B. J. HENDERSON[1], T. WALL[1], W. A. NICHOLS[2], R. SRINIVASAN[2], H. A. LESTER[1]
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Abstract: We report the first observation that chronic exposure to presumed smoking-relevant levels of menthol affects the properties and numbers of neuronal nicotinic acetylcholine receptors (nAChRs) *in vivo* and *in vitro*, even in the absence of nicotine. Using mice expressing fluorescent α4 and α6 nAChR subunits we show that menthol increases the numbers of α4* and α6* nAChRs in midbrain neurons. The α6* nAChRs on dopaminergic neurons of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) become more abundant during chronic treatment with menthol. We also found that chronic menthol increased numbers of α4* nAChRs in both VTA and SNc dopaminergic neurons; but α4* nAChRs in GABAergic neurons of the substantia nigra pars reticulata (SNr) did not change. To investigate effects of chronic menthol treatment in more detail, we studied cultured cells transiently expressing α4β2 or α6β2β3 nAChRs. In cells expressing α4β2 nAChRs, chronic treatment with menthol increases α4β2 nAChRs, but does not affect peak current amplitudes or desensitization kinetics of ACh-induced currents. We also found that chronic menthol treatment shifts the α4β2 nAChR stoichiometry from the usual mixed population (higher and lower sensitivity) toward the lower sensitivity using two metrics: 1) analysis of concentration-response curves and 2) the use of Förster resonance energy transfer (FRET). In cells expressing α6β2β3 nAChRs, chronic menthol treatment increased the population of α6β2β3 nAChRs, but decreased peak ACh-induced currents. This decrease in peak current amplitude resulted presumably from menthol-induced stabilization of α6β2(non-β3) nAChRs. Chronic menthol treatment reduced the FRET efficiency between fluorescent protein-labeled α6 and β3 nAChR subunits. This suggests that chronic menthol treatment does not favor (or destabilizes) assembled nAChRs pentamers containing the β3 subunit. Together, these data suggest that menthol, even in the absence of nicotine, increases nAChR numbers and preferentially stabilizes lower sensitivity assemblies of α4* and α6* nAChRs. These effects of chronic menthol on two populations of high-sensitivity nAChRs differ in detail from the effects of chronic nicotine. **Support:** DA017279; DA019375; DA033721.

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Poster

232. Cocaine: Neural Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 232.01/W1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R25NS080687

Title: Metabotropic glutamate receptor 5 modulation within NAc shell during environmental elicited cocaine conditioning

Authors: *K. TORRES, A. MARTÍNEZ-RIVERA, D. SANTIAGO-TRISTANI, I. CARRO-CRUZ, A. ROSADO, C. S. MALDONADO-VLAAR
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Abstract: The metabotropic glutamate receptor 5 (mGluR5) within the Nucleus Accumbens (NAc) have been implicated in modulating psychostimulant reward. Data from our laboratory demonstrate that mGluR5 blockade decreased environmental-elicited cocaine conditioning response. Our results imply that mGluR5 could be modulating the memory processes regulated within the NAc shell, essential for the association of environmental cues with cocaine effects. Different mechanisms can be driving this conditioning response. ERK1/2 have been related with cocaine addiction, both acute and repeated cocaine injections increase ERK phosphorylation (a measure of ERK activity) in projection areas of the mesocorticolimbic dopamine system. Moreover, studies have shown that mGluR5 activation leads to ERK1/2 phosphorylation and the up-regulation of CREB and Elk-1. We focused in elucidating which proteins have been affected during the expression of environmental elicited locomotion conditioning when we block mGluR5 with MPEP. Rats were implanted with cannula within NAc shell, and separate groups were exposed to a multimodal environment within activity chambers that signaled cocaine (paired) or saline (controls, unpaired). Prior to placing the animals in the chambers, rats received systemic injections of saline or cocaine for 10 consecutive sessions. On the test session (Day 12) separate groups of animals were infused within NAc shell with 25nmol/.5µl/side of MPEP. Preliminary data showed that the ratio of pERK/ERK 41-42 within NAc shell is not affected during the expression session after mGluR5 blockade. These results suggest that other proteins associated with mGluR5 cascade are modulating the expression of the conditioning. Furthermore, previous results report that mGluR5 positive allosteric modulators (PAMs) enhance synaptic plasticity, improve spatial learning and it's have a beneficial effects in the treatment of

cognitive impairment associated with schizophrenia. However, no study to date has investigated the therapeutic potential of other mGluR5 PAM novel compounds on specific aspects of cocaine addiction such as conditioned locomotor response. Our hypothesis is that the mGluR5 selective agonist CHPG will promote and enhance the expression of the cocaine conditioned response. Different groups of rats were microinjected with vehicle or the CHPG into the NAc shell and then they were placed in the activity chambers during Day 12. Interestingly, no change in the expression of the conditioned response were found in cocaine paired experimentally treated animals. Future studies are needed to further characterize these effects.

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Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS Grant P50 DA016511.

Title: Sex differences in hippocampal activation during initial abstinence from cocaine: Effects of the beta-adrenergic antagonist, propranolol

Authors: *A. S. KOHTZ, J. I. OSBORNE, M. E. SMITH, A. M. CASON, G. ASTON-JONES
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Abstract: Prior studies indicate that female rats exhibit greater drug-seeking behavior during initial abstinence following a period of cocaine self-administration when compared to male rats. The first day of extinction is important as it represents the initiation of abstinence, which is stressful due to absence from chronic drug. Locus coeruleus norepinephrine NE (LC-NE) neurons are involved in stress responses, including the ability of stress to drive relapse of drug-seeking. As well, LC-NE neurons are more sensitive to stress in females than males. One prominent target of LC-NE neurons is the dorsal hippocampus (DH), which has a number of structural and biochemical sex differences that modulate stress responsivity including axonal growth, granule cell density, cholinergic enzyme activity, as well as adrenergic-, corticosterone-, and GABA- receptor expression. Notably, the DH is required for context-dependent reinstatement of drug seeking, and drug relapse often occurs when addicts are re-exposed to

drug-associated contexts. Therefore, we hypothesize that the stress of initial abstinence may influence hippocampal neuron activity via beta-adrenergic receptors in a sex-dependent manner. Male and female Sprague-Dawley rats were implanted with indwelling jugular catheters and trained to lever-press for cocaine (iv, daily 2 hr sessions, 0.2mg/kg/ infusion) for 10 days of stable FR1 responding (>10 infusions/session). Rats were then administered 10mg/kg propanolol, a beta-adrenergic antagonist, or saline vehicle (IP), 20 minutes prior to being placed in the self-administration chamber during an initial abstinence/extinction session (ED1, 90 minutes, no cocaine infusions given), or remained in their home cages (HC) as controls. Rats were then deeply anesthetized, transcardially perfused with saline and 4% paraformaldehyde, and brains were removed and processed for immunohistochemical localization of Fos as a marker of neuronal activation. Results indicate that drug-seeking behavior on ED1 among both male and female rats is significantly decreased by administration of propranolol. Expression of Fos in hippocampal CA1 neurons is increased in female rats subjected to ED1 training compared to male rats, or compared to female HC controls. Administration of propranolol decreased CA1 Fos in both ED1 males and female rats. These findings indicate that hippocampus CA1 neurons may contribute to context driven drug-seeking behavior during initial abstinence.

Disclosures: **A.S. Kohtz:** None. **J.I. Osborne:** None. **M.E. Smith:** None. **A.M. Cason:** None. **G. Aston-Jones:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Program#/Poster#: 232.03/W3

Topic: C.17. Drugs of Abuse and Addiction

Support: AA007462

AA20908

AA0012262

AA007611

Title: The interaction between cocaine and ethanol within the nucleus accumbens shell

Authors: ***C. P. KNIGHT**[1], G. A. DEEHAN JR.[1], S. R. HAUSER[1], J. A. WILDEN[2], W. J. MCBRIDE[1], Z. A. RODD[1]

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Abstract: The co-abuse/use of cocaine and ethanol (EtOH) is common and occurs in 96% of cocaine users. Co-administration of alcohol and cocaine prolongs the euphoric effects of the drugs while diminishing the anxiogenic effects of cocaine. Cocaine and EtOH are directly self-infused into the nucleus accumbens shell (AcbSh). The current experiment was conducted to determine the interaction between ethanol and cocaine within the AcbSh to produce reward. In alcohol-preferring (P) rats, the threshold to establish self-infusion directly into the AcbSh is 100 mg% EtOH and 200 pmol/100nl cocaine. The current experiments examined co-infusion of combinations of sub-threshold doses of both drugs. Following one week of recovery for implanting guide cannulas aimed at the AcbSh, subjects were placed in standard two-lever (active and inactive) operant chambers. Test sessions were 60 min in duration and occurred every other day for a total of 7 sessions. Rats were randomly assigned to one of 17 groups (n=6-9/group) that self-infused (FR1 schedule) combinations of EtOH (0, 12.5, 25, 50, or 75 mg%) and combinations of cocaine (0, 6.25, 12.5, 25, 50, and 100 pmol/100nl) for the first 4 sessions. All rats received only aCSF in the infusate for sessions 5 and 6 (extinction), but received the original infusate again for session 7 (reinstatement). The results indicated that numerous sub-threshold doses of cocaine and EtOH are self-infused directly into the AcbSh. For example, 6.25 pmol/100nl cocaine + 50 mg% EtOH was self-infused directly into the AcbSh, but 6.25 pmol +25 mg% EtOH was not. In addition, 25 pmol/100 nl cocaine + 25 mg% EtOH was also self-infused into the AcbSh. The data indicate that cocaine would be self-infused at 32-fold lower concentrations if co-infused with EtOH than if given alone. Similarly, a 4-fold lower concentration of EtOH was co-infused with cocaine than if given alone. Overall, the data indicated that the combination of EtOH and cocaine acted synergistically within the AcbSh to produce reinforcement. The data provide a possible biological basis for the high rate of EtOH and cocaine co-abuse, and detail how the drugs interact to enhance their euphoric effects.

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Poster

232. Cocaine: Neural Mechanisms I

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Program#/Poster#: 232.04/W4

Topic: C.17. Drugs of Abuse and Addiction

Support: DA031429

Title: Retrospective carbon-14 birth dating demonstrates effects of cocaine and alcohol abuse on adult hippocampal cell turnover

Authors: H. DRUID[1], T. WARDI[1], K. ALKASS[1], S. BERNARD[2], *S. P. GARAMSZEGI[3], L. DUQUE[3], B. A. BUCHHOLZ[4], G. DHANABALAN[1], M. SALEHPOUR[1], K. SPALDING[1], D. C. MASH[3]
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Abstract: The global increase in atmospheric carbon-14 resulting from nuclear weapons testing affords a method for birth dating new neurons in the human brain. We used the retrospective carbon-14 birth dating technique developed at the Karolinska Institute in Stockholm to estimate when hippocampal cells were born to determine the effects of chronic cocaine and alcohol abuse on neuronal and nonneuronal cell turnover. Information about alcohol and drug use history, and medical conditions were obtained from structured interviews with relatives and informants and by review of forensic pathology investigations, police reports, and medical records. Archived brain specimens were sampled for FACS sorting and accelerator mass spectrometry of DNA extracted from the human hippocampus to compare levels of atmospheric carbon-14 during the birth year and across the lifetime of the alcoholic and cocaine dependent subjects. Mathematical modeling of carbon-14 concentrations was based on birth and death processes and renewal equations representing different scenarios for turnover of NeuN-positive cells (neurons) and NeuN-negative populations (oligodendrocytes, astrocytes, microglia, and endothelial cells) to quantify the overall extent of cell renewal. Analysis of the carbon-14 concentrations in hippocampal genomic DNA revealed that the majority of analyzed subjects had levels corresponding to time points after their birth, establishing postnatal cell generation in the human hippocampus. Weighted regression estimates of hippocampal cells were significantly lower in alcoholics as compared to controls ($p=0.015$, Wilcoxon rank-sum test, alcoholics vs. non-alcoholics). However, when comparing groups with or without cocaine dependence, the difference was not significant ($p=0.073$). The results show that although nonneuronal cells are exchanged in chronic cocaine abusers, the hippocampal neurons and nonneuronal fractions are as old or older in alcoholics, supporting the view that postnatal hippocampal cell turnover is damaged by chronic alcohol abuse.

Disclosures: **H. Druid:** Other; NIH Grant DA031429. **D.C. Mash:** Other; NIH Grant DA031429. **L. Duque:** Other; NIH Grant DA031429. **S.P. Garamszegi:** Other; NIH Grant DA031429. **K. Alkass:** Other; NIH Grant DA031429. **T. Wardi:** None. **S. Bernard:** None. **B.A. Buchholz:** None. **G. Dhanabalan:** None. **M. Salehpour:** None. **K. Spalding:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 232.05/W5

Topic: B.03. G-Protein Linked Receptors

Support: NIDA IRP funds

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CIBERNED

Title: Oligomers of orexin/hypocretin receptors, corticotropin-releasing factor receptors and σ1 receptors in the ventral tegmental area as targets for cocaine

Authors: *C. R. QUIROZ[1], G. NAVARRO[2], D. MORENO[2], A. SIERAKOWIAK[1], K. MCDOWELL[1], D. AGUINAGA[2], E. MORENO[2], F. HAUSCH[3], A. CORTES[2], J. MALLOL[2], V. CASADO[2], C. LLUIS[2], E. CANELA[2], P. MCCORMICK[2,4], S. FERRE[1]
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Abstract: Stress promotes relapse of drugs of abuse. In animal studies, stress reinstates drug-seeking behavior through the effects of CRF. The VTA is involved in the stress-induced and CRF-mediated reinstatement of drug self-administration. The orexigenic/hypocretin system drives cocaine reinstatement through activation of stress pathways and involves orexin receptors (OXR) localized in the VTA. In the present study we demonstrate the existence of allosteric interactions between ligands of CRF receptors (CRFR) and OXR in transfected cells and in the VTA that depend on CRFR-OXR heteromerization, and which modulate local dopamine somato-dendritic release. In transfected HEK-293T cells, CRF1R-OX1R and CRF2R-OX1R heteromerization was demonstrated with bioluminescent resonance energy transfer (BRET) and bi-molecular fluorescent complementation (BMFC) techniques. Negative crosstalk and cross-antagonism of CRFR and OXR ligands was observed with cell-signaling experiments. Synthetic peptides with the amino acid sequence of transmembrane 1 and 5 domains of OX1R (OX1R-TM1 and OX1R-TM5) disrupted BMFC and the negative crosstalk and cross-antagonism observed with MAPK signaling both in transfected cells and in rat VTA slices, demonstrating the presence of functional CRF1R-OX1R heteromers in the VTA. Also cocaine and the selective σ1

receptor agonist PRE-084 disrupted crosstalk and cross-antagonism of CRF1R and OX1R ligands in the VTA and modified the quaternary structure of CRF1R-OX1R heteromers, as demonstrated with BRET experiments in transfected cells. Sequential RET (SRET) experiments in transfected cells demonstrated the ability of σ1 receptor to complex with CRF1R-OX1R heteromers. *In vivo* microdialysis experiments in rats showed that local infusion of orexin A, but not CRF, induces dopamine release, which was antagonized by CRF. This negative crosstalk depended on CRF1R-OX1R heteromers, since it was counteracted by OX1R-TM1 and OX1R-TM5 peptides and by local perfusion with PRE-084. In the presence of the σ1 receptor agonist, CRF also induced VTA dopamine release. These results demonstrate that the CRF1R-OX1R heteromer is a target for cocaine and can explain the previously reported apparently independent effects of CRF and orexin A on stress-induced cocaine reinstatement.

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Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA025646

Title: CB1 receptor involvement in reconsolidation of context-cocaine memories that drive instrumental drug-seeking behavior

Authors: M. A. PRESKER[1], S. J. STRINGFIELD[1], *K. M. HARMON[2], J. A. HIGGINBOTHAM[2], A. A. ARGUELLO[2], R. A. FUCHS[2]
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Abstract: Exposure to drug-associated contextual stimuli precipitates relapse in cocaine users and cocaine-seeking behavior in rats. This phenomenon requires the maintenance of context-cocaine associative memories in long-term memory stores through the process of memory reconsolidation. Studies utilizing aversive or appetitive Pavlovian learning paradigms have

indicated a role for cannabinoid CB1 receptor-mediated signaling in memory reconsolidation. Here, we investigated the role of CB1 receptors in the reconsolidation of associative memories that promote drug context-induced cocaine-seeking behavior with focus on two brain regions implicated in this phenomenon: the basolateral amygdala and dorsal hippocampus. Male Sprague-Dawley ratswere trained to press a lever for intravenous cocaine infusions in a distinct context, followed by extinction training in a different context. On day 8 post cocaine, rats were re-exposed to the previously cocaine-paired context for 15 min in order to reactivate cocaine-related memories or remained in their home cages (no reactivation control). Immediately after this session, rats received systemic, intra-basolateral amygdala, or intra-dorsal hippocampal administration of the CB1 receptor antagonist, AM251. The rats were tested for reinstatement of extinguished cocaine-seeking behavior in the cocaine-paired context after at least two daily extinction sessions in the extinction context. Systemic administration of AM251 at the time of memory reconsolidation dose-dependently attenuated subsequent cocaine-seeking behavior in a memory reactivation-dependent fashion. Our preliminary findings indicate that post-memory reactivation AM251 administration into the basolateral amygdala disrupted, while AM251 administration into the dorsal hippocampus failed to alter, subsequent cocaine-seeking behavior. These findings suggest a requisite role for CB1 receptor stimulation in the reconsolidation of contextual cocaine memories that inform instrumental drug-seeking behavior.

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Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS grant P50 DA015369

NHMRC CJ Martin Fellowship

Title: The dopamine-prefrontal-accumbens circuit and cocaine reinstatement behavior

Authors: *M. H. JAMES, G. ASTON-JONES
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Background: Dopamine (DA) in medial prefrontal cortex (mPFC) is both necessary and sufficient for reinstatement of cocaine seeking (McFarland & Kalivas, 2001). Further evidence indicates that glutamatergic projections from mPFC to nucleus accumbens core (NAc) are involved in such reinstatement behavior. For example, reinstatement is blocked by optogenetic inhibition of mPFC terminals in NAc (Stefanik et al., 2013), as well as by intra-NAc microinfusions of an AMPA receptor antagonist (LaLumiere & Kalivas, 2008). The present study sought to provide evidence that dopamine input onto NAc-projecting mPFC cells is involved upstream of this circuit for reinstatement behavior, using a contralateral disconnection design. **Methods:** Sprague Dawley rats (n=27) were prepared with guide cannulae in the prelimbic area (PL) and NAc (contralateral hemispheres) and underwent cocaine self-administration training, followed by 7+ days of extinction. Prior to cue-induced reinstatement testing, animals received intra-PL microinfusions of either the D1/D2 antagonist fluphenazine (33.3mM/0.3µl) or vehicle, as well as either a cocktail of the NMDA/AMPA receptor antagonists AP-5 (33.8mM/0.3µl) and CNQX (3.3mM/0.3µl) or vehicle in NAc. One week following reinstatement testing, animals received similar treatments and were tested for locomotor activity. **Results:** Animals that received PL-directed fluphenazine and NAc-directed AP-5/CNQX in a contralateral fashion exhibited significantly attenuated reinstatement compared to vehicle-treated controls ($p<0.05$). Infusions of either fluphenazine or AP-5/CNQX alone in combination with vehicle in the contralateral hemisphere had no effect on reinstatement behavior. No differences were observed between treatment groups in terms of locomotor activity. **Conclusions:** These data provide evidence that dopamine acts on mPFC glutamate neurons that project to ipsilateral NAc to drive cue-induced reinstatement of cocaine seeking. These results extend those in the companion poster by McGlinchey et al that show that cue-induced reinstatement is associated with activation of PL neurons that project to NAc but not those that project to NA shell or ventral tegmental area. Studies are currently underway to characterize the electrophysiological properties of NAc-projecting mPFC cells that are responsive to dopaminergic input.

Disclosures: M.H. James: None. **G. Aston-Jones:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS award P50 DA015369

PHS award K99 DA035251

Title: Activated glutamatergic projections to the nucleus accumbens core vs. shell during cue-induced cocaine and sucrose seeking

Authors: *E. M. MCGLINCHEY, S. V. MAHLER, G. ASTON-JONES
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: The nucleus accumbens (NAc) is well established as an integral region in brain reward pathways. Glutamatergic inputs to the NAc are thought to promote motivated behaviors, including conditioned drug seeking. The three main brain regions that provide drug cue-related glutamatergic input to the NAc include the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and ventral subiculum of the hippocampus (VSub). We aimed to determine if mPFC (separated into prelimbic, PL, and infralimbic, IL, regions), BLA, or VSub neurons that project to the NAc core (NAcc) vs. NAc shell (NAcSh) are activated by cue-induced reinstatement of extinguished cocaine- or sucrose- seeking. We paired injections of the retrograde tracer cholera toxin B subunit (CTb) with Fos immunohistochemistry to investigate Fos activation of mPFC, BLA, and VSub neurons that project to NAcc and NAcSh. Rats trained to self-administer cocaine or sucrose by pressing a lever, then extinguished of this behavior. On test day, some rats received an additional extinction day, and other rats received a cue-induced reinstatement test in which cocaine- or sucrose-associated light/tone cues were presented on lever press without reward. We found that PL, BLA, and VSub neurons that project to the NAcc are activated during cued reinstatement of cocaine seeking compared to extinction of self-administration. No such differences were seen in PL neurons that project to the ventral tegmental area (VTA; Mahler 2012), in IL neurons that project to the NAcc, or in any of these regions that project to the NAcSh. We also found that the PL-NAcc circuit was uniquely activated during cocaine seeking, but not with sucrose seeking, whereas both cocaine and sucrose seeking activated NAcc-projecting BLA and VSub neurons. Moreover, the percentage of PL neurons projecting to NAcc that were also Fos+ correlated with active lever pressing during cocaine seeking, but not during sucrose seeking; such a correlation was not found for other projections studied. These results demonstrate differential activation of projections to NAcc vs. NAcSh with reinstatement of cocaine seeking. They also show marked selectivity for the PL-NAcc projection during cue-induced cocaine seeking, not seen in PL-VTA projection (Mahler, 2012). These results reveal important specificity in circuits underlying cue-induced relapse to cocaine seeking.

Disclosures: E.M. McGlinchey: None. **S.V. Mahler:** None. **G. Aston-Jones:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant R01-DA06214-25

Title: Orexin/hypocretin knockdown decreases cocaine seeking but does not alter cocaine taking

Authors: *B. A. ZIMMER, B. S. BENTZLEY, G. ASTON-JONES
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Studies indicate that hypothalamic orexin/hypocretin neurons play an important role in the psychostimulant addiction process, and that these cells are specifically involved in motivated responding for drugs of abuse. For example, orexin 1 receptor (OxR1) blockade significantly decreases behavior that requires high-effort responding such as progressive ratio performance, but not low-effort schedules such as fixed ratio-1 responding. It is thought that behavior under low-effort conditions is regulated primarily by consumptive (drug-taking) mechanisms, whereas high-effort responding is driven more by drug-seeking mechanisms. However, distinguishing between drug-taking and drug-seeking has historically been very difficult as both mechanisms are active at the same time under normal iv self-administration procedures. We used a novel within-session behavioral economics (BE) threshold procedure to quantitatively dissect and analyze both drug-taking and drug-seeking within a single self-administration session. In this study, we trained animals to self-administer cocaine and established baseline cocaine demand via behavioral economic analysis. We then microinjected vivo-morpholinos targeted to orexin into the lateral hypothalamus. This treatment has been shown to selectively knock down orexin production for several days. Rats continued to self-administer cocaine on a within-session threshold procedure during this treatment phase. BE analysis revealed that orexin knockdown attenuated cocaine demand at high effort but had no effect on hedonic setpoint (cocaine consumption under low-effort conditions). These results suggest that in a single session, the orexin system plays a crucial role in the motivational aspects of drug seeking under high-effort conditions, but is not necessary for mechanisms regulating low-effort drug consumption. These results combined with previous research indicate that the orexin system could be a productive target for clinical interventions.

Disclosures: B.A. Zimmer: None. **B.S. Bentzley:** None. **G. Aston-Jones:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Program#/Poster#: 232.10/W10

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA024038

Title: Incubation of cue-elicited cocaine-craving relates to sensitized glutamate release in the vmPFC

Authors: *C. B. SHIN, J. R. SHAHIN, M. M. SERCHIA, A. E. AGARONOVA, K. K. SZUMLINSKI
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Abstract: Relapse to drug-taking is a reoccurring phenomenon impairing addiction recovery that can be triggered by the elicitation of intense drug craving upon re-exposure to drug-paired cues. Cue-elicited drug craving increases in a time-dependent manner during drug abstinence - a phenomenon termed “incubation of craving”. The neural substrates of this phenomenon are not fully understood but may involve a sensitization of cue-elicited neurotransmitter release within the ventromedial prefrontal cortex (vmPFC). To test this hypothesis, male Sprague-Dawley rats were trained to lever-press for cocaine (0.25 mg/infusion; 6 h/day) or sucrose pellets (45 mg) and in both cases, reinforcer delivery was signaled by a light-tone compound stimulus. A control group was allowed the opportunity to lever-press for the compound stimulus only. After 10 consecutive days of self-administration training, animals were left undisturbed for either 3 or 30 days. Rats then underwent a 3-h *in vivo* microdialysis session in the operant chamber, during which animals were allowed to respond for presentation of the cues, in the absence of reinforcer delivery. As expected, cocaine-trained animals exhibited a time-dependent intensification of cue-reinforced responding that was selective for the lever that previously delivered cocaine. On the other hand, sucrose and control animals did not exhibit a difference in responding across withdrawal. The opportunity to lever-press for cocaine-paired cues elicited a time-dependent sensitization of vmPFC glutamate release during withdrawal, while the capacity of cocaine-cues to elevate extracellular dopamine dissipated with withdrawal. In contrast, the changes in vmPFC glutamate and dopamine elicited by sucrose-paired or neutral cues did not vary with the passage of time during withdrawal. These data provide novel evidence that the glutamate responsiveness of the vmPFC incubates during protracted withdrawal in parallel with the behavioral responsiveness to cocaine-paired cues. This heightened cortifugal glutamate drive likely contributes to the desensitization of vmPFC mGluR1/5 receptors underpinning extinction

learning deficits reported in “incubated” animals and pose pharmacotherapeutic strategies that curb corticofugal glutamate responsiveness to cocaine-paired cues as a viable strategy for facilitating addiction recovery.

Disclosures: **C.B. Shin:** None. **M.M. Serchia:** None. **J.R. Shahin:** None. **A.E. Agaronova:** None. **K.K. Szumlinski:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA031401

NIDA IRP

Title: A novel combination treatment of levo-tetrahydropalmatine (l-THP) and low dose naltrexone (LDN) for the prevention of cocaine relapse

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Abstract: l-THP is a promising agent for the treatment of substance abuse related disorders. It functions primarily as a modest dopamine antagonist and has been previously reported to be effective in attenuating cocaine-taking and cocaine-seeking behavior in rats. However l-THP’s dopamine antagonism produces some sedative side-effects. Interestingly when LDN is co-administered with l-THP, the sedative side-effects appear to be diminished. LDN (<0.5mg/kg) is proposed to regulate endogenous opioid release. Recent research suggests that long-term use of cocaine induces alterations in the mesolimbic dopamine pathway and the endogenous opioid system, which may play an important role in the pathophysiology of cocaine addiction. Based on these, we propose that the L-THP and LDN combination medication, which targets both the dopamine and endogenous opioid systems, may be more promising at preventing relapse. In the present study, we examined the combination treatment l-THP-LDN as a therapeutic agent for the prevention of cocaine relapse. Male Wistar rats were trained to self-administer cocaine under fixed ratio 2 (FR2) reinforcement to assess the effect of l-THP-LDN (5 mg/kg l-THP + 0.1 mg/kg LDN and 3 mg/kg l-THP + 0.1 mg/kg LDN) on cocaine self-administration. Additional

groups of rats were first trained to self-administer cocaine and then went to extinction to assess the effect of chronically administered l-THP-LDN on cocaine-induced reinstatement of drug-seeking behavior. We found that the combination of 5 mg/kg l-THP and 0.1 mg/kg LDN is more efficacious in attenuating intravenous cocaine self-administration and cocaine-induced reinstatement of drug-seeking behavior than l-THP or LDN alone. The combination of l-THP-LDN also attenuates l-THP-induced sedative effect as assessed by open field locomotor measurement. We conclude that the combination of l-THP and LDN could be a more effective pharmacotherapy with less unwanted sedative effect for the prevention of cocaine relapse in humans.

Disclosures: S.A. Sushchyk: None. **J. Wang:** None. **Z. Xi:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA 021261

Mark Diamond Research Fund Grant SP-14-16

Title: Neuropeptide Y attenuates cocaine-induced vocalizations and reinstatement behavior in the rat

Authors: *L. M. STRAND[1], C. P. KING[1], M. SUAREZ[1], E. NEILANS[1], D. HOLFOTH[1], J. M. DIPIRRO[2], M. L. DENT[1], A. C. THOMPSON[1]
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Abstract: Neuropeptide Y (NPY) is a neurotransmitter found within neural substrates associated with drug use and dependence (e.g., mesolimbicortical pathway) and known to modulate dopaminergic and glutamatergic neurotransmission. Our research has investigated the role of NPY in cocaine relapse. Here we tested the hypothesis that increasing central NPY attenuates (a) cocaine-prime induced reinstatement of cocaine self-administration (SA) and (b) cocaine-evoked 50 kHz vocalizations (USV) in rats with a history of cocaine use. Male Long-Evans rats were allowed to self-administer cocaine (0.5 mg/kg, IV) for 21 3-h sessions by snout-poking into the active hole which was a randomly-assigned hole in the wall. Each snout-poke into the active

(cocaine-paired) hole initiated a cocaine infusion and a cued 25-sec timeout period in which no cocaine infusions were allowed. After 3 weeks, snout poking in the active hole was extinguished by exposure to the SA procedure, without cocaine infusions, for 21 sessions. After extinction, rats were tested twice for reinstatement of the snout-poking response: once after a cocaine prime (10 mg/kg, IP) and once after a saline prime (1 ml/k, IP). NPY was administered (0 or 0.30 nmol, ICV) 30 min before each reinstatement test. Active and inactive snout-poking responses were measured to assess reinstatement of SA behavior. The number of 50 kHz USVs was measured during the first 30 min of the reinstatement session to quantify the USV intensity. As expected, the cocaine prime significantly increased the frequency of snout-poking into the active "cocaine-paired" hole relative to that observed during the last extinction session, that observed in saline-primed rats, and that observed for the inactive (non-cocaine paired) holes. Similarly, the cocaine-prime, but not the saline-prime, significantly increased the frequency of USVs during the reinstatement session (508 ± 67 vs 31 ± 11.4 50 kHz USVs respectively). ICV NPY significantly reduced both the number of active hole snout-pokes and the frequency of 50 kHz USVs during the cocaine primed reinstatement session but had no effect on these measures in the control saline-primed reinstatement session (50.3± 30.2 vs 6.7±1.2 50 kHz USVs respectively). These findings support the idea that central administration of NPY decreases the strength of cocaine and cocaine-associated cues to induce drug seeking behavior and decreases the intensity of 50 kHz USV induced by cocaine. As previous literature has suggested that 50 kHz USV are emitted in response to rewarding stimuli, these results suggest that NPY administered before cocaine, reduces its rewarding value.

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Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant T32 DA007027

NIH Grant R01 NS070714

Title: Effects of caffeine and its primary metabolite, paraxanthine, administered alone or in combination with cocaine on intracranial self-stimulation in rats

Authors: *M. F. LAZENKA[1], F. G. MOELLER[2], S. S. NEGUS[1]
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Abstract: Intracranial self-stimulation (ICSS) is one behavioral procedure for preclinical evaluation of abuse liability of drugs. Caffeine is the most widely used psychostimulant in the world, yet little research has been done to determine its effects on intracranial self-stimulation (ICSS). Further, the primary metabolite of caffeine, paraxanthine, has not been tested in ICSS, even though its efficacy to increase locomotor activity and cause dopamine release in the striatum is greater than that of caffeine. This study compared the potency and time course of effects produced by caffeine (3.2-32 mg/kg) and paraxanthine (3.2-32 mg/kg) in a frequency-rate ICSS procedure in rats. Effects of caffeine (32 mg/kg) or paraxanthine (32 mg/kg) in combination with cocaine (10 mg/kg) were also examined, because caffeine and paraxanthine are under consideration as candidate agonist medications that might attenuate abuse-related cocaine effects. Male Sprague-Dawley rats were equipped with electrodes targeting the medial forebrain bundle and trained to respond under a fixed-ratio 1 schedule for electrical stimulation that varied by frequency (56-158 Hz, 0.05 log increments) within each session. Each stimulation consisted of a 0.5 sec train of square-wave 100 μsec pulses at the designated frequency, and stimulation was accompanied by onset of stimulus lights located over the lever. These 0.5 sec periods of stimulation delivery served as time out periods, such that responding during a stimulation produced no scheduled consequences. Rates of reinforced responding (i.e. responses that produced stimulation) and time out responding (i.e. responses during a stimulation) were evaluated as a function of stimulation frequency. In the absence of drug, rats maintained a frequency-dependent increase in both reinforced and time out responding, with time out responding ~5-10% of reinforced response rates. Caffeine significantly facilitated both reinforced and time out responding at 10 mg/kg, as defined by a leftward shift of the ICSS curve, while 3.2 mg/kg and 32 mg/kg produced no effect. Paraxanthine did not alter reinforced responding, but 32 mg/kg paraxanthine significantly increased time out responding. Cocaine facilitated both reinforced and time out responding and pretreatment with either caffeine or paraxanthine failed to alter cocaine-induced facilitation of ICSS. These studies are consistent with the weak abuse liability of caffeine and paraxanthine. In addition, these studies did not yield evidence for a decrease in abuse-related cocaine effects following acute pretreatment with caffeine or paraxanthine.

Disclosures: **M.F. Lazenka:** None. **F.G. Moeller:** None. **S.S. Negus:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Support: MBRS-RISE R25-GM061838

Title: Role of Oxytocin on the anxiety environment-elicited cocaine seeking behavior and cocaine conditioning

Authors: *A. MORALES-RIVERA[1], J. PEREZ[2], C. M. COLON[3], C. PEREZ[2], C. S. MALDONADO[2]
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Abstract: Several clinical studies have revealed that the maintenance of cocaine addiction requires three key contributors: (a) the reinforcing effects evoked by cocaine intake, (b) the environmental stimuli associated with the drug, and (c) the stress (Sinha, 2003). Moreover, a stress stimulus triggers the reinstatement of cocaine seeking behavior, following long periods of abstinence in dependent subjects. The neuronal and anatomical basis of this behavioral phenomenon deserves further investigation. Oxytocin (OT), a neuropeptide produced within the hypothalamus-pituitary axis (HPA) axis, has been related to reward, learning, memory and stress, events that previously have been associated with cocaine addiction. Previously published data from our laboratory demonstrated an increase in mRNA OT levels within the NAc by acute and chronic cocaine exposure (Borrero 2009). Furthermore, it was demonstrated that OT treatments prior to cocaine conditioned stimuli resulted in a significant decrease in anxiety levels of the animals. These results support the notion that OT is involved in the anxiogenic effects of cocaine actions and might play a modulatory role in the rewarding properties of cocaine. The present set of experiments aims to examine the role of OT on environmentally elicited cocaine-seeking behavior and whether OT could reduce anxiety associated with this behavior. OT bind to its receptors distributed widely within several brain regions and upon binding OT triggers a cascade of intracellular events that mediate OT effects. OT receptor activation via PKC phosphorylate extracellular signal-regulated kinase 1 /2 (ERK1/2) within Hypothalamus pituitary axis (HPA). Thus we hypothesized that OT is modulating the stress/anxiety response triggered by environmentally elicited cocaine seeking behavior via MAPK/ERK1/2 within the amygdala and the NAc regions. Results showed that OT pre-treatment significantly reduced reinstatement of cocaine-seeking behavior. Most significantly, OT treatment reduced the anxiety triggered by cue-induced reinstatement conditions and cocaine-paired conditioned locomotion. The present studies demonstrate for the first time that OT actions within the brain mediate the anxiety response triggered by cues previously paired with cocaine intake.

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Poster

232. Cocaine: Neural Mechanisms I

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Program#/Poster#: 232.15/W15

Topic: C.17. Drugs of Abuse and Addiction

Title: Potentiation of behavioral sensitization by the co-administration of methylenedioxypyrovalerone (MDPV) and cocaine in rodents

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Abstract: Reports of abuse and fatal intoxication associated with synthetic cathinones known as "bath salts" are on the rise nationwide. Methylenedioxypyrovalerone (MDPV), one of the primary constituents of bath salts, is commonly used in combination with other drugs such as cocaine (COC) to enhance or prolong stimulant effects. Recent studies have indicated MDPV is a catecholamine transport inhibitor similar to but more potent than COC. Co-administration of these substances may therefore result in additive or synergistic effects. The combined behavioral effects of MDPV and COC have not yet been thoroughly examined. Behavioral sensitization following repeated psychostimulant exposure is considered an index of neuroadaptive changes in brain dopamine reward pathways. The present study sought to investigate the effects of repeated, concomitant administration of MDPV and COC on the development of sensitization and on cross-sensitization to COC. Four groups of male Sprague-Dawley rats were treated with subcutaneous injections of 0.5 mg/kg MDPV, 5 mg/kg COC, 0.5 mg/kg MDPV + 5 mg/kg COC, or saline. Treatments were conducted once daily for 7 consecutive days with locomotor assessments on the first and last day of treatment. Following a 10-day washout period, all groups were administered a challenge dose of 5 mg/kg COC. Behavioral indices evaluated were horizontal activity, vertical activity, and stereotypy counts. Horizontal activity and stereotypy counts in both the MDPV alone and MDPV+COC groups were significantly higher on Day 7 compared to Day 1 with a more pronounced effect in the MDPV+COC group, and vertical activity was significantly higher in the MDPV+COC group only. Furthermore, COC administration following the 10-day washout period significantly increased horizontal activity and stereotypy counts in animals previously treated with MDPV+COC compared to animals

previously treated with saline. These results indicate the development of sensitization and cross-sensitization to COC is enhanced by concurrent administration of MDPV+COC. Moreover, these findings warrant further assessment of the enhanced risk of developing addiction with concurrent MDPV and COC use.

Disclosures: **M.M. Peet:** None. **E.L. Harvey:** None. **J.L. Walters:** None. **L.E. Baker:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Title: Substance-specific influences of setting on drug reward: An ultrasonic vocalization study in rats self-administering heroin and cocaine

Authors: *R. AVVISATI[1], L. CONTU[1], E. STENDARDO[1], C. MONTANARI[1], M. L. SCATTONI[2], A. BADIANI[1]
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Abstract: Clinical and preclinical evidence indicate that the setting of drug use affects drug reward in a substance-specific manner. When heroin and cocaine co-abusers, for example, report on the circumstances of drug use, they indicate distinct settings for the two drugs: heroin being used preferentially at home and cocaine preferentially outside the home (Caprioli et al. 2009; Badiani and Spagnolo 2013). Similar results were obtained in rats that were given the opportunity to self-administer intravenously both heroin and cocaine. Rats living in the self-administration (SA) chambers (Resident rats) exhibited in fact a preference for heroin vs. cocaine, whereas rats that were transferred to the SA chambers only for the test sessions (Non-Resident rats) tended to prefer cocaine to heroin (Caprioli et al. 2007, 2008, 2009). It has been hypothesized that the emotional appraisal of drug effects varies as a function of the setting (Badiani 2013). The sympathomimetic and arousing effects of cocaine, for example, would be appraised as unsuitable to a familiar home environment whereas the sedative effects of heroin would be appraised as unsuitable to novel, potentially dangerous environments. Hence, cocaine would be less rewarding at home than outside the home whereas heroin would be less rewarding outside the home. The hypothesis that cocaine produces a more positive affective state when rats self-administer it outside the home relative to the home environment (and vice versa for heroin)

was investigated here by recording ultrasonic vocalizations (USVs) during drug SA. It has been reported that rats emit USVs in the range of 50-kHz when exposed to rewarding stimuli, suggesting that 50-kHz USVs reflect positive affective states (Burgdorf et al. 2000, 2001, 2008). We found that, overall, Non Resident rats emitted more 50-kHz USVs than Resident rats, indicating a state of heightened arousal during exposure to a novel environment. Most important, we found that Non-Resident rats emitted more 50-kHz USVs when they self-administered cocaine than when self-administered heroin whereas Resident rats emitted more 50-kHz USVs when self-administering heroin than when self-administering cocaine. Differences in USVs in Non Resident rats were more pronounced during the first SA session (78±22,3 for cocaine vs. 43±23,5 for heroin), that is when the SA chambers were completely novel to them. In contrast, the differences in USVs in Resident rats were more pronounced during the last SA sessions (38±16,9 for cocaine vs. 100±45,7 for heroin). These findings indicate that the setting of drug taking exerts a substance-specific influence on the ability of drugs to induce positive affective states.

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Poster

232. Cocaine: Neural Mechanisms I

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Topic: C.17. Drugs of Abuse and Addiction

Title: The sensitivity of preclinical abuse potential models to detect reinforcing signals of the local anesthetic procaine

Authors: *D. B. HORTON, E. DUNN-SIMS, A. FOOTE, A. N. MEAD
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Abstract: Local anesthetics, such as procaine, have an extensive history of medical use and act primarily through sodium channel inhibition. While procaine is not scheduled and widespread misuse of procaine does not exist, data from preclinical and clinical studies reveal weak reinforcing signals. Furthermore, the novel sodium channel blocker lacosamide was recently designated a schedule V drug, consistent with a drug thought to possess low potential for abuse. The purpose of the current study was to further characterize the discriminative stimulus and reinforcing properties of procaine in two “classic” preclinical models of abuse potential

assessment, drug discrimination and self-administration. Specifically, the discriminative stimulus properties of procaine (0-60 mg/kg, IP) and cocaine (0-10 mg/kg, IP) were examined in rats which had previously been trained to perform an operant discrimination task based on the interoceptive cue induced by cocaine (9 mg/kg, IP). Additionally, the ability of procaine (0-10 mg/kg/infusion) to support IV self-administration in rats previously trained to self-administer cocaine (0.75 mg/kg/infusion) was determined. As expected, cocaine engendered partial generalization at 2.5 and 5 mg/kg and full generalization at 10 mg/kg. Procaine (30 and 60 mg/kg) produced partial generalization to the cocaine discriminative cues and examination of individual animal data revealed two populations, with some animals displaying full generalization to cocaine and the remaining animals showing little to no generalization. Furthermore, results showed that procaine (1 and 3 mg/kg/infusion) robustly supported self-administration with increases in both infusion rates and active lever responses compared to vehicle. The dose response followed an "inverted U-shape" pattern, similar to that induced by many drugs of abuse in this model. Furthermore, an increase in active lever selectivity was observed at all procaine doses. Analysis of response patterns revealed that procaine maintained inter-infusion intervals with a regularity similar to those maintained by cocaine, suggesting clear control of drug intake. In conclusion, the current study expands on existing data showing that drugs which are not widely abused can display clear reinforcing or discriminative signals in models in which experimental parameters are optimized to detect such signals. These findings are consistent with the perception that preclinical models of abuse potential can be exquisitely sensitive to the reinforcing or discriminative effects of drugs with minimal abuse potential, and that data from such experiments should be interpreted with this information in mind.

Disclosures: **D.B. Horton:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **E. Dunn-Sims:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **A. Foote:** A. Employment/Salary (full or part-time):; Pfizer, Inc. **A.N. Mead:** A. Employment/Salary (full or part-time):; Pfizer, Inc..

Poster

232. Cocaine: Neural Mechanisms I

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Use of facilities at the Michael E. DeBakey VA Medical Center, Houston, TX

Title: Cocaine addiction pharmacogenetics of buprenorphine therapy

Authors: *D. A. NIELSEN[1,2], R. WALKER[3], S. C. HAMON[4], M. H. HARDING[1,2], W. LING[5], T. R. KOSTEN[1,2]
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Abstract: The aim of this study was to identify genetic markers that modulate therapeutic response to buprenorphine/naloxone (Suboxone) treatment of cocaine addiction. Since buprenorphine is a μ-opioid, δ-opioid, and nociceptin receptor full agonist, as well as a κ-opioid receptor partial agonist, we have focused our analysis to several genes of the opioidergic system. A clinical trial, the CTN-0048 Cocaine Use Reduction with Buprenorphine, was conducted with 302 cocaine-dependent subjects who had past-year opioid dependence/abuse, or past-year opioid use and a history of lifetime opioid dependence. Subjects were randomly assigned for 8 weeks to one of three daily medication arms on a platform of naltrexone: 4 mg buprenorphine, 16 mg buprenorphine, or placebo. Subjects also received once-weekly cognitive behavioral therapy. DNA was obtained from 234 subjects. Six genes were genotyped for a total of 13 variants. Treatment efficacy was evaluated by the percent cocaine positive urines per two week period. Modest reductions in cocaine positive urines were observed in both the 4mg and 16 mg buprenorphine groups. Since these reductions were similar, the two buprenorphine groups were combined and compared with the placebo group. Using repeated measures ANOVA, significant interactions of variant x treatment were observed for two variants in the *proopiomelanocortin* (*POMC*) gene (both experiment-wise p=0.003). Buprenorphine decreased positive urines from 43% to 39% and placebo increased positive urines from 57% to 60% in the *POMC* rs1009388 C allele-carrier group. No difference was observed in the GG genotype group between those receiving placebo and buprenorphine. These results suggest that POMC peptides may be targets for subsequent studies on cocaine addiction pharmacotherapy.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA-027115

NIH DA-027525

Title: Cocaine self-administration results in a distinct epigenetic state in the dorsal medial prefrontal cortex

Authors: *K. PLOENSE[1], A. CARR[2], D. BAKER-ANDRESEN[3], N. WOODWARD[2], X. LI[3], Y. SUN[4], T. BREDY[3], T. KIPPIN[2]
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Abstract: DNA methylation is a key determinant of gene expression and is implicated in neuroplasticity relevant to addiction. Here, we examine DNA methylation and mRNA expression for two genes, Homer2 and NPAS4, within the dorsal medial prefrontal cortex (dmPFC) following saline, short-access (1h), or prolonged-access (6h), to cocaine during self-administration. As reported previously, only 6h rats exhibited escalated cocaine intake across sessions. Initial MeDIP-CHIP analyses of dmPFC tissue by ChIPMonk software revealed dramatic and extremely gene-specific changes in DNA methylation/demethylation within promoter regions and flanking the transcription start site (TSS). The Homer2 and NPAS4 promoters showed active changes in DNA methylation following 6h and 1h cocaine self-administration compared to saline. A follow-up analysis, via sodium bisulphite conversion followed by mass spectrophotometry interrogating a 1000bp sequence of the Homer2 promoter, confirmed increased methylation of CpG7 in the 6h rats, which contains putative consensus sequences for GATA-1 and p300. Additionally, MeDIP followed by qPCR revealed an increase in DNA methylation for both 1h and 6h rats within the 457-645bp upstream of the NPAS4 transcription start site (TSS).qPCR revealed an increase in Homer2 mRNA levels in the 6h versus 1h and saline conditions, and a decrease in NPAS4 mRNA in 1h and 6h versus saline conditions. These results demonstrate that prolonged cocaine self-administration alters DNA methylation of the Homer2 and NPAS4 gene promoters within the dmPFC in a distinct manner. Additionally, the altered DNA methylation leads to decreases in NPAS4 mRNA and increases in

Homer2 mRNA expression within the dmPFC. Given the known roles of Homer2 as a scaffolding protein for the metabotropic glutamate 1 and 5 (mGluR1/5) receptors and NPAS4 as a transcription factor promoting inhibitory synapse formation, these data indicate that excessive cocaine intake induces changes in gene regulation for inhibitory and excitatory synapses. Thus, excessive cocaine intake induces a distinct change in DNA methylation which may contribute the long-term alterations in brain function associated with addiction.

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Poster

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The Adelson Medical Research Foundation (MJK)

Title: Predisposing genetic differences in opioid peptides and receptors mRNA levels contribute to the escalation of cocaine intake in rats

Authors: *M. VALENZA, R. PICETTI, V. YUFEROV, M. KREEK
Rockefeller Univ., New York, NY

Abstract: Vulnerability to drug addiction is influenced not only by environmental factors but also by genetic heritability. Since endogenous opioids play a central role in mediating the reinforcing properties of drugs of abuse, including cocaine, the expression of genes involved in those pathways may contribute to the vulnerability to abuse. Therefore aim of the present study was to investigate on the expression of genes coding opioid peptides and receptors in reward-related brain regions to determine their contribution to the vulnerability to escalate in cocaine consumption. We compared two inbred strains known to behave differently when exposed to drugs of abuse, such as Fischer and Lewis rats. Rats were daily exposed to cocaine for 18 h per day, and were allowed to choose the dose to self-administer, closely modelling the human condition of cocaine exposure. Fischer rats had a stable intake while Lewis escalated

progressively their consumption over 14 days. Rats were sacrificed after 24 h withdrawal. Saline-yoked rats were used as control. By quantitative PCR, we found interesting differences between strains in the basal mRNA levels of some genes, e.g. in saline-yoked Lewis rats, never exposed to cocaine, the basal hypothalamic prodynorphin, proenkephalin and delta opioid receptor gene expression were higher than in Fischer rats. The basal delta opioid receptor mRNA levels were found significantly higher in the core of the nucleus accumbens of Lewis compared to Fischer rats. Lewis control rats showed also a lower level of mu opioid receptor gene expression than Fischer saline rats in the nucleus accumbens shell. Prodynorphin mRNA levels were found increased in both the nucleus accumbens shell and in the hypothalamus of Fischer rats exposed to cocaine compared to yoked-saline rats. Such changes were not found in Lewis rats which escalate their cocaine intake. The present results corroborate the hypothesis that predisposing genetic factors are crucially important in the escalation of intake in rodent models of cocaine addiction.

Disclosures: **M. Valenza:** None. **R. Picetti:** None. **V. Yuferov:** None. **M. Kreek:** None.

Poster

232. Cocaine: Neural Mechanisms I

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Program#/Poster#: 232.21/W21

Topic: C.17. Drugs of Abuse and Addiction

Support: Howard Hughes Medical Institute

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Title: Neuron-subtype specific transcriptional changes following cocaine self-administration in mice

Authors: ***E. C. ANDRADE**[1], E. F. SCHMIDT[1], J. L. WARNER-SCHMIDT[2], J. ALVAREZ[1], S. B. PICKETT[1], P. GREENGARD[2], N. HEINTZ[1]
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Abstract: Studies aimed at understanding the molecular basis of drug addiction have focused largely on the mesolimbic dopamine system and related structures. From these studies, long-lasting alterations in transcription are a clear consequence of repeated exposure to drugs of

abuse. What remain elusive are the specific contributions of neuronal subtypes within these regions to addiction. To better understand the contributions of different cell-types, bacterial artificial chromosome (BAC) transgenic mice which drive the expression of EGFP-tagged ribosomal protein L10a in defined cell populations (known as bacTRAP transgenic lines) were trained to self-administer cocaine. Cell-types of interest include those found in the mesolimbic dopamine system, such as striatal and cortical neurons. Following 3 weeks of cocaine self-administration, polysomal mRNA's were isolated by affinity purification and analyzed by RNA-seq to identify translational profiles of each cell type. Results from these studies provide insight into the neurocircuitry most affected by cocaine use as well as classes of genes most affected.

Disclosures: E.C. Andrade: None. **E.F. Schmidt:** None. **J.L. Warner-Schmidt:** None. **J. Alvarez:** None. **S.B. Pickett:** None. **P. Greengard:** None. **N. Heintz:** None.

Poster

232. Cocaine: Neural Mechanisms I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 232.22/W22

Topic: C.17. Drugs of Abuse and Addiction

Title: Nicotine and Cocaine induce temporally-regulated, drug-specific and genome-wide changes in nucleosome occupancy

Authors: *A. N. BROWN[1], J. H. DENNIS[2], P. G. BHIDE[3]
[1]Florida State Univ. Col. of Med., Ctr. for Brain Repair, Biomed. Sci., Tallahassee, FL; [2]Dept. of Biol. Sci., Florida State Univ., Tallahassee, FL; [3]Ctr. for Brain Repair, Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: Drugs of abuse alter the expression of numerous genes throughout the brain that ultimately manifest as behavioral alterations. DNA methylation and histone modifications resulting from drug use have been shown to repress or permit transcription of specific genes by altering local chromatin structure and DNA accessibility. These epigenetic changes may be transient or persist long after drug-use has ceased. In the last several years, changes in DNA methylation and histone modifications following exposure to drugs such as nicotine or cocaine, in correlation with corresponding transcriptional and behavioral alterations, have been well characterized for a variety of genes, *in vitro* and *in vivo* models. However, the underlying molecular mechanisms facilitating these drug-induced epigenetic changes, specifically the masking or unmasking of the DNA to the action of transcription factors and regulatory

machinery, have remained elusive. We exposed human neuroblastoma cells (SH-SY5Y) to nicotine or cocaine, two of the most highly abused drugs in our society today, to examine changes in nucleosome occupancy. Nucleosome occupancy also restricts accessibility of DNA; nucleosome-bound DNA is unavailable for binding by other trans-acting factors, and sets the stage for alterations in transcriptional capability. Using Comparative Genome Hybridization (CGH) to examine the genomic distribution of nucleosomes following exposure to either nicotine or cocaine has revealed widespread, drug- and time-dependent changes in nucleosome occupancy. These drug-induced changes can be predicted based on DNA sequence and often coincide with transcription factor binding sites. However, presence/absence of nucleosomes does not regulate transcription *de facto*. Based on our data, we propose that the effects of drugs of abuse on nucleosome positioning represent an initial genome-wide alteration of the intracellular transcriptional capability, followed by cell-type specific responses in the cascade of downstream signaling or gene expression events. Detailed characterization of the mechanisms and effects of these drug-specific alterations in chromatin structure will further our understanding of drug-induced behavioral responses, pathologies, mechanisms of transgenerational inheritance and potential therapeutic targets for future studies.

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Poster

232. Cocaine: Neural Mechanisms I

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 232.23/W23

Topic: C.17. Drugs of Abuse and Addiction

Title: Alterations in behavioral regulation and Pavlovian conditioning in cadherin-13 (Cdh13) knock-out rats

Authors: C. P. KING[1], C. VERSAGGI[1], L. MILITELLO[2], J. CATLIN[1], A. A. PALMER[3], J. B. RICHARDS[2], *P. MEYER[1]
[1]Psychology, Univ. at Buffalo, Buffalo, NY; [2]Res. Inst. on Addictions, Univ. at Buffalo, Buffalo, NY, NY; [3]Dept. of Human Genet., Univ. of Chicago, Chicago, IL

Abstract: Variants of the cadherin-13 (*CDH13*) gene have been associated with the subjective response to amphetamine, drug addiction, and attention deficit hyperactivity disorder (ADHD). To examine the role of CDH13 in rodent behaviors related to these disorders, *Cdh13* knockout rats were tested in five paradigms: sensory reinforcement, responding for saccharin, choice

reaction time task, Pavlovian conditioned approach, and cocaine-induced conditioned place preference. Knockout rats responded significantly more for a saccharin reinforcer. Knockout rats also acquired the choice reaction time task more slowly than wild types, but had similar reaction times and number of premature responses once the task was learned. There was also a significant effect of genotype on conditioned place preference, where knockout rats failed to develop a preference for a cocaine-paired cue. There were no differences between knockout and wild-type rats in sensory reinforcement, responding for water, or Pavlovian conditioned approach. Taken together, these data suggest that *Cdh13* is involved in the sensitivity to palatable rewards as well as the conditioned effects of cocaine. Future studies will examine whether these differences depend on sex and extend to drug self-administration phenotypes.

Disclosures: C.P. King: None. **C. Versaggi:** None. **L. Militello:** None. **J. Catlin:** None. **A.A. Palmer:** None. **J.B. Richards:** None. **P. Meyer:** None.

Poster

233. Catecholamines and Behavior

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: C.18. Behavioral Pharmacology

Support: NSERC Discovery Grants 385732-2012

Title: Behavioural consequences of monoamines signaling alterations in the new conditional VMAT2 mutant mice

Authors: *Q. RAINER, E. ISINGRINI, L. PERRET, M.-E. DESAULNIERS, L. MOQUIN, A. GRATTON, B. GIROS
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Abstract: Investigations of the precise role of monoaminergic signaling, norepinephric (NE), serotoninergic (5HT) and, dopaminergic (DA) has led to the creation of VMAT2 (Vesicular Monoaminergic Transporter) knockout (KO) mice. While it results in death within few days after birth, VMAT2 heterozygous (Het) mice are viable and display 50% reduction in VMAT2 expression associated with a specific behavioural phenotype. To unravel the specific implication of NE, 5HT and DA in the behavioral effects observed in the constitutive VMAT2-HET mice in which the 3 systems are compromised, we created conditional VMAT2 models. The floxed gene

VMAT2 is specifically spliced in the NE, 5HT or DA neurons by the cre-recombinase expressed under the control of the DBH, SERT or DAT genes respectively. In KO mice, an absence of VMAT2 is revealed respectively and specifically in the LC, in the raphe and in the VTA and SN. A whole brain HPLC study indicates a drastic decrease of NE, 5HT or DA respectively. While complete depletion of DA results in death after 3 weeks, mice with 5HT or NA depletion are viable. Behavioral study of the conditional VMAT2-HET mice specific to NE, 5HT or DA neurons allow us to shed light on the individual and collective contributions of DA and NA in locomotion and the one of DA and 5HT in drugs response. However, independent alteration of only one system is not sufficient to induce depression-like behaviour. This study allows determining the exact contributions of NE, 5HT and DA in specific behaviour associated with psychiatric disorders.

Disclosures: Q. Rainer: None. **E. Isingrini:** None. **L. Perret:** None. **M. Desaulniers:** None. **L. Moquin:** None. **A. Gratton:** None. **B. Giros:** None.

Poster

233. Catecholamines and Behavior

Location: Halls A-C

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Topic: C.18. Behavioral Pharmacology

Support: NSERC Discovery Grant 385732-2012

Title: Norepinephrine (NE) function in basal and acute stress conditions with a new NE depletion model: the conditional VMAT2 knockout mice

Authors: *L. C. PERRET, E. ISINGRINI, L. MOQUIN, M.-E. DESAULNIERS, A. GRATTON, B. GIROS
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Abstract: The noradrenergic (NE) neurons, mainly located in the locus coeruleus (LC), have been linked to psychiatric disorders involving anxiety and depression. Previous studies have shown that changes in NE transmission from the LC influence anxio/depressive-related behaviors. The purpose of this study was to develop a viable model with NE depletion in the central nervous system without affecting the periphery to investigate NE functions. The NE depletion model was developed using the cre recombinase expressed by the dopamine β-hydroxylase (DBH) genes, which splices out the floxed VMAT2 gene (Vesicular

Monoaminergic Transporter). The VMAT2DBHcre KO mice were validated by a lack of VMAT2 mRNA expression specifically in the LC and NE depletion in the entire brain. The assessment of development and motor abilities confirmed VMAT2DBHcre KO as viable subjects for further testing. VMAT2DBHcre KO mice demonstrated less anxiety- and depression-related behaviors. Moreover, tissue level of DA and 5HT were altered in different brain structures associated with mood and motivational behavior. While basal corticosterone levels were not affected in the VMAT2DBHcre KO mice, acute stress through physical restraint revealed a faster return to basal corticosterone levels in VMAT2DBHcre KO mice. However, prior to the restraint, dexamethasone injections did not yield further differences between wildtype and KO mice. All together, these observations showed a predisposition for VMAT2DBHcre KO mice to exhibit an altered susceptibility to developing anxiety and depression related psychiatric disorders.

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Poster

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Topic: C.18. Behavioral Pharmacology

Support: NSERC grant discovery 385732-2012

FRQS

Title: Unravelling norepinephrine function in cognition with the new conditional VMAT2 knock-out mice with brain-specific norepinephrine depletion

Authors: *E. ISINGRINI, L. PERRET, N. EILSTEIN, V. GORGIEVSKI, E. GUMA, M.-E. DESAULNIERS, L. MOQUIN, A. GRATTON, B. GIROS
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Abstract: The norepinephrine (NE) system extensively modulates cognitive functions, in particular via its ascending projections to the prefrontal cortex, the hippocampus and the limbic circuitry. In order to unravel the specific role of NE in learning, memory and executive functions, we took advantage of a new conditional knock-out (KO) mouse model for the

Vesicular Monoamine Transporter type-2 (VMAT2) gene. To generate VMAT2DBHcre mice, a floxed VMAT2 gene is specifically spliced in NE neurons by the cre recombinase that have been introduced in a BAC-DBH (dopamine β-hydroxylase) cassette. Because the VMAT2 is almost not expressed at the periphery (where VMAT1 is present), this conditional KO is brain-specific. Contrarily to the DBH-KO mice that died shortly after birth, VMAT2DBHcre mice are viable and show no obvious developmental deficits. In the VMAT2DBHcre-KO mice, autoradiographic *in situ* hybridization confirmed the absence of VMAT2 expression specifically in the locus coeruleus (LC) and whole brain HPLC showed a drastic decrease of NE. An exhaustive characterization of learning and memory processes was performed. Our results indicate that NE depletion does not alter 1) spatial memory in the Morris water maze paradigm, including both long term and working memory, and 2) recognition memory both in the object and place recognition test. In the fear conditioning test, while no alteration was found in the cued test, VMAT2DBHcre-KO mice exhibited a significant increase in freezing behavior during the contextual recall test, indicating a specific impairment in emotional memory. Finally, executive functions were studied in the attentional set shifting task including a reversal, an intra-dimensional and an extra-dimensional shifting stage. While VMAT2DBHcre-KO and WT mice had similar performances in the reversal and intra-dimensional shift stages, VMAT2DBHcre-KO mice exhibited an increased tendency in the number of trials needed to rich criteria in the extra-dimensional shift stage. With this new model of NE depletion in the whole brain, we demonstrated the specific implication of NE in contextual emotional memory recall and in executive functions such as shifting behavior. Increased fear reactivity and maladaptive shifting behaviors are symptoms frequently associated with affective disorders, especially anxiety, panic disorders and post-traumatic stress disorders. Based on these observations, this model of complete NE depletion constitutes a valuable tool in order to shed light on the neurocircuitry and mechanisms implicated in such disorders.

Disclosures: E. Isingrini: None. **L. Perret:** None. **N. Eilstein:** None. **E. Guma:** None. **V. Gorgievski:** None. **L. Moquin:** None. **A. Gratton:** None. **B. Giros:** None. **M. Desaulniers:** None.

Poster

233. Catecholamines and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 233.04/W27

Topic: C.18. Behavioral Pharmacology

Title: Binge-eating behaviour produces alterations in dopaminergic neurochemistry in the brains of rats

Authors: *S. C. CHEETHAM[1], J. GOSDEN[1], M. R. PROW[1], P. H. HUTSON[2], D. J. HEAL[1]
[1]RenaSci Ltd, Nottingham, United Kingdom; [2]Shire Develop. Inc, Wayne, PA

Abstract: Binge-eating disorder (BED) is a common psychiatric condition, affecting ~2% of adults. It presents as compulsive, excessive consumption of highly palatable foods. BED is associated with obesity, but a significant proportion of BED sufferers are normal weight. We developed a rodent model where freely-fed rats are given irregular, limited access to chocolate. Rats develop robust, intermittent, hyperphagia of palatable food with concomitant reductions in consumption of normal chow and as a result maintain normal bodyweight. This rodent model mirrors human BED without obesity [Vickers et al, 2013 SfN, Abstract 236/03.]. Recent evidence linked eating disorders with CNS dopaminergic dysregulation [Geiger et al, 2009 Neuroscience 159, 1193; Johnson & Kenny, 2010 Nat Neurosci 13, 635]. We have, therefore, determined the effects of binge-eating in rats on dopamine neurochemistry and D1 and D2 receptors in various brain regions. Forty-five adult, lean, female Wistar rats were housed individually on reversed-phase lighting with free access to standard diet and water. Ground milk chocolate was offered to rats for 2 hr periods at irregular intervals to establish binge eating. Control rats were treated identically except empty glass jars were placed in their cages during the binge sessions. Rats were killed 1 hr after the final binge. Dopamine (DA) and its metabolites (DOPAC, HVA and 3 MT) were measured in striatum (STR), prefrontal cortex (PFC) and hypothalamus (HPT) by HPLC ECD. D1 and D2 receptors were quantified by saturation binding analysis in STR membranes using [3H]SCH23390 and [3H]raclopride. Acquired binge-eating did not alter the concentration of DA or its metabolites in STR, PFC or HPT. Compared with controls, DA turnover (DA/DOPAC ratio) was increased by 18% ($p<0.05$) in HPT, but it was not altered in STR or PFC. STR D1 receptors were reduced by 39% in BED rats (Bmax [fmol/mg protein]: BED = 176 ± 26 [n=10]; Control = 287 ± 25 [n=10]; $p<0.01$), but D2 receptors were unaltered (Bmax [fmol/mg protein]: BED = 303 ± 8 [n=6]; Control = 289 ± 21 [n=8]). The affinity (Kd) values of D1 and D2 receptors for [3H]SCH23390 and [3H]raclopride were unchanged. Binge-eating decreased STR D1 receptors without altering the size of the DA neuronal pool or rate of DA turnover. Together, they indicate binge eating is associated with decreased dopaminergic signalling via D1 receptors in STR. There were no neurochemical changes in PFC suggesting that dopaminergic neurotransmission was unaltered. The HPT is an important regulator of food intake and increased DA turnover suggests dopaminergic signalling is also dysregulated in this region. This study was funded by Shire Pharmaceuticals, UK.

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Poster

233. Catecholamines and Behavior

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Topic: C.18. Behavioral Pharmacology

Support: NIH R01 DA 027222

Title: Selective dopamine and noradrenergic antagonist oppositely modulate the PFC neuronal activity following chronic MPD exposure

Authors: *N. DAFNY, C. CLAUSSEN
Univ. Texas Med. Sch., Houston, TX

Abstract: The illicit use of methylphenidate (MPD), a psychostimulant, among students and adults has steadily increased. The prefrontal cortex (PFC) has been shown to play a role in the response to MPD exposure. Specifically, a subset of D1 and D2 DA receptors and noradrenergic alpha 2 receptors are essential in the mechanism of MPD action. The objective of this study was to determine the role of different dopamine and noradrenergic alpha 2 receptors in the mechanism of MPD action. Therefore the neuronal activity in the PFC units were recorded on awake freely behaving animals previously implanted with 4 permanent semi microelectrodes. Neuronal recordings were obtained following acute and chronic MPD and following selective (SCH 23390) D1 DA antagonist, (Raclopride) D2 DA antagonist and Yohimbine the selective alpha 2 antagonist. On experimental day 1 (ED1) animals were exposed to an acute 0.6 or 2.5 or 10.0 mg/kg MPD single injection and continued with daily MPD maintenance injections given for ED2-ED7, followed by a 3 day washout (ED7-9) and then a rechallenge of 0.6 or 2.5 or 10.0

mg/kg MPD at ED10. On ED11 animals after the chronic effect of MPD was established animals were exposed to their respective selective antagonist and activity was recorded for 30 minutes proceeded by a rechallenge of either a 0.6, 2.5 or 10.0 mg/kg MPD. The findings showed that the D1 DA antagonist given prior to MPD rechallenge elicited a significant decrease in neuronal firing rates in 67%, 72%, 84% of PFC neurons in the groups exposed to chronic 0.6, 2.5, and 10.0 mg/kg chronic MPD, respectively. The D2 DA antagonist given prior to MPD rechallenge was able to slightly modulate the PFC neuronal response; however those results were not significant. The 1.0 mg/kg Yohimbine elicited a significant increase of the MPD effect in 45%, 60% and 69% of neuronal firing activity in PFC neurons. These results demonstrate the involvement of dopamine D1 and noradrenergic alpha 2 receptors in the electrophysiological effects of MPD on PFC neurons.

Disclosures: N. Dafny: None. **C. Claussen:** None.

Poster

233. Catecholamines and Behavior

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Ministry of Science and Education (SF0180125s08)

European Regional Development Fund

Title: Differential expression of dopamine signaling proteins in Wfs1-deficient mice

Authors: *T. VISNAPUU, K.-L. ESKLA, R. REIMETS, H. LUUK, A. TERASMAA, C. A. HUNDAHL, E. VASAR
Univ. of Tartu, Tartu, Estonia

Abstract: Behavioral and biochemical studies have shown that homozygous Wfs1-deficient mice have blunted dopamine release. At the same time, dopamine receptors seem to be functional in these animals. Therefore, we here studied the expression of pre- and postsynaptic proteins which mediate dopaminergic signaling. We dissected the dorsal striatum (caudate-putamen) after amphetamine (5 mg/kg) or saline administration (20, 45 and 120 min following the injection). By using western blot, we determined the relative expression levels of dopamine

transporter (Dat), tyrosine hydroxylase (TH), p-Akt (Thr308), p-Akt (Ser473) and p-DARPP-32 (Thr34). Wild-type mice exhibited a rise in Dat expression at 120 min after amphetamine injection, whereas this effect of amphetamine did not appear at any of the time points in Wfs1-deficient mice. In addition, TH levels were significantly higher in amphetamine-treated wild-type and Wfs1-deficient mice at 120 min when compared with the respective saline-treated group of mice of the same genotype. The basal levels of relative p-Akt (Thr308) expression were higher in Wfs1-deficient mice than in their corresponding wild-type littermates and amphetamine failed to inhibit p-Akt (Thr308) in Wfs1-deficient animals at 20 min and 45 min. In wild-type animals, amphetamine significantly inhibited p-Akt (Thr308) at every time point. We did not observe an effect of amphetamine on the expression level of p-Akt (Ser473) in either of the genotypes and the relative baseline values were similar for this protein. The basal levels of relative p-DARPP-32 (Thr34) expression were similar when comparing the genotypes and amphetamine significantly increased the phosphorylation of this protein in wild-type animals at 20 min. Homozygous animals did not exhibit an elevation of this phosphoprotein in response to amphetamine. These data show that major proteins mediating dopaminergic signaling are differentially expressed in Wfs1-deficient mice and these findings shed light to the nature of dopaminergic impairment caused by lack of functional Wfs1 protein.

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Poster

233. Catecholamines and Behavior

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Program#/Poster#: 233.07/W30

Topic: C.18. Behavioral Pharmacology

Title: Phenotypical, behavioral and pharmacological characterization of a novel Knockout Rat model lacking the Dopamine Transporter

Authors: P. ILLIANO[1], D. LEO[1], I. SUKHANOV[1], L. MUS[1], S. ESPINOZA[1], T. D. SOTNIKOVA[1], M. C. HOENER[2], *R. R. GAINETDINOV[1]
[1]Inst. Italiano Di Tecnologia, Genova, Italy; [2]Neurosci. Research, Pharmaceuticals Division, F. Hoffmann-La Roche Ltd., Basel, Switzerland

Abstract: The major function of Dopamine Transporter (DAT) is the reuptake of dopamine (DA) from the synaptic cleft into presynaptic nerve terminals. Wistar Han rats in which the gene

encoding the DAT (SLC6A3, belonging to the family of Solute Carrier Transporter genes) has been disrupted, were produced by means of Zinc Fingers Nucleases technology. DAT homozygous knockout rats (DAT-/-) do not show any pre- and postnatal impairment, since they feed, develop and reach adult stage as their wildtype (DAT+/+) and heterozygotes littermates (DAT+/-). Nevertheless, (DAT-/-) show an impaired weight gain during developmental stage; naïve homozygotes have a pronounced Spontaneous Locomotor Activity (LMA), as detected throughout development up to adulthood, at different ages and over different periods of observation. Preliminary tissue content and fast scan cyclic voltammetry analysis suggest that the increase in the LMA is a direct consequence of the extended length of time that DA spends in the extracellular space following release. Preliminary pharmacological characterization of (DAT-/-) with amphetamine and a Tyrosine Hydroxylase reversible inhibitor show further support for the hyperdopaminergic functional state induced by the knockout of DAT, key protein for the homeostasis of dopaminergic terminals. The DAT knockout rats should be an excellent and improved tool for the study and development of drugs used in the management of dopaminergic dysfunctions. Our goal is to provide a complex and translational model for several human diseases involving aberrant DA function or mutations affecting DAT or altered DAT regulatory mechanisms *in vivo* such as schizophrenia, ADHD and newly discovered Dopamine Transporter Deficiency Syndrome (DTDS).

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Poster

233. Catecholamines and Behavior

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Topic: C.18. Behavioral Pharmacology

Support: NIH R01 DA 027222

Title: Dopamine D1 antagonist prevents and D2 antagonist attenuates methylphenidate eliciting behavioral sensitization

Authors: C. CLAUSSEN, *J. ARONOWSKI, N. DAFNY
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Abstract: Methylphenidate (MPD) is a readily prescribed drug for the treatment of attention deficit hyperactivity disorder (ADHD) and moreover is used illicitly by youths for its cognitive enhancing effects and recreation. Repetitive MPD exposure leads to an augmented behavioral response referred to as behavioral sensitization, an experimental marker for a drugs ability to elicit dependence. There is evidence that dopamine (DA) is a key player in the acute and chronic MPD effect, however the exact role of DA in the effects elicited by MPD is still debated. The objective of this study was to investigate the role of D1 and/ or D2 DA receptors in the acute and chronic effect of MPD. Seven groups of adult male SD rats were used with control group, three groups treated with SCH 23390 the 0.3 mg/kg D1 DA antagonist and three groups treated with 0.3 mg/kg D2 DA antagonist given before and after acute and chronic 2.5 mg/kg MPD administration during a twelve day protocol as follows: Group I saline only, group II and III received a D1 and D2 antagonist injection at ED2 prior to the initial MPD exposure, group IV and V received either a D1 and D2 antagonist at ED3 prior to the second MPD exposure and group VI and VII received either a D1 or D2 injection prior to MPD exposure at ED12 following six daily MPD exposures (ED2 to 7) three washout days (ED8-10) and MPD rechallenge at ED11. The D1 antagonist was able to significantly ($P<0.05$) attenuate the locomotor activity when given at experimental day 2 (ED2) prior to the initial MPD exposure (group II), at ED3 following a single MPD injection (group IV) and at ED12 before the MPD rechallenge administration group (VI). The D2 antagonist did significantly attenuate the locomotor activity only when given at ED3 before the second MPD exposure. The results show the role, at least in part, of the D1 DA receptor in the mechanism of behavioral sensitization whereas the D2 DA receptor only partially modulates the response of the MPD effects.

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Poster

233. Catecholamines and Behavior

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Support: NIH R21 DA034192 (S.G.N)

Title: DREADD'ed addiction: Investigating the effect of DREADD-mediated modulation of G-protein coupled signaling in lateral habenula neurons projecting to the ventral tegmental area on cocaine self-administration and reinstatement in rats

Authors: *S. G. NAIR[1], D. SMIRNOV[2], J. F. NEUMAIER[2]
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Abstract: The lateral habenula (LHb), part of the habenular complex in the dorsal diencephalon, is an important regulator of midbrain dopaminergic systems that are known to be involved in the reinforcing properties of cocaine. The LHb projects to the ventral tegmental area (VTA) and the rostromedial tegmental nucleus (RMTg) among other brain regions. Here, we examined the role of the LHb neurons projecting to the VTA in cocaine reinforced operant responding, and cocaine-priming induced reinstatement. A Cre-recombinase dependent viral vector based flip-excision method was employed that involved injecting a combination of floxed, inverted Gi/o coupled DREADD (hM4Di) into LHb neurons and a canine adenovirus 2 (CAV-2) engineered to express Cre recombinase into the VTA. CAV-2 efficiently infected VTA axon terminals and was retrogradely transported to the neuronal cell bodies in the LHb, resulting in the expression of hM4Di receptors exclusively in LHb neurons that project to the VTA. Approximately 10-14 days after viral infusions, male, Long-Evans rats were trained to self-administer cocaine (0.75 mg/kg/infusion) on a fixed ratio 1, 20 second timeout reinforcement schedule. Activation of hM4Di by the otherwise inert synthetic ligand clozapine-N-oxide CNO (1 and 3 mg/kg, i.p) had no effect on cocaine reinforced operant responding. Secondly, a distinct cohort of rats was infused with viral vectors as described above and trained to self-administer cocaine (0.75 mg/kg/infusion) on a progressive ratio reinforcement schedule. Interestingly, preliminary data indicate that increased Gi/o-coupled signaling in LHb neurons projecting to the VTA increases operant responding on a progressive ratio schedule. Finally, rats were trained to self-administer cocaine and the operant responding was extinguished over 10-14 days. Exposure to a single injection of cocaine (10 mg/kg, ip) reinstated operant responding. Preliminary data indicate that increasing Gi/o-coupled signaling in LHb neurons projecting to the VTA decreases cocaine-priming induced reinstatement. Taken together, these results suggest a differential role of activation of Gi/o-coupled signaling in LHb neurons projecting to the VTA in the motivation to self-administer cocaine versus reinstatement of cocaine seeking in rats.

Disclosures: S.G. Nair: None. **J.F. Neumaier:** None. **D. Smirnov:** None.

Poster

233. Catecholamines and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 233.10/W33

Topic: C.18. Behavioral Pharmacology

Support: NIMH Grant 1K01MH100644-01A1

NIDA Grant 5R01DA002925-30

Title: Selective D1 agonists induce head twitch response in mice: Implications for modeling comorbid Tourette's syndrome and obsessive-compulsive disorder

Authors: *L. M. KLEIN[1], M. A. GEYER[2], A. L. HALBERSTADT[2]
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Abstract: Tourette's syndrome (TS) and obsessive-compulsive disorder (OCD) demonstrate remarkable comorbidity, with up to 60% of TS patients exhibiting OCD-like behaviors. Several independent rodent models for OCD and TS have been proposed. For instance, the perseverative grooming induced by the D1 receptor partial agonist SKF 38393 has been used to model aberrant behaviors found in OCD patients, and the head twitch response (HTR) induced by 5HT2A receptor agonists represents a possible model for the tics associated with TS. However, few reliable animal models exist for comorbid TS and OCD. Interestingly, we found that the D1 agonist SKF 38393 induces perseverative grooming and HTR in C57BL/6J mice (Halberstadt & Geyer, Psychopharmacology 227:737 (2013)), providing a potential model for comorbid OCD and TS. Since the HTR is typically associated with activation of 5HT2A rather than D1, we investigated the pharmacology of the HTR induced by D1 agonists in C57BL/6J mice. Using a head-mounted magnet and a magnetometer coil to detect head movements, we tested whether SKF 38393 and the highly selective D1 full agonist (+)-doxanthrine induce the HTR. We also assessed the involvement of D1 and 5HT2A receptors in mediating the response to SKF 38393 and (+)-doxanthrine. Administration of SKF 38393 (5-40 mg/kg, IP) produced a dose-dependent increase in HTR. Treatment with (+)-doxanthrine (1 and 5 mg/kg, IP) similarly induced a significant increase in the frequency of HTR. The D1 antagonist SCH 23390 (0.025 and 0.1 mg/kg, SC) dose-dependently blocked the HTR induced by SKF 38393 (40 mg/kg) and (+)-doxanthrine (5 mg/kg). Pretreatment with the selective 5-HT2A antagonist M100907 (0.01 and 0.1 mg/kg, SC) completely blocked the HTR induced by SKF 38393 and (+)-doxanthrine. The ability of SCH 23390 to block the HTR induced by SKF 38393 and (+)-doxanthrine suggests that these effects are mediated by D1 receptor activation. This conclusion is supported by the fact that (+)-doxanthrine is highly selective for D1 over 5-HT2A. However, our results also suggest that the HTR induced by D1 agonists is dependent on 5-HT2A receptor activation. Future studies will investigate whether D1 agonists induce the HTR by stimulating the release of 5-HT, which consequently activates 5-HT2A receptors. The combination of perseverative grooming and HTR behaviors induced by D1 agonists in mice represents a potential model of comorbid TS and OCD. Further investigation of the mechanisms for these behavioral effects may reveal new insights into the neural underpinnings of these disorders.

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collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Intracellular Therapeutics, Johnson & Johnson, NIDA, NIMH, U.S. Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); San Diego Instruments. F. Consulting Fees (e.g., advisory boards); Abbott, Acadia, Addex, Cerca, Lundbeck, Merck, Neurocrine, Omeros, Takeda, Teva. **A.L. Halberstadt:** None.

Poster

233. Catecholamines and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 233.11/W34

Topic: C.18. Behavioral Pharmacology

Support: NIDA Intramural Research Program

Title: Behavioral economics in dopamine D2-like receptor mutant mice

Authors: *P. L. SOTO[1], T. HIRANITA[2], M. XU[3], D. K. GRANDY[4], J. L. KATZ[5]
[1]Educational Psychology, Texas Tech. Univ., Lubbock, TX; [2]U.S. Food and Drug Admin., Jefferson, AR; [3]Univ. of Chicago, Chicago, IL; [4]Oregon Hlth. & Sci. Univ., Portland, OR; [5]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Dopamine receptors play an important role in reinforcement processes however the precise roles played by different dopamine receptor subtypes are not known. Previous research demonstrated that demand curves for food were steeper (i.e., more elastic) for mice lacking dopamine (DA) D2 receptors (D2R knockout [KO] mice) compared to wild-type (WT) littermates, suggesting a role of D2Rs in the reinforcing effectiveness of food. The current studies investigated whether the economic conditions (i.e., whether the mice received food outside of the experimental sessions) affected the differences in food's reinforcing effectiveness between DA D2R KO and WT. Additionally, the current studies extended the study of food's reinforcing effectiveness to DA D3R and D4R KO and WT mice. Mice of all genotypes responded for food under fixed-ratio (FR) schedules of food reinforcement. FR values were varied over a wide range under short 30-min open economy conditions, wherein mice were fed outside of the experimental sessions, and long 11-hr closed economy conditions, wherein mice were not fed outside of the experimental sessions. Demand curves for D2R KO mice were more elastic than demand curves for D2 WT mice under both open and closed economies. Demand

curves for D3R KO mice did not differ from demand curves obtained in their WT littermate counterparts, whereas demand curves for D4R KO mice decreased more steeply under open, but not closed economy conditions, compared to demand curves obtained in D4R WT mice. Demand curves decreased more steeply under open versus closed economy conditions in all genotypes, consistent with food having a higher reinforcing effectiveness under closed versus open economy conditions. The present findings replicate previous results and indicate that the role of D2Rs in the reinforcing effectiveness operates in both open and closed economies. Further, these results suggest that of the D2-like receptor subtypes, the D2R subtype is the primary receptor subtype involved in mediating food's reinforcing effectiveness.

Disclosures: **P.L. Soto:** None. **T. Hiranita:** None. **M. Xu:** None. **D.K. Grandy:** None. **J.L. Katz:** None.

Poster

233. Catecholamines and Behavior

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Topic: C.18. Behavioral Pharmacology

Support: NIH Grant DA031734

Title: Increased mesocorticolimbic dopamine before, during, and after social defeat stress: Role of corticotropin releasing factor receptors in the ventral tegmental area

Authors: *E. N. HOLLY, J. F. DEBOLD, K. A. MICZEK
Dept. of Psychology, Tufts Univ., Medford, MA

Abstract: Stress potently excites dopamine (DA) neurons in the ventral tegmental area (VTA), promoting phasic DA increases in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc). In rats, many stressors acutely cause a phasic DA increase in both the NAc and mPFC, while repeated aversive experience can produce long term changes in DAergic tone in these regions. The neural mechanisms which activate VTA DA during stress may provide clues as to how stress can interact within the reward system to result in stronger behavioral and neural responses to drugs of abuse. Due to their key function in the physiological stress response and localization within the mesolimbic DA system, corticotropin releasing factor (CRF) and its receptors (CRFR1 and CRFR2) are proposed to be critical in the behavioral and neural interactions of stress and reward. This study examines the influence of VTA CRF receptors on

monoamine concentrations in response to acute and repeated social defeat as well as neural cross-sensitization to cocaine. Long Evans rats were implanted with bilateral microinjection cannulae in the VTA as well as unilateral microdialysis cannulae aimed at both the mPFC and NAc. After 1 week recovery, rats were microinjected with a CRFR1 antagonist (CP376395), CRFR2 antagonist (Astressin2B), or vehicle (aCSF) 20 min prior to social defeat on days 1, 4, 7, and 10. Extracellular monoamine (norepinephrine (NE), serotonin (5HT), and DA) concentrations in the mPFC and NAc were assessed via *in vivo* microdialysis on days 1 and 10. On day 20, rats again underwent microdialysis and were assessed for neural cross-sensitization to cocaine (10 mg/kg, ip). Consistent with prior findings using other stressors, acute social defeat (day 1) significantly elevated extracellular DA levels in both the NAc and mPFC, and the increased DA was sustained for the duration of exposure to social stress, with no effects on NE or 5HT. Repeated social defeat altered the DAergic response, such that tonic DA concentrations were reduced on day 10, although there was no difference in percent change from baseline. These aCSF pretreated animals also exhibited neural cross-sensitization to cocaine, in the form of augmented extracellular DA in the NAc. Intra-VTA CRFR1 and CRFR2 antagonism had no immediate effects on NAc or mPFC monoamine concentrations and were both able to prevent the induction of neural cross-sensitization. These data indicate VTA CRFR activation is necessary for promoting stress-induced increased phasic downstream DA (presumably through increased neuronal firing) and neural cross-sensitization to cocaine. As such, CRFRs continue to be important targets for therapeutic intervention in addiction.

Disclosures: E.N. Holly: None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

233. Catecholamines and Behavior

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Program#/Poster#: 233.13/W36

Topic: C.18. Behavioral Pharmacology

Title: Pharmacology in sexually sluggish male Wistar rats: A new animal model to examine serotonin 5-HT_{1A} and dopamine disorders?

Authors: *J. S. CHAN[1], E. Y. BIJLSMA[1], M. F. H. VAN SCHAIK[1], E. KOOIJMAN[1], F. N. VAN HASSELT[1], J. TITULAER[1], R. E. VERDONSCHOT[1], M. D. WALDINGER[1], A. NEWMAN-TANCREDI[2], M. VARNEY[2], R. S. OOSTING[1], B. OLIVIER[1]
[1]Pharmacol., Univ. Utrecht, Utrecht, Netherlands; [2]Neurolixis, Inc., Dana Point, CA

Abstract: Male Wistar rats with consistently low or no sexual behaviors are unusual in an otherwise healthy population of rats and can constitute a model of sexual dysfunction. Drugs that activate serotonin 5-HT_{1A} and/or dopamine systems typically facilitate male rat sexual behaviors. Therefore, various compounds were examined in normal (N), delayed ejaculating (DE), and non-copulating (NC) rats in a 30 minute test session with an estrus female rat. 8-OH-DPAT, a prototypical 5-HT_{1A} receptor agonist, increased ejaculation frequencies in N and DE animals to normal to fast levels and drastically reduced mounts and intromissions before the 1st ejaculation, while NC rats remained sexually inactive. Buspirone, a non-specific partial 5-HT_{1A} agonist, increased ejaculation frequency and lowered mounts for DE rats. NC rats remained inactive. F13714 and F15599, a pre- and post- synaptic 5-HT_{1A} receptor 'biased' agonist, respectively, facilitated sexual behaviors in DE, with F13714 acting more potently; suggesting that the facilitating effects are mediated through presynaptic receptors. The facilitating effects of F13714 are blocked by WAY100,635, a selective 5-HT_{1A} antagonist. Quinpirole, a selective D_2 and D_3 receptor agonist, reduced mounts in DE rats and increased ejaculation frequencies in DE and NC. GBR12909, a selective dopamine reuptake inhibitor, did not significantly affect any sexual behaviors in either type of rats, however NC rats again showed some sexual behaviors. Bupropion, a noradrenalin and dopamine reuptake inhibitor, and Apomorphine, a dopamine agonist, did not significantly affect the sexual behaviors of DE rats. Tadalafil, a PDE5 inhibitor, reduced the number of mounts before the 1st ejaculation in DE rats while NC rats remained inactive. Overall, DE rats appear to have a blunted response to all dopamine-challenging treatments (only high doses of quinpirole induced a facilitating effect). NC rats may have a perturbed serotonin/dopamine system since 8-OH-DPAT normally elicits a strong ejaculatory response in other rats. DE and NC rats are also heavier than N, with NC rats heaviest; an interesting find since serotonin and dopamine plays an important role in food intake. No differences in the open field test and in serum testosterone levels were found. Together, DE and NC rats mimic the human symptoms of sexual dysfunctions with low libido, delayed ejaculation, and anorgasm. The number of mounts before the first ejaculation were reduced in some compounds which may indicate an improvement in genital sensitivity. This, in conjunction with the lowering of the ejaculation threshold in some compounds, may be the cure for human sexual dysfunctions.

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Poster

233. Catecholamines and Behavior

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Program#/Poster#: 233.14/X1

Topic: C.18. Behavioral Pharmacology

Support: NIH R21 DA034192

Brain and Behavior Young Investigator Award A82234

Title: Effect of activation of Gi/o-coupled signaling in the lateral habenula on food reinforced operant responding and cue-induced reinstatement of food seeking

Authors: *J. F. NEUMAIER, D. SMIRNOV, S. NAIR
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Abstract: The lateral habenula (LHb) is part of the habenular complex in the dorsal diencephalon. Uniquely positioned anatomically to participate in reward-related brain circuits, this nucleus is an important regulator of the midbrain dopaminergic system that is known to be involved in the neuronal circuitry underlying various feeding behaviors. However, very little is known about the precise role of this nucleus in operant food self-administration and relapse to food seeking. Here, we utilized the DREADD (Designer Receptors Exclusively Activated by Designer Drugs) technology that we have successfully used to bidirectionally manipulate LHb neuronal activity associated with motivated behaviors. Male, Long Evans rats were infused with adeno-associated virus expressing Gi/o coupled human M4 (hM4Di) receptors into the LHb bilaterally. Approximately 10-12 days following viral infusion, rats were trained to self-administer food pellets for 10 days on a fixed ratio 1, 20 second timeout reinforcement schedule; food pellet delivery was paired with a compound tone and light cue. Activation of hM4Di by the pharmacologically inert synthetic ligand clozapine-N-oxide (CNO) activates GIRK currents and results in hyperpolarization of neuronal activity. Our initial data indicate that activation of hM4Di in the LHb with CNO (1 and 3 mg/kg, i.p) had no influence on food reinforced operant responding. Studies are currently underway to determine the effect of activation of Gi/o-coupled signaling in the LHb on the motivation to self-administer food pellets on a progressive ratio schedule of responding as well as cue-induced reinstatement of food seeking.

Disclosures: **J.F. Neumaier:** None. **D. Smirnov:** None. **S. Nair:** None.

Poster

233. Catecholamines and Behavior

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Topic: C.18. Behavioral Pharmacology

Support: NIH grant DA020041

UCSD Chancellor's Interdisciplinary Collaboratory grant

NSF GRFP (KKH, SLS)

Title: Point mutations in the dopamine transporter reveal an obligatory role in learning and memory

Authors: *S. A. CARMACK[1], S. L. SCUDDER[1], K. K. HOWELL[1], G. N. PATRICK[1], H. H. GU[2], S. G. ANAGNOSTARAS[1]
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Abstract: Monoaminergic neurotransmission, such as dopamine and norepinephrine neurotransmission, is traditionally thought to play a modulatory, rather than essential, role in learning and memory. Previous research investigating the role of the dopamine transporter in learning and memory using knockout mice or pharmacological methods has largely been uninterpretable due to profound confounding effects on locomotion. Here we show that novel knock-in mice with highly selective point mutations in the dopamine transporter show dramatic memory deficits on several memory tasks, independent of any effects on locomotion and without any apparent effect on hippocampal synaptic plasticity. Our results suggest an obligatory role for the dopamine transporter in learning and memory.

Disclosures: S.A. Carmack: None. **S.L. Scudder:** None. **K.K. Howell:** None. **G.N. Patrick:** None. **H.H. Gu:** None. **S.G. Anagnostaras:** None.

Poster

233. Catecholamines and Behavior

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Program#/Poster#: 233.16/X3

Topic: C.18. Behavioral Pharmacology

Support: NIH Grant MH094955

NIH Grant GM103436

Title: The effects of early-life risperidone administration on behavior in the social interaction test during adulthood

Authors: *C. BROWN[1], M. A. GANNON[2], M. E. BARDGETT[2]
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Abstract: Antipsychotic drugs, such as the atypical antipsychotic drug, risperidone (Risperdal®), have been approved for use in young children with autism, a disorder marked by deficits in social interaction. Recent work in our laboratory has found that risperidone administration to young rats decreases social play immediately after injection due to drug-induced sedation. In addition, qualitatively different decrements in social play can be observed at 23 hours post-risperidone administration when the drug is absent from the blood. The purpose of this study was to determine if early-life administration of risperidone led to changes in social interaction during adulthood, long after cessation of drug administration.Thirty-six Long-Evans rats received subcutaneous daily injections of one of four doses of risperidone (vehicle, 0.3, 1.0, or 3.0 mg/kg) from postnatal day 14 through 42. Beginning on postnatal day 60, each rat was tested over four phases of a standard, three-chamber social interaction test. During the last two phases, rats were allowed to make contact with a novel male rat (phase 3), or with a novel rat or the same rat from phase 3 (phase 4). In each phase, the target rats were confined to a small cage placed in one of the apparatus chambers. All rats spent more time and travelled a greater distance in the chamber that contained the novel rat during phase 3 but more evenly split their time and distance travelled between the two occupied chambers in phase 4. Early-life risperidone administration did not modify any of these behaviors during either phase. Early-life risperidone administration may alter social play in rats during the course of treatment but does not appear to have dramatic, long-term effects on social contact during adulthood. Future studies in this area should consider more nuanced assessments of social behavior that allow for direct interaction between rats. Such research, along with the present results, could provide a more fully informed picture of the short- and long-term impact of early-life antipsychotic treatment on social behavior.

Disclosures: C. Brown: None. **M.A. Gannon:** None. **M.E. Bardgett:** None.

Poster

233. Catecholamines and Behavior

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Program#/Poster#: 233.17/X4

Topic: C.18. Behavioral Pharmacology

Support: CHRP

Schlumberger Foundation Faculty for the Future Fellowship

Title: Modulation of *C. elegans* electrotactic swimming behavior by dopamine signaling

Authors: *S. SALAM[1], R. K. MISHRA[2], P. SELVAGANAPATHY[3], B. P. GUPTA[1]
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Abstract: Dopamine (DA) is an important neuromodulator which is involved in controlling movement and psychological functions such as cognition and reward processing. Alteration in DA levels is associated with various neurological disorders and behavioral changes. The nematode Caenorhanbditis elegans is an established model organism for study of neuronal regulation of behaviors. The *C. elegans* nervous system consists of only 302 neurons including eight dopamine neurons which has conserved dopamine pathways. DA modulates a range of behaviors such as egg laying, feeding and locomotion. In an electrotaxis behavior assay the worms show movement toward cathode in low external DC electric field. However, the mechanism of how DA signaling modulates electrotactic swimming behavior is elusive. To investigate the role of DA signaling in electrotactic swimming behavior, we employed a novel microfluidic platform that facilitates on-demand control of worm movement using electric fields. In this study, we demonstrate how DA modulates the electrotactic swimming speed in a microfluidics channel. The DA deficient mutant cat-2 which encodes a tyrosine hydroxylase has a wild type like electrotactic swimming speed. DA transporter mutant dat-1 has a slow electrotactic swimming. DA deficient mutant bas-1 and cat-1 encode for aromatic amino acid decarboxylase and vesicular monoamine transporter respectively and these mutants showed a faster electrotactic speed which is partially rescued by the exogenous DA treatment. Similar to mammalian system, *C. elegans* DA mediates extrasynaptic signaling through the DA receptors that are expressed in different cells including cholinergic motor neurons and muscle cells. The D2 like receptor DOP-3 is expressed in the cholinergic motor neurons. dop-3 showed a faster speed suggesting that DOP-3 is the primary receptor through which DA acts on to slow movement. The D1 like receptors dop-1 and dop-4 and D2 like dop-2 mutant showed a wild type like electrotactic swimming speed. To demonstrate the antagonistic signaling of D1 and D2 type receptors we examined the dop-2;dop-1dop-3. We also reveal that longer exposure of *C. elegans* in the electric field induces slow electrotactic swimming speed which is regulated by DA. The induce slow speed is mediated by the D2 type DA signaling. We also examined the role of DA neurons for their function in electrosensory behavior. DA neuron ablated worms demonstrated a slow speed without any impaired electrosensory behavior.

Disclosures: **S. Salam:** None. **R.K. Mishra:** None. **P. Selvaganapathy:** None. **B.P. Gupta:** None.

Poster

233. Catecholamines and Behavior

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Topic: C.18. Behavioral Pharmacology

Title: The effect of acutely inhaled toluene on locomotor activity and extracellular dopamine is moderated by gaba in the mouse caudate putamen

Authors: *S. CALLAN, A. A. APAWU, T. A. MATTHEWS, S. E. BOWEN
Wayne State Univ., Detroit, MI

Abstract: Toluene is a ubiquitous solvent commonly abused for intoxication. Despite its frequency of misuse, there is little understanding of how toluene acts within the brain. Previous in-vivo examinations of dopamine (DA) levels during toluene inhalation have been mixed with some investigations showing increases in extracellular DA during toluene exposure with other studies showing little to no effect of toluene on DA levels. Additionally, the majority of in-vivo microdialysis studies have focused on the rat, specifically the nucleus accumbens. Previous neurochemical work has also suggested that toluene is active at GABA sites, and that DA levels in the caudate are mediated by increases in GABA, suggesting that toluene's action on DA levels may be related to GABA levels. In the present study, we employed an acute solvent inhalation model to observe the impact of toluene exposure on DA release in the mouse caudate in-vivo using microdialysis techniques in Swiss Webster mice. This project represents the first *in vivo* examination of toluene-DA dynamics using a mouse model. This project employed a novel dynamic vapor exposure chamber to allow for freely moving animals during dialysate collection. Toluene doses of 4000ppm and 8000ppm increased locomotor activity, while neurochemically toluene exposure increased DA levels at only the highest dose (8000ppm) examined. In a second experiment, animals pretreated with 10μM bicuculline, a GABAA antagonist, 30 min before toluene exposure via reverse-dialysis displayed elevated DA release as compared to controls. In addition, animals in the bicuculline/toluene group showed potentiated locomotor activity compared to all other groups. These preliminary neurochemical findings suggest that acute toluene exposure potentiates the release of DA in the caudate and that this effect is enhanced

with bicuculline pretreatment. Taken together, these effects provide evidence that the action of toluene on DA levels in the caudate may be mediated by the action of GABA.

Disclosures: **S. Callan:** None. **S.E. Bowen:** None. **A.A. Apawu:** None. **T.A. Matthews:** None.

Poster

233. Catecholamines and Behavior

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Topic: C.18. Behavioral Pharmacology

Support: Western Michigan University Graduate Student Research Grant

Title: The neurobehavioral consequences of gestational and chronic atrazine exposure in male and female Sprague-Dawley rats

Authors: ***J. L. WALTERS**[1], T. A. LANSDELL[3], K. LOOKINGLAND[3], L. E. BAKER[2]
[2]Psychology, [1]Western Michigan Univ., Kalamazoo, MI; [3]Pharmacol. and Toxicology, Michigan State Univ., Lansing, MI

Abstract: Atrazine is an herbicide used extensively around the world to control weeds on crops such as corn, sorghum, and sugarcane. The adverse risks of exposure to this herbicide in humans are not fully understood. Although numerous studies have demonstrated atrazine to be an endocrine disrupter, the neurobehavioral consequences of developmental atrazine exposure have not been thoroughly examined. The aim of this study was to investigate the effects of environmentally-relevant levels of gestational followed by continued chronic atrazine exposure on rodent locomotor activity, motor coordination, and striatal dopamine function. Male and female Sprague-Dawley rats were mated and upon detection of the vaginal plug, 12 dams were randomly assigned to one of three treatment groups. Dams received daily oral feedings of corn oil or atrazine (100 µg/kg or 10 mg/kg) dissolved in corn oil throughout pregnancy and lactation. Within 1 to 2 days of birth, pups were culled to 5 males and 5 females per litter. Upon weaning, the offspring continued daily corn oil or atrazine feedings for an additional six and a half months. During this time, rats were subjected to a series of behavioral assays, including locomotor activity assessments and a walking-beam task to assess motor coordination. Twenty-four hours after the last treatment, animals were euthanized by rapid decapitation and brains were immediately frozen for tissue analysis. Tissue punches were performed in the striatum, nucleus accumbens, and median eminence and samples were analyzed for dopamine (DA) and

3,4dihydroxyphenylacetic acid (DOPAC) by high performance liquid chromatography with electrochemical detection (HPLC-EC). Results revealed increased locomotor activity at 1 month of age in males exposed to 100 μg/kg atrazine compared to controls as well as impaired walking beam performance on PND 37-46 in both males and females exposed to 100 μg/kg and 10 mg/kg atrazine. At 6 months of age, males exposed to 10 mg/kg atrazine displayed decreased locomotor activity compared to controls. Neurochemical assays revealed decreased striatal DA and DOPAC in males and females exposed to 10 mg/kg atrazine and in males exposed to 100 μg/kg atrazine. However, females exposed to100 μg/kg had decreased striatal DA with no changes in DOPAC compared to controls. These findings provide evidence that developmental exposure to environmentally relevant levels of atrazine significantly impairs motor coordination with associated reductions in striatal DA in rodents. Further investigation of the potential impact of atrazine exposure on neurological development in humans and increased risks for Parkinson disease may be warranted.

Disclosures: J.L. Walters: None. **T.A. Lansdell:** None. **K. Lookingland:** None. **L.E. Baker:** None.

Poster

233. Catecholamines and Behavior

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Topic: C.18. Behavioral Pharmacology

Support: SIP-IPN

Title: Antinociceptive effect of tizanidine and tramadol combinations in rats: Isobolographic analysis

Authors: *K. BELTRÁN-VILLALOBOS[1], M. DÉCIGA-CAMPOS[2]
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Abstract: The use of more than one drug to achieve a desired effect has been a common practice in pharmacologic testing and in clinical practice. For example, combinations of analgesics are frequently prescribed with a view to enhancing pain relief and reducing adverse effects. It is also well established that administration of more than one drug may give effects that are greater than, or less than, the additive effect of each drug given individually. This study sought to evaluate the

nature of the antinociceptive interaction of systemic administration of a combination of the tramadol with tizanidine, by isobolographic analysis in the formalin pain test of rat. Concentration-response curves were generated in rats for tramadol, tizanidine alone or their combination at the fixed ratios of 1:1 and 3:1 respectively. The study was carried out in female rats weighing 180-200 g, and the protocol was to test each drug (at dosages of 0.1-100 mg/paw of tramadol and 0.01-10 mg/paw of tizanidine) alone and in combination. Tizanidine (CE50= 0.125 ± 0.026 μg) was more potent than tramadol (CE50=16.45 ± 6.4 μg). Combination of tramadol-tizanidine at the fixed ratios 1:1 (CE50exp = 67.43 ± 11 μg.12; CE50teo = 8.28 ± 3.2 μg) and 3:1 (CE50exp = 31.25 ± 9.49 μg; CE50teo =12.36 ± 4.8 μg) generated a subadditivity effect (antagonism). Bassed on the current preclinical data, the pharmacological profile of combinations of tramadol-tizanidine evaluated with isobolograms produce antagonism. In this sense, it's important to utmost caution is requires during the use of this combination in clinical practice, because their present antagonism interaction.

Disclosures: K. Beltrán-Villalobos: None. **M. Déciga-Campos:** None.

Poster

233. Catecholamines and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 233.21/X8

Topic: C.18. Behavioral Pharmacology

Support: NIH Grant MH094955

NIH Grant GM103436

Title: Early-life risperidone administration enhances locomotor responses to amphetamine during adulthood

Authors: B. L. STUBBEMAN, C. J. BROWN, *M. E. BARDGETT
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Abstract: Antipsychotic drug prescriptions for pediatric populations have increased tremendously over the past 20 years, particularly the use of atypical antipsychotic drugs such as risperidone. In rats, forebrain dopamine receptor densities are elevated upon cessation of early-life risperidone administration. This finding suggests that adult rats administered risperidone early in life should display enhanced behavioral sensitivity to drugs that elevate dopamine

neurotransmission. This hypothesis was tested by measuring locomotor responses to amphetamine - a drug that releases forebrain dopamine - in adult rats administered risperidone early in life. Thirty-five Long-Evans rats received one of four doses of risperidone (vehicle, 0.3, 1.0, or 3.0 mg/kg) daily from postnatal day 14 through 42. Beginning on postnatal day 75, locomotor activity was recorded for 30 minutes once a week for four weeks. After 30 minutes, each rat received a subcutaneous injection of one of four doses of amphetamine (saline, 0.3, 1.0, or 3.0 mg/kg) in a counter-balanced order across the four weeks. Locomotor activity was measured for 27 hours after amphetamine administration. Activity levels did not differ between the vehicle and risperidone groups for six hours after saline injection. Rats administered risperidone early in life displayed significantly greater locomotor activity for six hours after amphetamine injection. This effect was most prominent in the first two hours after injection of the 0.3 and 1.0 mg/kg amphetamine doses, and was seen in all groups administered risperidone early in life, but was most marked in the risperidone 3.0 mg/kg group. The results suggest that the development of forebrain dopamine systems is permanently altered by early-life antipsychotic drug administration. The data raise concerns about possible increases in sensitivity to recreational and therapeutic drugs that target dopamine in adults treated with antipsychotic drugs during childhood.

Disclosures: B.L. Stubbeman: None. **M.E. Bardgett:** None. **C.J. Brown:** None.

Poster

233. Catecholamines and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 233.22/X9

Topic: C.18. Behavioral Pharmacology

Support: 2012 NARSAD Young Investigator Grant

Penn State Parkinson's Disease Research Fund

Title: Functional selectivity at the dopamine D1 receptor and its role in mediating antiparkinson effects

Authors: *Y. YANG, S.-M. LEE, X. HUANG, C. A. LIEU, T. SUBRAMANIAN, V. MURTHY, R. B. MAILMAN
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Abstract: Dopamine D1 receptors are expressed highly in basal ganglia and cerebral cortex, and modulate movement and cognition, both of which are impaired in neurodegenerative disorders like Parkinson's disease. Dopamine D1 agonists directly activate D1 receptors in these brain areas to reduce symptoms dramatically. The activation of D1 receptors is known or hypothesized to be associated with the changes in several intracellular signaling pathways, including cAMP, β-arrestin, and ion channels. There is, however, minimal knowledge of the role of each signaling pathway. Using a combination of pharmacological and behavioral techniques, we investigated how biased D1 agonists may activate these signaling pathways to modulate movement and cognition. We used dihydrexidine (DHX), EFF0311 (EFF) and CY208243 (CY) as test compounds, and dopamine and the partial agonist SKF38393 (SKF) as reference drugs. The intrinsic activity (IA) of these compounds was evaluated for *in vitro* functional selectivity using activity at cAMP and β-arrestin signaling. Phospholipase C signaling was not examined as we have recently shown this is likely an off-target effect of D1 agonists. Compared to dopamine, both DHX and EFF showed almost 100% IA at adenylate cyclase vs. 88% for CY. SKF had the expected partial agonist activity (IA =ca. 50%). At β-arrestin signaling, both DHX and EFF had somewhat higher activity than dopamine (138% and 122%, respectively), whereas CY and SKF had ca. 50% activity. Thus, DHX and EFF appear to be true full agonists, whereas CY has high IA, but is biased towards cAMP signaling. Several mechanisms may explain why DHX, and to a lesser extent EFF, showed slightly higher intrinsic β-arrestin activity than dopamine. Previous studies in the MPTP primate model of Parkinson's disease have shown that DHX caused profound antiparkinson effects that are greater than levodopa. We now report that EFF also causes dramatic antiparkinson effects in non-human primates without inducing dyskinesias. Conversely, CY has been reported to be somewhat less effective. These data suggest that full activation of both cAMP and β-arrestin signaling pathways is necessary for maximal antiparkinsonian effects. The discovery of highly biased D1 agonists will be very important in elucidating the relative roles of these pathways. It will be important to determine if the D1 signaling responsible for antiparkinson effects is the same as that which improves various aspects of learning and memory.

Disclosures: Y. Yang: None. **S. Lee:** None. **X. Huang:** None. **C.A. Lieu:** None. **T. Subramanian:** None. **V. Murthy:** None. **R.B. Mailman:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.01/X10

Topic: C.19. Drug Discovery and Development

Support: NIH Grant U01-AG043415

Title: Attenuation of synaptic dysfunction by a novel experimental therapeutic that targets a single kinase present in both neurons and glia

Authors: O. ARANCIO[1], L. J. VAN ELDIK[2], F. SAEED[1], A. BACHSTETTER[2], J. P. SCHAVOCKY[3], J. C. PELLETIER[3], S. M. ROY[3], *D. WATTERSON[4]
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Abstract: The p38α MAPK is a stress-activated, serine/threonine protein kinase (S/T PK) that mediates increased production of neurotoxic substances by glia as well as being directly involved in stress related neuronal dysfunctions. For example, the pathophysiological upregulation of proinflammatory cytokine production and other neuroinflammatory responses through p38α MAPK in activated glia can be injurious to neurons, which is compounded by neuronal p38α MAPK involvement in the dysfunction of fast axonal transport and synaptic homeostasis. The parallel process provides a novel pharmacological opportunity to modulate a biological systems wide response by targeting a single molecular entity. Prior art from preclinical animal model studies has shown that dosing with CNS drugs of appropriate design can attenuate the excessive proinflammatory cytokine production back towards homeostasis in the absence of immunosuppression, indicating the promise of this novel therapeutic approach. Our recent development of specific p38α MAPK inhibitor probes for *in vivo* investigations has allowed extension to the attenuation of synaptic and cognitive dysfunction (PLOS ONE, 2013). We report here further preclinical drug development advances, with the successful medicinal chemistry optimization of the *in vivo* molecular probes driven by ADMET (adsorption, distribution, metabolism, excretion, toxicology) considerations. MW 150 is a new chemical entity that has survived initial de-risking that renders it appropriate for investigational new drug (IND) enabling development.

Disclosures: O. Arancio: None. **J.P. Schavocky:** None. **J.C. Pelletier:** None. **S.M. Roy:** None. **D. Watterson:** None. **F. Saeed:** None. **L.J. Van Eldik:** None. **A. Bachstetter:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.02/X11

Topic: C.19. Drug Discovery and Development

Title: Characterization of pharmacological effects of a novel LRRK2 inhibitor

Authors: *J. M. THOMAS, X. HE, F. XUE, T. LI, S. ZHONG, D. YANG, J. LIU, L. KONG, P. VOULALAS, H. E. HASSAN, J.-S. PARK, A. D. MACKERELL JR., W. W. SMITH
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Abstract: Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene cause autosomal-dominant Parkinson's disease (PD) and contribute to sporadic PD. LRRK2 contains GTPase and kinase activities that have been implicated in the neuronal degeneration in PD pathogenesis and therefore LRRK2 becomes a potential drug target. To date, there is no disease-modifying drug to slow the neuronal degeneration of PD. Here, we characterized a novel LRRK2 inhibitor, FX2149, which was synthesized by chemical approaches. It altered LRRK2 GTP domain activity and reduced LRRK2 kinase activity *in vitro* in cell culture. It penetrated into the blood brain barrier and altered the LRRK2 GTP domain and kinase activities in brains of LRRK2 transgenic mice. Moreover, FX2149 significantly reduced the mutant LRRK2-induced neuronal toxicity in human neuroblastoma SH-SY5Y cells. These studies not only provide a pharmacological tool to further study LRRK2 biological functions but also provide a lead compound for further development of novel therapeutics for PD.

Disclosures: J.M. Thomas: None. **X. He:** None. **F. Xue:** None. **T. Li:** None. **S. Zhong:** None. **D. Yang:** None. **J. Liu:** None. **L. Kong:** None. **P. Voulalas:** None. **H.E. Hassan:** None. **J. Park:** None. **A.D. MacKerell Jr.:** None. **W.W. Smith:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.03/X12

Topic: C.19. Drug Discovery and Development

Support: NIA 5U01AG032969-05

Title: Blood-brain barrier permeable Hsp90 inhibitor reduces soluble tau burden in mouse models of Alzheimer's disease

Authors: *J. KOREN, III, C. INDA, M. UDDIN, P. PANCHAL, G. CHIOSIS
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Abstract: Current Alzheimer's disease (AD) medications attempt to address the symptoms associated with this disorder. However, there are no FDA approved AD medications which can act on the biochemical mechanisms which drive toxic pathology including tau (MAPT, tau) accumulation. Toxic tau aggregates are a hallmark AD pathology and are also the primary pathogenic species in a collection of neurodegenerative disorders, similar to AD, described as "tauopathies." In these disorders, tau accumulation is driven, in part, by abnormal phosphorylation progressing to a state of hyperphosphorylation. One avenue for treatment is the inhibition of the 90kDa heat shock protein (Hsp90); a molecular chaperone found to be capable of inducing the formation of neurotoxic species of the microtubule associated protein tau. Normally, aberrant protein accumulation is regulated by the molecular chaperones. However, AD presents a disease-specific population of Hsp90; a population which preserves disease driving tau accumulation. Here, we present data obtained through *in vivo* studies utilizing two different transgenic mouse strains treated with a blood-brain barrier permeable Hsp90 inhibitor, PU-HZ151, designed by our lab. PU-HZ151 capably reduces tau species associated with neurotoxicity, ameliorates strain-specific tau-related phenotypes, and significantly improves memory as assessed through behavioral analysis. Mice treated display no signs of toxicity; confirmed through chronic administration. Additionally, PU-HZ151 induced the expression of the pro-survival chaperone Hsp70, a chaperone also associated with tau degradation. Together, these findings demonstrate the potential for Hsp90 inhibition in the treatment of neurodegenerative diseases involving tau accumulation; namely, Alzheimer's disease.

Disclosures: J. Koren: None. **C. Inda:** None. **M. Uddin:** None. **P. Panchal:** None. **G. Chiosis:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.04/X13

Topic: C.19. Drug Discovery and Development

Support: Else Kröner Fresenius Foundation (Translational Research Innovation Pharma (TRIP) graduate school, I.T.)

Title: R-flurbiprofen attenuates experimental autoimmune encephalomyelitis in mice

Authors: K. SCHMITZ[1], *N. DE BRUIN[2], J. MÄNNICH[1], J. LÖTSCH[1], M. J. PARNHAM[2], G. GEISSLINGER[1], I. TEGEDER[1]
[1]Inst. of Clin. Pharmacol., Pharmazentrum Frankfurt, Frankfurt, Germany; [2]Transl Med. & Pharmacol (TMP), Fraunhofer Inst. Mol Biol and Appl Ecol (IME), Frankfurt Am Main, Germany

Abstract: R-flurbiprofen is the non-cyclooxygenase inhibiting R-enantiomer of the non-steroidal anti-inflammatory drug flurbiprofen, which was assessed as a remedy for Alzheimer's disease. Because of its anti-inflammatory, endocannabinoid-modulating and antioxidative properties, combined with low toxicity, we assessed R-flurbiprofen in experimental autoimmune encephalomyelitis (EAE) models of multiple sclerosis in mice. Oral R-flurbiprofen prevented and attenuated primary progressive EAE in C57BL6/J mice and relapsing-remitting EAE in SJL mice, even if the treatment was initiated after the first flare of the disease. R-flurbiprofen reduced immune cell infiltration and microglia activation and inflammation in the spinal cord, brain and optic nerve and attenuated myelin destruction, which was investigated via immunofluorescence studies and *in vivo* imaging. Also the EAE-evoked hyperalgesia was attenuated in the R-flurbiprofen treated mice compared with the vehicle group. FACS analyses revealed that R-flurbiprofen treatment increased CD4+CD25+FoxP3+ regulatory T-cells, CTLA4+ inhibitory T-cells and interleukin-10, whereas the EAE-evoked upregulation of pro-inflammatory genes in the spinal cord was strongly reduced. The effects were associated with an increase of plasma and cortical endocannabinoids but decreased spinal prostaglandins, the latter likely due to R- to S inversion. The promising results suggest potential efficacy of R-flurbiprofen in human MS and its low toxicity may justify a clinical trial.

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Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.05/X14

Topic: C.19. Drug Discovery and Development

Support: MEFOPA - Grant EU MEFOPA project HEALTH-2009-241791

Title: Protective role of olesoxime against wild type alpha-synuclein-induced toxicity in human neuronally differentiated SHSY-5Y cells

Authors: C. GOUARNE[1], J. TRACZ[1], M. GIRAUDON-PAOLI[1], V. DELUCA[1], M. SEIMANDI[1], G. TARDIF[1], M. XILOURI[2], L. STEFANIS[2,3], T. BORDET[1,4], *R. M. PRUSS[1]
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Abstract: Parkinson's disease (PD) is usually diagnosed clinically based on classical motor symptoms, while definitive diagnosis is made post-mortem, based on the presence of Lewy bodies and nigral neuron cell loss. Alpha-synuclein (ASYN), the main protein component of Lewy bodies, appears to play an important role in the onset of neurodegeneration that characterizes PD. Additionally, mutations in the ASYN gene or copy number variations are associated with some forms of familial PD. Olesoxime is a promising neuroprotective drug demonstrating in a pivotal phase II/III study, a protective effect on motor function loss and an improvement in overall health status in spinal muscular atrophy patients. Here, the objective was to evaluate whether olesoxime can prevent ASYN-mediated neurotoxicity by using a novel and attractive cellular model based on the inducible over-expression of human wild-type ASYN in neuronally differentiated neuroblastoma cells. This model demonstrates gradual cellular degeneration coinciding temporally with the appearance of soluble and membrane-bound ASYN oligomers and eventually cell death combining both apoptotic non-apoptotic pathways. We find that olesoxime fully protects differentiated SHSY-5Y cells from neurite retraction, cytoplasmic shrinkage and cell death induced by moderate ASYN overexpression. This protection was associated with a reduction in cytochrome c release from mitochondria and caspase-9 activation suggesting that olesoxime prevents ASYN toxicity by preserving mitochondrial integrity and function. In addition, we show that olesoxime displays neurotrophic effects on neuronally differentiated SHSY-5Y cells regardless of ASYN expression, by promoting their differentiation. Since ASYN-triggered neurodegeneration may be a common underlying factor in PD, olesoxime could be a promising therapy to treat PD, but also for other related disorders, termed synucleinopathies.

Disclosures: **C. Gouarne:** A. Employment/Salary (full or part-time):; Trophos. **J. Tracz:** A. Employment/Salary (full or part-time):; Trophos. **M. Giraudon-Paoli:** A. Employment/Salary (full or part-time):; Trophos. **V. Deluca:** A. Employment/Salary (full or part-time):; Trophos. **M. Seimandi:** A. Employment/Salary (full or part-time):; Trophos. **G. Tardif:** A. Employment/Salary (full or part-time):; Trophos. **M. Xilouri:** None. **L. Stefanis:** None. **T. Bordet:** A. Employment/Salary (full or part-time):; Trophos. **R.M. Pruss:** A. Employment/Salary (full or part-time):; Trophos.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.06/X15

Topic: C.19. Drug Discovery and Development

Title: Correlation between HSP90 target engagement and HSP70 level in mice brain

Authors: K. THIRSTRUP[1], *L. C. RONN[2], S. M. NIELSEN[3]
[1]Neurodegeneration 1, [3]Mol. Pharmacol., [2]Lundbeck, Valby, Denmark

Abstract: HSP90 (Heat shock protein 90) is a molecular chaperone protein ubiquitously expressed throughout all tissues in the body. As many of the HSP90 client proteins are oncogenic, HSP90 has been considered a promising chemotherapeutic target. In addition, HSP90 has also been proposed as a target to clear pathological proteins leading to neurodegeneration in Huntington's disease, Parkinson's disease and Alzheimer's disease. The mechanism of how HSP90 inhibition leads to clearance of misfolded proteins is not fully understood. It may involve direct effect of inhibiting ATPase function, indirect effects by inducing the Heat-shock-response resulting in upregulation of other chaperone proteins like HSP70 or a combination of both. Although HSP70 induction is routinely used as readout for HSP90 inhibition, the relationship between target engagement and HSP70 induction has only been studied to a limited extent *in vitro* and *in vivo*. In the current study we use pharmacological tools to establish the link between binding affinities, target occupancies and heat-shock upregulation effects of different HSP90 inhibitor tool compounds. In particular we describe how a simple and general method using ^{3}H-17AAG as tracer can be used to measure target engagement *in vitro* and *ex vivo* in brain tissue to correlate HSP90 target engagement with the down-stream upregulation of HSP70 using an ELISA assay. The current developed assay methodologies may first of all be of significant importance in the further elucidation of the mechanism involved in the *in vivo* effect of HSP90 inhibition in models for neurodegeneration, but may also be highly useful tools in any *in vivo* disease model, where HSP90 inhibition display positive effect.

Disclosures: K. Thirstrup: None. **L.C. Ronn:** None. **S.M. Nielsen:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.07/X16

Topic: C.19. Drug Discovery and Development

Title: Enhancement of protein clearance via activation of the 20S proteasome: A therapeutic approach for neurodegenerative diseases

Authors: S. KOIRALA[1], *J. GRAY[2], S. TALREJA[1], H. DOU[1], M. CUEVA[1], O. MURPHY[1], J. DANAO[1], Q. CHENG[1], K. LABITZKE[3], P. JAECKEL[3], S. MATERNA-REICHELT[3], S. SCHNEIDER[4], S. JOHNSTONE[1], N. SHARKOV[1], J. TANG[1], J. YE[1], C. PLEWA[5], P. CAO[1], S. ZHAO[1], P. ANDREWS[4], H. BECKMANN[3], Z. WANG[1], B. FOX[1], S. SAMBASHIVAN[1], S. KAMB[5], S. WANG[1]
[1]Amgen, S SAN FRANCISCO, CA; [2]Amgen, S SAN FRAN, CA; [3]Amgen, Regensburg, Germany; [4]Amgen, Cambridge, MA; [5]Amgen, Thousand Oaks, CA

Abstract: Impairments in protein clearance are a hallmark of multiple neurodegenerative diseases including Alzheimer's, Parkinson's, Huntington's and Amyotrophic Lateral Sclerosis (ALS). It is thought that intracellular accumulation of proteins such as tau in Alzheimer's and α-synuclein in Parkinson's may cause neuronal dysfunction and death. Enhancing protein clearance and reducing levels of potentially toxic proteins could have therapeutic benefits. One novel approach to increase endogenous protein clearance is to identify activators of the 20S proteasome, which is a core molecular complex involved in protein degradation. Here we describe a strategy to identify small molecule activators of 20S function. This includes a set of biochemical and cell-based assays to support target validation, screening, and post-screen evaluation of compounds. Biochemical assays that we describe, and validate using established proteasome inhibitors such as epoximicin, involve 20S-mediated proteolysis of various peptide substrates as well as native protein substrates such as α-synuclein. Cell based assays measure the turnover of proteins thought to be 20S substrates, including α-synuclein. In addition to assay platforms, we also describe refinement of methods to generate purified human 20S and detect small molecule binding using microscale thermophoresis. Using the array of tools at our disposal, we implemented a high throughput screening campaign for small molecule activators of 20S function, and identified compounds that increased the proteolysis of peptides, and in some cases, full length α-synuclein, by 20S.

Disclosures: **S. Koirala:** A. Employment/Salary (full or part-time):; Amgen. **J. Gray:** A. Employment/Salary (full or part-time):; Amgen. E. Ownership Interest (stock, stock options,

royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen. **S. Talreja:** A. Employment/Salary (full or part-time):; Amgen. **H. Dou:** A. Employment/Salary (full or part-time):; Amgen. **M. Cueva:** A. Employment/Salary (full or part-time):; Amgen. **O. Murphy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen. **J. Danao:** A. Employment/Salary (full or part-time):; Amgen. **Q. Cheng:** A. Employment/Salary (full or part-time):; Amgen. **K. Labitzke:** A. Employment/Salary (full or part-time):; Amgen. **P. Jaeckel:** A. Employment/Salary (full or part-time):; Amgen. **S. Materna-Reichelt:** A. Employment/Salary (full or part-time):; Amgen. **S. Schneider:** A. Employment/Salary (full or part-time):; Amgen. **S. Johnstone:** A. Employment/Salary (full or part-time):; Amgen. **N. Sharkov:** A. Employment/Salary (full or part-time):; Amgen. **J. Tang:** A. Employment/Salary (full or part-time):; Amgen. **J. Ye:** A. Employment/Salary (full or part-time):; Amgen. **C. Plewa:** A. Employment/Salary (full or part-time):; Amgen. **P. Cao:** A. Employment/Salary (full or part-time):; Amgen. **S. Zhao:** A. Employment/Salary (full or part-time):; Amgen. **P. Andrews:** A. Employment/Salary (full or part-time):; Amgen. **H. Beckmann:** A. Employment/Salary (full or part-time):; Amgen. **Z. Wang:** A. Employment/Salary (full or part-time):; Amgen. **B. Fox:** A. Employment/Salary (full or part-time):; Amgen. **S. Sambashivan:** A. Employment/Salary (full or part-time):; Amgen. **S. Kamb:** A. Employment/Salary (full or part-time):; Amgen. **S. Wang:** A. Employment/Salary (full or part-time):; Amgen.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.08/X17

Topic: C.19. Drug Discovery and Development

Support: Doktor Robert Pfleger - Stiftung

Title: Acidic dual γ-secretase modulators/PPARγ agonists improve the mitochondrial dysfunction in a cellular model of Alzheimer's disease

Authors: *M. POHLAND[1], S. HAGL[1], M. WURGLICS[2], M. SCHUBERT-ZSILAVECZ[2], G. P. ECKERT[1]
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Abstract: Alzheimer's disease (AD) is a progressive, neurodegenerative disorder leading to dementia. Deposits of beta amyloid protein (Aβ) and intracellular neurofibrillary tangles are pathological hallmarks of AD. Increasing evidences indicate mitochondrial dysfunction as an early event in AD pathogenesis. Mitochondria are essential for the supply of energy but are also involved in oxidative stress and apoptosis. Current drugs act merely symptomatic and new disease modifying drugs against AD have almost failed in human clinical trials recently. We investigated the efficacy of a novel class of acidic γ-secretase modulators/PPARγ agonists with a dual mechanism of action against mitochondrial dysfunction in HEK293-APP_{695} cells expressing neuronal APP. Dimebon, DAPT, and Pioglitazone were used as controls. The basic structure of the new compounds, derivated of pirinixic acid, is thiobarbituric acid. Optimization of pharmacological activities leaded to new molecules with a significantly increased activity at both pharmacological targets. All examined compounds were active and influenced the cell biology of the studied HEK293-APP_{695} cells. First, the cell viability was measured by MTT assay in the range of 0,03 - 30 μM. For the non-toxic concentrations we measured changes in mitochondrial membrane potential (MMP) and levels of adenosine triphosphate (ATP) to determine alterations of mitochondrial efficiency and function. The compounds MH49, MH73, MH84 and MH163 were able to exhibit significant protective effects against NO-releasing drugs like sodium nitro prusside (SNP). Aβ-induced changes in mitochondrial enzyme activities are leading to oxidative stress and enhanced apoptosis. The rate of mitochondrial respiration was investigated and especially in the complex-IV respiration, which is decreased by Aβ, we were able to show a significant improvement after treatment with compound MH84 compared to control group. To get a closer look insight, we measured the citrate synthase (CS) activity, used as a marker enzyme for the mitochondrial mass. CS activity in HEK293-APP_{695} cells was increased by the GSM MH84, indicating an increased mitogenesis. In summarizing review substances MH84 and MH73 in our in-vitro experiments were the most convincing compounds in the nM range. Further experiments will reveal the molecular consequences of γ-secretase/PPARγ modulation and to test the compounds in mouse models of Alzheimer`s disease.

Disclosures: **M. Pohland:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Doktor Robert Pfleger - Stiftung. **S. Hagl:** None. **G.P. Eckert:** None. **M. Wurglics:** None. **M. Schubert-Zsilavecz:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.09/X18

Topic: C.19. Drug Discovery and Development

Support: NIMH R01 MH052711

Title: Role of oxidative redox shift on STEP activity

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Abstract: Protein tyrosine phosphatases (PTPs) are enzymes that dephosphorylate phospho-tyrosine in target proteins. STEP (Striatal-enriched protein tyrosine phosphatase; or Ptpn5) is a brain-specific phosphatase associated with cognition and which is upregulated in several neuropsychiatric disorders including Alzheimer's disease (AD), Parkinson's disease (PD), fragile X syndrome (FXS) and schizophrenia. We previously reported that genetically decreasing STEP activity restores cognitive deficits in an AD mouse model (3xTg-AD). Recently, our lab reported the identification and characterization of a STEP inhibitor that improved cognitive function in these mice. The mechanism of action of the inhibitor was to form a covalent bond with the catalytic cysteine (Cys). Addition of glutathione (GSH) decreased the inhibitory capacity of this compound, suggesting an oxidative mechanism for STEP inhibition. PTPs, including STEP, have a conserved redox-active Cys residue in the catalytic center and the active thiol (SH) moiety in this Cys makes it prone to oxidation. Since the effect of reactive oxygen species (ROS) on STEP remains largely unexplored, here we investigated the role of oxidative stress on STEP protein level and activity, as well as the Tyr phosphorylation of downstream targets: pNR2B, pERK, and pPyk2. We hypothesized that free radicals would initiate redox changes in the active cysteine that would reversibly decrease STEP expression and activity. We exposed neuronal cultures to either 1) a more oxidative environment by increasing levels of ROS with H2O2 in a dose and time dependent manner or 2) a more reducing environment by decreasing ROS with catalase and superoxide dismutase, and observed the effects on STEP levels and substrate phosphorylation. Using several biochemical and molecular approaches, we show direct effects of oxidative stress on STEP activity. Moreover, our findings will give us a better understanding of whether increased ROS in neurodegenerative disorders function, at least in part, through modulation of STEP activity.

Disclosures: D. Ghosh: None. **J. Ellman:** None. **A.C. Nairn:** None. **P.J. Lombroso:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.10/X19

Topic: C.19. Drug Discovery and Development

Support: NIMH R01 MH052711

Title: Characterization of TC-2153 as a potent inhibitor of STriatal Enriched protein tyrosine Phosphatase

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Abstract: STEP (STriatal-Enriched protein tyrosine Phosphatase), a neuron-specific phosphatase is overactive in several neuropsychiatric conditions including Alzheimer's disease, fragile X syndrome, schizophrenia, Parkinson's disease as well as in age-related memory deficits. Genetic reduction of STEP in models of several of these diseases improved their cognitive function. Based on these results, STEP represents a novel target for cognitive disorders. Here we characterize a small molecule inhibitor, benzopentathiepin 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6 amine hydrochloride (known as TC-2153), as an inhibitor of STEP. We tested TC-2153 in *in vitro* para-nitrophenyl phosphate assay and in cell-based secondary assays along with *in vivo* wild-type (WT) mice experiments by measuring the increased tyrosine phosphorylation of STEP substrates ERK1/2, Pyk2, and GluN2B that occur with inhibition of STEP activity. We tested TC-2153 for its specificity in WT and STEP KO mice and cognitive improvement in in 6- month old triple transgenic AD (3xTg-AD) mice. We present data indicating that the mechanism for STEP inhibition involves oxidative attack of the catalytic cysteine residue in the phosphatase domain. Our results indicate that TC-2153 is a potent inhibitor of STEP *in vitro* with an IC50 of 24.6 nM, and that TC-2153 was able to inhibit STEP at 0.1 μM in neuronal cell and at 10 mg/kg, i.p in WT mice. Moreover, TC-2153 does not target homologous PTPs known to dephosphorylate ERK1/2 and Pyk2 when tested *in vivo* and showed no cytotoxicity response at doses 10 times higher than the effective dose in neuronal cultures. Administration of TC-2153 improves cognitive performance of AD mice in Y-maze alternation, the Morris water maze, and the novel object recognition task. These findings demonstrate that TC-2153 is capable of reversing cognitive deficits in a mouse model of AD.

Disclosures: **M. Chatterjee:** None. **J. Xu:** None. **T. Baguley:** None. **J. Brouillette:** None. **P. Kurup:** None. **D. Ghosh:** None. **J. Kanyo:** None. **J. Gresack:** None. **P. Greengard:** None. **T. Lam:** None. **L. Tautz:** None. **J. Ellmann:** None. **A.C. Nairn:** None. **P. Lombroso:** None.

Poster

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Support: The Michael J. Fox Foundation

NIMH R01 MH052711

DOD

Title: STEP61 is a novel substrate for the E3 ligase parkin, and is implicated in Parkinson's disease

Authors: *E. FOSCUE[1], P. KURUP[1], R. ALEXANDRA VIDEIRA[3], J. XU[1], C. ONONENYI[1], G. BALTAZAR[3], A. C. NAIRN[2], P. LOMBROSO[1]
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Abstract: Parkinson's disease (PD) is characterized by degeneration of substantia nigra pars compacta (SNc) dopamine neurons that leads to severe motor and cognitive dysfunction. The E3 ubiquitin ligase parkin is involved in both genetic and sporadic forms of PD and inactivation of parkin by genetic mutations or by toxins leads to accumulation of parkin substrates in the brain. $STEP_{61}$ (STriatal-Enriched protein tyrosine Phosphatase) is a protein tyrosine phosphatase highly enriched in striatum. Previous studies showed that $STEP_{61}$ is regulated by ubiquitin-mediated proteasomal degradation and it is up-regulated in several neuropsychiatric illnesses. Here we demonstrate that $STEP_{61}$ is a novel substrate of parkin, and that $STEP_{61}$ levels are regulated by ubiquitin-mediated proteasomal degradation. $STEP_{61}$ is increased in human PD brain and MPTP-induced PD toxin models. The increases in $STEP_{61}$ levels are associated with a decrease in the phosphorylation of ERK1/2 and the downstream target of ERK1/2, CREB. These results indicate that parkin inactivation leads to accumulation of $STEP_{61}$ and altered ERK1/2 and CREB signaling in striatum, which may have potential implications in striatal synaptic plasticity and

symptoms associated with PD. Funding: The Michael J. Fox Foundation, the National Institute of Health, and the Department of Defense.

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Poster

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Motac Holding, Inc.

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Title: The tyrosine phosphatase STEP is implicated in age-related memory decline across different species

Authors: *J. BROUILLETTE[1], C. MÉNARD[2], R. QUIRION[3], B. BONTEMPI[4], J. S. SCHNEIDER[5], C. M. NORRIS[6], G. FERLAND[2], E. BÉZARD[4], P. GAUDREAU[2], P. J. LOMBROSO[1]
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Abstract: Age-associated memory deficits are observed across many species, but the underlying molecular mechanisms remains to be determined. Here we report elevations in the levels and activity of striatal-enriched protein tyrosine phosphatase (STEP) in aged memory-impaired mice

and rats. We also observed increased STEP expression in aged rhesus monkeys and humans with mild cognitive impairments (MCI) compared to age-matched controls. These increases are linked to enhanced dephosphorylation of the STEP substrates GluN2B and ERK1/2, as well as increased internalization of GluN2B-containing NMDARs. STEP accumulation with aging appears to involve dysfunction of the ubiquitin-proteasome system (UPS). Genetic reduction of STEP levels alleviates age-related memory decline and restores active phospho-CREB and BDNF levels, both implicated in memory consolidation. Elevated STEP levels that occur with advancing age constitute a molecular mechanism common to several species that contributes to age-related cognitive deficits, and may represent a target for pharmacological therapy to enhance cognition in the elderly and MCI patients.

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Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

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Topic: C.19. Drug Discovery and Development

Support: NIMH R01 MH052711

FRAXA Research Foundation

Title: Effects of inhibiting STEP activity on synaptic connections in Alzheimer's Disease

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Abstract: Dendritic complexity is reduced in Alzheimer's disease (AD). The altered morphology of AD neurons causes changes in neuronal firing and contributes to the cognitive deficits observed in the disease. Previous studies have shown that STEP (striatal-enriched protein tyrosine phosphatase) is upregulated in AD. The increase in STEP expression is due to a beta amyloid (Aβ)-mediated inhibition of the proteasome, resulting in a decrease in STEP degradation. We demonstrated that genetic reduction of STEP in 3xTG-AD mice restores memory deficits associated with the pathology. These studies validated STEP as a target for drug

discovery and we recently identified a pharmacological inhibitor of STEP, TC-2153 Here we test the hypothesis that administration of the STEP inhibitor TC-2153 to neuronal cultures will improve dendritic and synaptic connections in cortical cultures derived from a mouse model of AD. We have tested this hypothesis by quantification of dendritic length, arborization, and number and location of nodes. Neuronal cultures were grown from wild type and triple transgenic (3xTG-AD) mice. The wild type cultures were treated with Aβ peptide as well as vehicle or TC-2153. In addition, a control group was treated with vehicle alone, as well as drug vehicle or TC-2153. Treatment with TC-2153 rescued some of the Aβ-induced deficits in neurons. This research demonstrates a physiological mechanism that may explain, at least in part, how TC-2153 treatment alleviates cognitive deficits in 3xTG-AD mice. Moreover, these results suggest a role for STEP in the development of synaptic connections, a new hypothesis that will be tested in future studies.

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Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

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Topic: C.19. Drug Discovery and Development

Support: NIMH R01 MH052711

Title: Effects of orally administered TC-2153, an inhibitor of the tyrosine phosphatase STEP, on cognitive deficits in an Alzheimer's mouse model

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Abstract: STriatal-Enriched protein tyrosine Phosphatase (STEP) has been implicated in Alzheimer's disease (AD). Increased levels of STEP61 were noted in the prefrontal cortex of patients with AD, as well as in four different mouse models of the disease. In addition, earlier studies demonstrated that beta amyloid (Aβ) mediates the increased expression of STEP61 through inhibition of the ubiquitin proteasome pathway. The increased level of STEP61 results in greater NMDA and AMPA receptor internalization. Thus a current model of STEP function is that it normally opposes the development of synaptic strengthening, and that increased expression of STEP disrupts synaptic function and contributes to cognitive deficits. Consistent

with this model, we previously reported that genetic reduction of STEP in 3xTg-AD mice increased the Tyr phosphorylation of STEP substrates, improved hippocampal synaptic plasticity, and rescued cognitive deficits. These results validated STEP as a promising target for AD drug development. We recently characterized TC-2153 as a potent STEP inhibitor with therapeutic potential (see nearby poster). Results were obtained in a mouse model via intraperitoneal injection of TC-2153. It is not known, however, whether TC-2153 is similarly efficacious when administered orally, which may be preferable as a non-invasive long-term treatment. In this study, we tested this by administration of TC-2153 to 3xTg-AD and wild-type (WT) mice via oral gavage. The Tyr phosphorylation of ERK1/2, Pyk2, and GluN2B was examined in the cortices of WT mice 3 hours after oral administration. In addition, behavioral tests including the Y-maze alternation task for spatial working memory and the novel object recognition test were performed for the WT and 3xTg-AD mice. Findings from this study will elucidate the potential of TC-2153 as an orally administered therapeutic agent in AD, in animal models of the disorder.

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Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

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Program#/Poster#: 234.15/X24

Topic: C.19. Drug Discovery and Development

Title: SUVN-D4010: Novel 5-HT4 receptor partial agonist for the treatment of Alzheimer's disease

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and AD patients have impaired cognitive skills. 5-HT4 receptors are densely present in the hippocampus and frontal cortex suggesting the role of this receptor in memory and cognition. 5-HT4 ligands have the ability to induce neurogenesis in various brain regions such as hippocampus. These compounds replace the degenerated cells by inducing production of neurons. 5-HT4 partial

agonists offer both symptomatic and disease-modifying effects hence may be beneficial in treatment of AD. 5-HT4 ligands may offer improved clinical efficacy and/or tolerability relative to acetylcholinesterase inhibitors which are being currently used in the treatment of AD. SUVN-D4010 a potent, selective and orally bioavailable and brain penetrant 5-HT4 partial agonist, was evaluated for its procognitive property. Modulation of acetylcholine levels by SUVN-D4010 was evaluated using brain microdialysis technique. Striatal 5-HT4 receptor occupancy was measured using non-radiolabeled tracer in rats. Cortical sAPPα levels and CSF amyloid-β protein levels were also evaluated in the preclinical species using ELISA kits. Safety, toxicity and mutagenic potential of SUVN-D4010 were evaluated in rodents/non rodents and *in vitro* models. SUVN-D4010 reversed time induced memory deficits using object recognition task. Alleviation in working and emotional memory deficits induced by scopolamine in radial arm maze task and fear conditioning assay were also observed. Oral administration of SUVN-D4010 significantly increased the brain acetylcholine levels. The above observed improvement in cognition was blocked by selective 5-HT4 antagonist. SUVN-D4010 showed a dose dependent increase in receptor occupancy in rat brain. A significant increase in cortical sAPPα and decrease in CSF amyloid-β protein levels were noted in non-transgenic animal models. SUVN-D4010 was well tolerated in preclinical toxicity studies and did not show any mutagenic potential *in vitro*. SUVN-D4010 is a novel, potent, selective, orally bioavailable, brain penetrant, efficacious and safe 5-HT4 receptor partial agonist. IND enabling studies have been completed and US IND filing is in progress.

Disclosures: **N. Muddana:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Subramanian:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Medapati:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Abraham:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Gadi:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **V. Benade:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Palacharla:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **A. Manoharan:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **V. Goyal:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **S. Pandey:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **A. Mohammed:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **S. Ravella:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA. **R. Nirogi:** A. Employment/Salary (full or part-time):; SUVEN LIFE SCIENCES LTD., HYDERABAD, INDIA.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: C.19. Drug Discovery and Development

Title: Effect of gender and food on the single dose pharmacokinetics of SUVN-502, a potent and selective 5-HT6 receptor antagonist

Authors: *R. V. NIROGI, K. MUDIGONDA, K. PENTA, G. BHYRAPUNENI, D. AJJALA, N. MUDDANA, V. BENADE, V. CHOWDARY PALACHARLA, V. GOYAL, S. PANDEY, R. ABRAHAM, P. JAYARAJAN, R. BADANGE, R. KAMBHAMPATI
Suven Life Sci., Hyderabad, India

Abstract: Efficacy studies conducted in animal models of cognition and early clinical studies suggest that 5-hydroxytryptamine receptor [5-HT6] antagonists improve cognition by releasing neurotransmitters acetylcholine and glutamate. SUVN-502 is a potent and selective 5-HT6 receptor antagonist exhibiting cognitive enhancement in rodent models. SUVN-502 is being developed for the treatment of Alzheimer's disease (AD). SUVN-502 was investigated in a single-center, multiple-faceted, phase 1 clinical study (US IND) to evaluate its safety and effects of gender and food on the pharmacokinetics following a single dose of 100 mg oral tablet in healthy subjects. To evaluate the gender effect 12 healthy male and female subjects between 18 to 45 years of age were given a single dose under fasted conditions. Similarly food effect was evaluated in 12 healthy male subjects between 18 to 45 years of age. Subjects were given a single dose of 100 mg tablet on Day 1 and 8 with and without food in a crossover manner. SUVN-502 and its active metabolite M1 of SUVN-502 were quantified using a validated LC-MS/MS method. There were no clinically relevant or serious adverse events reported by any subject during the Phase I study. No subject was withdrawn from the study for safety reasons. Pharmacokinetic parameters were comparable between the male and female groups. Mean AUC(0-inf) and Cmax in the fed group were approximately 20% higher and 15% lower than that in the fasted group, respectively. Median tmax was slightly delayed in the fed group (3.5 h in fed vs 2.5 h in fasted) while mean t1/2 was similar between the fasted and fed groups at approximately 10 hours. Mean AUC(0-inf) and Cmax of M1 of SUVN-502 in the fed group were approximately 13% higher and 27% lower than that in the fasted group respectively. SUVN-502 has favorable safety and pharmacokinetic profile after single dose administration. Gender and food did not have any affect on pharmacokinetic parameters of SUVN-502.

Disclosures: **R.V. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **K. Mudigonda:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **K. Penta:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **D. Ajjala:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **N. Muddana:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **V. Chowdary Palacharla:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **V. Goyal:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **S. Pandey:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **R. Abraham:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **R. Badange:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **R. Kambhampati:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

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Topic: C.19. Drug Discovery and Development

Support: nih grant R21 NS 074062-A1 NIH/NINDS

Title: *In vitro* inhibition of glutamate/cystine transporter in microglial cells reduces excitotoxic conditions

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Abstract: Virtually every pathological condition that disrupts brain homeostasis, including neurodegenerative disorders such as Parkinson's, Alzheimer's and HIV-associated neurocognitive disorders, can trigger microglial cell activation. This event is characterized by the release of glutamate, nitric oxide, and cytokines, and changes in cell morphology and behavior. System xC- is an antiporter that imports extracellular cystine and exports intracellular glutamate leading to an increase in the extracellular levels of this neurotransmitter and neuronal cell death

via excitotoxicity. We show that system xC- activity increases upon lipopolysaccharides (LPS) activation of rat primary microglia, leading to a ≥3-fold change in extracellular glutamate. Inhibition of system xC- with prototype inhibitors significantly reduced glutamate release from LPS- and HIV Tat protein-treated microglia. Erastin, also known to be an anti-cancer agent, was the most effective compound tested (IC50 = 14 ± 3 nM) and its effects were selective for glutamate, since a different marker of microglia activation, tumor necrosis factor alpha release, was not affected. An inactive analog, erastin A8, showed no effects modulating glutamate levels, while more potent analogs showed enhanced inhibitory activity. No cell toxicity was observed with erastin at the effective concentration and it did not induce pro-apoptotic or endoplasmic reticulum stress-associated gene expression profile changes. However, inhibition of system xC- by erastin decreased the redox capability of microglial cells as a direct consequence of lower cystine uptake. Excess extracellular glutamate could be decreased by inhibiting system xC- in microglial cells with specific and potent small molecule inhibitors such as erastin, consequently protecting neurons from excitotoxic insults.

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Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

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Program#/Poster#: 234.18/Y3

Topic: C.19. Drug Discovery and Development

Title: Characterization of MARP1, a novel positive allosteric modulator of the AMPA receptor that amerliorates age-related cognitive deficits in rats

Authors: ***J. A. MORROW**[1], L. H. SMITH[2], F. MARTIN[2], S. NEALE[2], N. ABROUS[3], P. V. PIAZZA[3], H. M. MARSTON[2]
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Abstract: AMPA receptors are ligand-gated ion channels that mediate the majority of fast excitatory amino acid transmission in the CNS and also participate in forms of synaptic plasticity

underlying learning and memory. Drugs that positively modulate AMPA receptor function hold promise for the treatment of cognition related dysfunction in disorders such as Alzheimer's disease and schizophrenia. Here, we describe the characterization of MARP1, a novel small molecule positive allosteric modulator of the AMPA receptor. The effect of MARP1 at native AMPA receptors was determined by whole-cell patch-clamp recordings in rat cultured cortical neurons. Glutamate (0.5 mM) was applied for 1s either alone or with the test compound using a semi-rapid drug application system. Co-application of MARP1 (1-100μM) with glutamate decreased desensitisation leading to increases in steady-state current in a reversible and concentration dependent manner: maximum increases were 680±115% (n=9) and the EC_{50} value was 2.5μM. The *in vitro* effects of MARP1 on synaptic transmission and plasticity were studied in submerged hippocampal slices from rats. Field excitatory postsynaptic potentials (fEPSPs) were recorded from stratum radiatum of CA1 area in response to Schaffer-commissural pathway stimulation. To induce LTP, presynaptic fibres were stimulated with a theta burst stimulation in which 10 bursts of stimulation pulses were delivered at a frequency of 5Hz; each burst contained three pulses at 100Hz. Bath application of MARP1 (10μM) increased the slope of fEPSPs by 34±4% (mean±S.E.M) ($p<0.01$, n=8). The effect on fEPSPs was rapid and peaked within 10 minutes of drug applications. 100nM MARP1 was effective in inducing LTP with three pulse theta burst stimulation. While synaptic potentials were unchanged in all 8 control experiments, 6 out of 6 experiments performed in the presence of MARP1 (100nM) showed stable LTP with an average potentiation of 224±119% in fEPSP slope, ($p<0.05$, n=6). 10 μM MARP1 was also demonstrated to ameliorate LTP deficits following four pulse theta stimulation in hippocampal slices from aged rats. In a series of tests designed to determine the impact of MARP1 on behavioral functioning in rats, MARP1 was demonstrated to ameliorate age-related cognitive deficits in spatial learning and memory in a water maze task and cognitive flexibility in a serial reversal learning task. These results indicate that MARP1 is a positive allosteric modulator of recombinant and native rat AMPA receptors. Thus, it represents an interesting and informative tool for investigating the therapeutic potential of glutamatergic enhancement in psychiatric and neurological disorders.

Disclosures: **J.A. Morrow:** A. Employment/Salary (full or part-time):; Full time employee of Merck & Co. **L.H. Smith:** A. Employment/Salary (full or part-time):; Full time emplyee of Merck & Co. **F. Martin:** A. Employment/Salary (full or part-time):; Full time emplyee of Merck & Co,. **S. Neale:** A. Employment/Salary (full or part-time):; Full time emplyee of Merck & Co,. **N. Abrous:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Principal investigator of drug study. **P.V. Piazza:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Principal Investigator of drug study. **H.M. Marston:** A. Employment/Salary (full or part-time):; Full time emplyee of Merck & Co..

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

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ALSAM Foundation

Title: Targeting nicotinic acetylcholine receptors with the designed multiple ligand (DML) approach

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Abstract: The designed multiple ligand (DML) approach allows the development of compounds with desired poly-pharmacological profiles for the treatment of many disorders, including complex central nervous system (CNS) diseases. Recently, we designed and synthesized DMLs targeting the nicotinic acetylcholine receptor (nAChR) family (JPET, 2013, 347:424-437) and we have now expanded this approach. Diaza(bi)cyclic moieties, which are known to be important scaffolds for nAChR ligands (nAChRL) and for a variety of other CNS drugs, are attached or merged with other CNS active compounds or derived scaffolds. Example compound libraries including nAChRL/nAChRL, and nAChRL/riluzole hybrids will be presented. Riluzole has been approved by the US FDA for the treatment of amyotrophic lateral sclerosis (ALS) and interacts with diverse ion channels. It displays a privileged scaffold - benzothiazole - which provides a hydrogen bond acceptor motif. nAChRL based scaffolds were attached to, or merged with, riluzole in two different ways to alter its poly-pharmacological properties while attempting to retain a favorable CNS druggability profile and create a designed multiple ligand (DML) lead

with combined or amplified activities. These compound libraries were tested against diverse nAChRs to obtain insight into their structure activity relationships. Radioligand binding assays were performed with [3H]epibatidine, [3H]methyllycaconitine, [3H or 125I]alpha-bungarotoxin, [3H]NS14492 and [3H]NS10743 using membrane fractions of rat brains, pig brains, pig adrenals, and Torpedo californica electroplax to evaluate the affinity of these compounds for nAChR subtypes. A broad spectrum of affinities (Ki values: < 10 nM to > 10,000 nM) and subtype selectivity provided important hints for further "designing in", "balancing", and "designing out" strategies to obtain DML lead candidates.

Disclosures: I. Tomassoli: None. **A.J. Reinhart:** None. **M.R. Picciotto:** None. **D. Gundisch:** None. **T.T. Talley:** None. **R.L. Papke:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.20/Y5

Topic: C.19. Drug Discovery and Development

Support: Boehringer Ingelheim Ulm University BioCenter (BIU)

Title: Investigation of selective monoacylglycerol lipase (MAGL) inhibition and selective fatty acid amide hydrolase (FAAH) inhibition in comparison to simultaneous FAAH/MAGL inhibition on catalepsy and brain endocannabinoid, arachidonic and prostaglandin levels in mice

Authors: *B. FERGER[1], C. PORAZIK[1,2], N. PASQUARELLI[1,2], A. WITTING[2]
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Abstract: Monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) are the principal enzymes for endocannabinoid metabolism and are of particular interest to study behavioral and neurochemical alterations mediated by the endogenous cannabinoid receptor ligands 2-arachidonoylglycerol (2-AG) and anandamide (AEA). Here, we investigate the effects of the commercially available selective MAGL inhibitor KML29 and the commercially available selective FAAH inhibitor PF3845 in comparison to the simultaneous MAGL/FAAH inhibition by JZL195 or a combination of KML29 + PF3845 on the potential induction of cataleptic behavior and endocannabinoid pathway biomarker correlates. Male adult C57BL/6JRj mice were orally treated with KML29 (10,20mg/kg), PF3845 (10mg/kg), JZL195 (10mg/kg) or vehicle.

Haloperidol treatment (1mg/kg) served as positive control for catalepsy which was determined in the bar test 0, 30, 60, 90 and 120 min after compound administration. After the catalepsy test brains were removed and the tissue was processed for liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis (for details of LC-MS/MS please see poster of Porazik et al., this SFN meeting). Neither the selective MAGL inhibitor nor the selective FAAH inhibitor produced any cataleptic behavior at any time. In contrast, a simultaneous MAGL and FAAH inhibition either by the dual MAGL/FAAH inhibitor or by a combination of the MAGL and FAAH inhibitors showed a significant and time dependent increase of catalepsy. 2-AG levels were highest in the brains of mice which received the KML29 + PF3845 combination followed by KML29 and by JZL195 and were unchanged after PF3845 and haloperidol treatment. The ranking order of AEA increase was led by PF3845 followed by the combination of KML29 + PF3845 and JZL195 and no change was observed after KML29 and haloperidol treatment. Interestingly, the 2-AG and AEA metabolite arachidonic acid and PGE_2 were only decreased by KML29, JZL195 and the combination of KML29 + PF3845 but not by the selective FAAH inhibitor PF3845 alone. In conclusion, a simultaneous inhibition of FAAH and MAGL was not well-tolerated in mice and did not produce any synergistic effects on 2-AG or AEA brain levels. Thus, selective MAGL or FAAH inhibition is less prone to a cataleptic motor phenotype and probably less prone to cannabimetic effects in comparison to simultaneous FAAH/MAGL inhibition.

Disclosures: **B. Ferger:** A. Employment/Salary (full or part-time):; B.F. is a full time employee of Boehinger Ingelheim Pharma GmbH & Co. KG.. **C. Porazik:** None. **N. Pasquarelli:** None. **A. Witting:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.21/Y6

Topic: C.19. Drug Discovery and Development

Title: Pharmacological characterization of TASP0428980, a dual orexin receptor antagonist

Authors: J.-I. KARASAWA[1], *T. AOKI[1], D. KAMBE[1], T. SHIMAZAKI[1], H. HIKICHI[1], D. NOZAWA[2], H. KAWAMOTO[2]
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Abstract: The orexin system plays an important role in the regulation of sleep and wakefulness by activating two G-protein coupled receptors, orexin 1 (OX1) and orexin 2 (OX2) receptors. Many clinical and pre-clinical studies have shown that the dual orexin receptor antagonists at OX1 and OX2 might be a promising drug candidate for the treatment of insomnia. Here we show pharmacological profiles of TASP0428980, a newly synthesized and potent dual orexin receptor antagonist. TASP0428980 showed potent antagonist activity at both human and rat OX1/OX2 receptors, and shifted the dose-response curve of an orexin A analog-induced Ca2+ mobilization rightward without altering the maximal response. In contrast, TASP0428980 at 10 μM had no apparent affinity for 72 other receptors, transporters, and ion channels. In the electroencephalogram studies using freely moving rats, administration of TASP0428980 (3, 10, 30 mg/kg, ip) significantly shortened the non-REM sleep latency, and increased the time spent in both non-REM sleep and REM sleep. In contrast, TASP0428980 did not exhibit impairment of the motor performance at 30 mg/kg (ip), which was commonly observed by treatment of pharmacological effective dose of GABA-A receptor positive modulators, assessed by rotarod test in rats. These results show that TASP0428980 is the potent and selective dual orexin receptor antagonist with sleep-promoting effects, and that this compound can be used as a tool to explore pharmacological significance of blockade of orexin receptors.

Disclosures: J. Karasawa: A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co. LTD. **T. Aoki:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co. LTD. **D. Kambe:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co. LTD. **T. Shimazaki:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co. LTD. **H. Hikichi:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co. LTD. **D. Nozawa:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co. LTD. **H. Kawamoto:** A. Employment/Salary (full or part-time):; Taisho Pharmaceutical Co. LTD.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.22/Y7

Topic: C.19. Drug Discovery and Development

Support: KAKENHI25340104

MEXT-Supported Program for the Strategic Research Foundation at Private Universities 2013-2018

Title: Induced expression of Nor1 is not important for neurite outgrowth in PC12 cells

Authors: *K. SHIMOKE[1], T. TOMIOKA[1], H. AOYAMA[1], Y. NISHIHATA[1], H. MARUOKA[2], T. IKEUCHI[1]
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Abstract: Histone deacetylase (HDAC) inhibitors can induce neuronal differentiation in the central nervous system (CNS) or peripheral nervous system (PNS). The effect of HDAC inhibitor is not limited to neuronal differentiation but to the cell survival. We have reported that HDAC inhibitor promotes neurite outgrowth and cell survival via expression of Nur77, one of the immediate early gene (IEG), in PC12 cells. While Nur77 has a family protein, for instance, Nor1, the function of Nor1 by HDAC inhibitor is still unclear. Thus, we analyzed whether Nor1 is essential gene product for neurite out growth by HDAC inhibitor, comparing with expression of Nur77 by forskolin (FSK) because FSK is well-known to induce neurite outgrowth. We used tricostatin A (TSA) as a HDAC inhibitor and FSK as a positive control for neurite outgrowth. We also added siRNA to deplete the expression of Nur77. Then, the neurite was photographed using phase-contrast microscopy and the length was measured using analysis software (BZ-9000). As a result, we found that TSA, a potent HDAC inhibitor, or FSK induced neurite outgrowth accompanied with expression of Nur77 when TSA or FSK was treated to the cells for 24 hours. We also observed that only FSK induced the expression of Nor1. In addition, the experiment using siRNA against mRNA for nur77 gene demonstrated that TSA did not induce neurite outgrowth. These results suggested that the expression of Nor1 is not essential but the expression of Nur77 is necessary for neurite outgrowth by TSA.

Disclosures: **K. Shimoke:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; KAKENHI25340104, MEXT-Supported Program for the Strategic Research Foundation at Private Universities 2013-2018. **T. Tomioka:** None. **H. Aoyama:** None. **Y. Nishihata:** None. **H. Maruoka:** None. **T. Ikeuchi:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.23/Y8

Topic: C.19. Drug Discovery and Development

Title: Cathepsin s: a novel therapeutic target for alzheimer's disease

Authors: ***B. YI**[1], A. K. EVANS[1], L. J. HOLSINGER[2], R. BOOTH[2], M. SHAMLOO[1]
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Abstract: Cathepsin S is a cysteine protease exhibiting both extra- and intracellular activities important for regulation of MHC Class II antigen presentation and neuroinflammation. During neuronal injury, cathepsin S is secreted from microglia and cleaves membrane bound fractalkine (FKN), releasing soluble FKN. The released FKN activates the fractalkine receptor (CX3CR1), which modulates microglial activation, release of inflammatory mediators, and lymphocyte recruitment. Chronic neuroinflammation and elevation of microglial-derived cytokines are a key hallmark of Alzheimer's Disease (AD). In mouse models of AD, deletion of the FKN receptor prevents neuronal loss, microglial activation, beta-amyloid deposition, and loss of cognitive function. Moreover, an overexpression of cathepsin S and FKN is detected in postmortem human AD brains. In line with this, we have also shown an over-expression and activation of cathepsin S in a mouse model of AD along with a concomitant activation of microglia. In a preclinical study using the Thy1-APP$^{Lond/Swe+}$ (T41B) transgenic mouse model of AD, we have shown that once-daily administration for 6 weeks of VBY-50365, a selective cathepsin S inhibitor, which possesses good central nervous system penetration, leads to a significant improvement in working memory in a Y-maze test. Moreover, a trend for improvement was also seen in passive avoidance and fear conditioning tests. A reduced level of amyloid-beta was also detected in the treated animals. These results suggest that modulation of cathepsin S may have tremendous therapeutic value and VBY-50365 may be an efficacious therapeutic for the treatment of AD with a novel mechanism of action. Further preclinical and biochemical studies are ongoing to confirm these findings.

Disclosures: **B. Yi:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Virobay Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Virobay Inc. **A.K. Evans:** None. **L.J. Holsinger:** A. Employment/Salary (full or part-time):; Virobay Inc. **R. Booth:** A. Employment/Salary (full or part-time):; Virobay Inc. **M. Shamloo:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.24/Y9

Topic: C.19. Drug Discovery and Development

Title: The use of stereological analysis in non-clinical models of neurological disease and neurodegeneration

Authors: M. CERRADA-GIMENEZ, P. SWEENEY, A. NURMI, O. KONTKANEN, *T.-K. STENIUS, T. AHTONIEMI, N. VARTIAINEN
Charles River Discovery Res. Services Finland, Kuopio, Finland

Abstract: Stereology is a technique used for the morphological analysis of tissue samples in biological research, it is gaining increased importance in the study of preclinical neurological disease models as it is currently the only methodology capable of providing unbiased and accurate results for a wide variety of morphological analysis. The employment of stereology is particularly important for analysis of a variety of unique morphological biomarkers biomarkers in rodent models of neurodegeneration such as Huntington's disease (HD), Amyotropic Lateral Sclerosis (ALS) and for various models of Parkinson's disease (PD). We have performed basic characterization of the R6/2 and zQ175KI mouse models for Huntington's disease by analyzing the levels of inflammatory microglia (Iba-1 or Ferritin-positive) and GFAP-positive astroglia in the striatum. In the case of the Amyotropic Lateral Sclerosis, the stereological analysis focused on the determination of Acetylcholine Esterase-positive fibers in the sciatic nerve. For the Parkinsons's disease models we have performed accurate counts on the Tyrosine Hydroxylase-positive cells in the *Substantia nigra pars compacta* of rats that had been injected with an AAV harboring the human α-synuclein gene with the A53T mutation. Stereology may be considered a rather more labour intensive component of preclinical studies relative to standard immunohistochemistry but due to the fact that it readily provides unbiased and accurate results for the biomarkers being analyzed like, such as Iba-1 positive microglia, tyrosine hydroxylase-positive neurons, or Acetylcholine Esterase-positive fibers among others, preclinical neurobiologists, cell biologists and pathologists are beginning to employ this methodology as an adjunctive end-point along with behaviour, immunohistochemisty and imaging end-points in preclinical proof-of-concept studies in neurodegeneration.

Disclosures: M. Cerrada-Gimenez: None. **T. Stenius:** None. **P. Sweeney:** None. **A. Nurmi:** None. **O. Kontkanen:** None. **T. Ahtoniemi:** None. **N. Vartiainen:** None.

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.25/Y10

Topic: C.19. Drug Discovery and Development

Support: Coins for Alzheimer's Research Trust (CART)

University of North Carolina President's Strategic Initiative

Title: Further development of PADK for Alzheimer's disease: CNS lysosomal modulation by oral dosing and initial safety evaluation

Authors: S. RUIZ[1], M. C. PAIT[1], L. H. ELLIOTT[1], C. T. LONG[1], C. TIRLA[2], H. ROMINE[1], *B. A. BAHR[3], U. S. IKONNE[4]
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Abstract: Alzheimer's disease (AD) is characterized by pathological assembly states of Aβ42, tau pathology, and associated synaptotoxicity. Inefficient clearance contributes to AD accumulation events, thus drug discovery efforts have targeted the lysosomal pathway of autophagy to enhance protein clearance. Positive modulation of the lysosomal enzyme cathepsin B (CatB) was previously found to be linked to PHF-tau clearance in the hippocampal slice model of protein accumulation (Bendiske & Bahr 2003: JNEN 62:451). Recently, CatB was discovered to degrade Aβ42 via C-terminal truncation, and its modulation effectively reduced higher orders of Aβ assemblies (Mueller-Steiner et al. 2006: Neuron 51:703; Butler et al. 2011: PLoS One 6:e20501; Wang et al. 2012: JBC 287:39834). In addition to clearing intracellular Aβ in transgenic AD models, CatB modulator Z-Phe-Ala-CHN2 (PADK, also called ZPADK or Z-FA-DMK; MW = 394.4) and its derivatives were found to reduce Aβ deposits, ameliorate behavioral deficits, and, as in the brain slice model, restore synaptic markers (Bahr et al. 2012: Rejuvenation Res 15:189; Viswanathan et al. 2012: ACS Med Chem Lett 3:920). Here, multiple dosing by stressful oral gavage was avoided by using an alternative formulation of compound mixed to a homogenous suspension in peanut butter that was fed to rats twice daily for 11 days. The lowest PADK dose tested, 3 mg/kg/0.5d, produced significant 1.5- to 2-fold increases in the active CatB isoform and CatB activity in hippocampus, frontal cortex, midbrain, and cerebellum as compared to rats fed peanut butter with Z-FA-OH (non-modulator control compound). The same regions as well as neocortex and brainstem exhibited 2- to 4.5-fold increases in CatB at higher PADK doses, a selective effect since cathepsin D activity was unchanged as were GluR1 and actin levels. Behavior assessment also found no difference between PADK and Z-FA-OH groups in open field exploration and passive avoidance learning. A higher MW derivative with similar

CatB modulation as PADK in the slice model was less effective by oral dosing. For safety tests in mice, 15-40 mg/kg/d PADK i.p. produced a dose-dependent increase in active CatB in brain samples, while pre- and postsynaptic markers were unchanged. BUN and ALT blood markers were also unchanged, tissue from major organs scored normal by a pathologist, and no adverse effects on motor coordination or spatial memory learning were found. *In vitro*, 5-100 μM PADK did not cause bacterial cytotoxicity, at 3800 times the IC50 of mitomycin C, nor did PADK cause mutagenicity in the Ames test. PADK's safe toxicity profile and lysosomal modulatory efficacy after oral dosing support its potential to treat AD.

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Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.26/Y11

Topic: C.19. Drug Discovery and Development

Title: The novel M1/M4-selective muscarinic agonist NSX-0527 for the treatment of Alzheimer's disease

Authors: J. C. OCKULY, S. A. HANSON, J. D. BECK, *M. L. HENDRICKSON
Neurosci., NeuroSolis, Inc., Madison, WI

Abstract: Selective M1 muscarinic agonists have long held promise as potential treatments for Alzheimer's disease (AD). However, compounds investigated in early clinical trials suffered from a lack of efficacy, likely due to low potency. The M1/M4-preferring agonist xanomeline, a more recent compound that has undergone significant clinical assessment, did show efficacy in AD patients in a Phase II trial, but was abandoned due to poor tolerability. NSX-0527 is an M1/M4-selective orthosteric muscarinic agonist showing good bioavailability (~75%) and brain penetration (~60%). A terminal half-life of one hour has been observed in rats following oral and IV dosing, with excellent metabolic stability as determined by incubation in human liver microsomes. NSX-0527 displayed potent activation of central M1 receptors as demonstrated by

an increase in hippocampal inositol-1-phosphate (IP1) concentration following oral dosing in rats with significantly greater intrinsic efficacy and potency compared to xanomeline. In the novel object recognition behavioral test, mice receiving subcutaneous doses of NSX-0527 as low as 0.1 mg/kg showed improved cognitive performance compared to controls. Finally, NSX-0527 produced an acute reduction of hippocampal A-Beta1-42 in Tg2576 transgenic mice as measured by microdialysis, indicating the potential of NSX-0527 for disease-modifying activity in Alzheimer's disease.

Disclosures: J.C. Ockuly: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis, Inc. **S.A. Hanson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis, Inc. **J.D. Beck:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis, Inc. **M.L. Hendrickson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroSolis, Inc..

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.27/Y12

Topic: C.19. Drug Discovery and Development

Support: Auburn University IGP Award

Title: Selective PPARγ activation improves memory deficits and increases BDNF expression

Authors: *J. BLOEMER[1], G. NANAYAKKARA[1], K. PARAMESHWARAN[2], M. DHANASEKARAN[1], V. SUPPIRAMANIAM[1], R. AMIN[1]
[1]Dept. of Drug Discovery and Develop., [2]Col. of Vet. Med., Auburn Univ., Auburn, AL

Abstract: Alzheimer's disease (AD) is the sixth leading cause of death in United States, suggesting the limitations of the current therapies to prevent the advancement of the disease. Continual increases in mortality rates due to AD indicate the urgent need for establishing novel molecular targets for therapeutic potential. Many reports verify direct pathological links between AD and diabetes, which highlights the contribution of diabetes to the development of AD. Insulin sensitizing agents that activate the nuclear receptor peroxisomal proliferator activating

receptor gamma (PPARγ) such as rosiglitazone (rosi) and pioglitazone (pio), have been recognized as promising agents for improving cognition in diabetic and AD rodent models by reducing amyloid beta (Aβ) levels. However, these agonist display poor blood brain barrier (BBB) permeability and produce a number of adverse effects in humans such as ectopic lipid accumulation and increased plasma volume leading to increased incidence of heart failure and weight gain. Further, there is even less information on the effects of PPARγ agonists in the hippocampus in relation to synaptic plasticity and memory formation. We have developed a library of selective PPARγ modulators (SPPARγMs) based specifically upon computational evaluation of the crystal structure of PPARγ. These compounds have enhanced BBB permeability, and our lead compound, SPPARγM 9, was found to improve cognitive deficits in diabetic type 2 leptin deficient (db/db) mice. This compound also displays positive effects upon cellular metabolic regulation while displaying no harmful effects upon the heart or increased ectopic lipid accumulation. Compared to rosi, SPPARγM 9 lacks interaction with Tyr473 of the PPARγ ligand binding domain thus signifying the importance of interaction with Tyr473 for the off target effects of full PPARγ agonism. Our findings support that selective PPARγ modulators ameliorate cognitive deficits and lack off-target toxicity in peripheral tissue. Recently, we determined that SPPARγM 9 acted similarly to intracerebroventricular (ICV) rosi by inducing the expression of brain derived neurotrophic factor (BDNF) in the hippocampus of diabetic mice. Further, both rosi and SPPARγM 9 transcriptionally regulate BDNF expression as demonstrated by promoter activity assays (luciferase) in hippocampal neuronal cells as well as increased the expression of post synaptic receptor (GluA1 and GluN2A). These studies are novel as they will provide greater insights into activity of selective PPARγ agonism and delineate roles of partial vs. full agonism in diabetes and AD models for improving cognitive deficits.

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Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.28/Y13

Topic: C.19. Drug Discovery and Development

Support: NIH Grant 2R44AG029777-04

Title: Drug discovery for Alzheimer's disease targeting tau oligomers

Authors: *P. K. KRISHNAMURTHY, G. PAPIANI, P. LOPEZ, D. ROMERO, E. DAVIDOWITZ, J. MOE
Oligomerix, Inc., New York, NY

Abstract: Small molecule drugs are being developed targeting tau oligomers for the treatment of Alzheimer's disease (AD) and related tauopathies. We and other laboratories have studied the role of tau oligomers in memory formation and disease progression. Oligomeric forms of tau are highly correlated with neuronal loss and memory impairment. Moreover, extracellular tau oligomers cause impairment of memory formation in mice and induce synaptic dysfunction. Tau oligomers are specifically taken up by neurons and are vectors for transmission of pathology to healthy neurons. The tau oligomer target has been validated for drug discovery in mouse models of tauopathy using an immunotherapeutic approach. Here, we present the progress of our small molecule program in validating our drug discovery approach *in vivo* and in lead optimization for drug development. Validation studies are being performed using tool compounds discovered from screening a highly diverse library of 100,000 drug-like small molecules. The primary assay measured tau self-association using a proximity-based method of detection. There are no mutations in tau associated with AD, therefore, full-length tau without mutations was used in the assay optimized for tau oligomer formation. Hits were validated and dose response and neurocytotoxicity assays were performed. Medicinal chemistry analysis was used to organize the hits into chemical series and to select hits for secondary assays. Although drug development will utilize a mouse model expressing tau without mutations, the *in vivo* validation study is being performed with the JNPL3 tauopathy mouse model provided by Taconic to complete the validation within a shorter timeframe. Similarly, cell models for tau oligomer formation were developed with tau constructs with and without tauopathy mutations facilitating aggregation. Pilot validation studies are currently being performed in mice to select a tool compound for assessing in a full validation study. Fifty-seven drug-like molecules predicted to have good CNS penetration were selected from 11 chemical series and 19 singletons. The most active hits of 8 series were chosen as tool compounds and for lead development. Some of these compounds showed good dose response in the cell assay. Acute toxicity evaluation *in vivo* showed no adverse effect in wild type mice at high, medium and low doses. Brain homogenates from our first pilot validation study are currently being evaluated. Analysis of lysates from mice cortex tissue indicates a dose-dependent reduction in tau aggregates, supporting the validation of this approach for drug discovery.

Disclosures: P.K. Krishnamurthy: A. Employment/Salary (full or part-time):; Oligomerix, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **G. Papiani:** A. Employment/Salary (full or part-time):; Oligomerix, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **P. Lopez:** A. Employment/Salary (full or part-time):; Oligomerix, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Oligomerix, Inc. **D. Romero:** A. Employment/Salary (full or part-time):; Oligomerix, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **E. Davidowitz:** A. Employment/Salary (full or part-time):; Oligomerix, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **J. Moe:** A. Employment/Salary (full or part-time):; Oligomerix, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc..

Poster

234. Drug Discovery and Development: Neurodegenerative Diseases I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 234.29/Y14

Topic: C.19. Drug Discovery and Development

Support: FUI (French Fonds Unique Interministériel)

Alsace Region and Mulhouse

Title: An in silico simulator to study oscillopathies and drug effects in various neurological disorders

Authors: A. LEGENDRE[1], M. BEDEZ[1], M. SARMIS[1], A. F. KELLER[1], N. AMBERT[1], R. GREGET[1], F. LALOUE[1], J.-M. C. BOUTEILLER[1,2], M. BAUDRY[1,3], *S. BISCHOFF[1], S. MOUSSAOUI[1]
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Abstract: Several neurological disorders, including Alzheimer's disease and epilepsy, are characterized by early changes in cerebral electrical activities in various brain areas. These changes in electrical activity may represent biomarkers of high value for early diagnosis, based on their correlation with clinical symptoms, and for monitoring the clinical efficacy of new therapeutic strategies. Using anatomical data as well as a variety of experimental data, we developed computational models of intracellular pathways, synapses, neurons and neuronal networks in order to simulate the effects of drugs at multiple levels of analysis. At the molecular level, our library includes kinetic models of glutamatergic, GABAergic, cholinergic,

dopaminergic, ionotropic and metabotropic receptors, as well as diverse ion channels, transporters and enzymes. At the cellular level, we have built and validated morphologically and biophysically realistic neuronal models (e.g. hippocampal CA1 pyramidal cell), incorporating key molecular players involved in action potential generation, dendritic integration, and neurotransmission. At the network level, we implemented models of neuronal circuits capable of reproducing oscillatory activities observed experimentally and clinically. One of the key implemented neuronal circuits includes CA1 and CA3 neurons, septal cholinergic and GABAergic neurons as well as fast-spiking basket cells, and stratum oriens interneurons. In addition to simulating intracellular neuronal firing activity, the simulator also generates extracellular field potential, which can be compared to experimental data obtained with microelectrode arrays, or linear probes. Results of the simulations were validated by reproducing changes in electrical activity observed under physiological and pathological conditions as well as following treatment with a variety of drugs acting on various molecular targets. In particular, the effects of a variety of procognitive and antiepileptic compounds were investigated. The simulator represents a powerful tool for the identification of new drug-able targets and for screening and profiling new drug candidates, and to identify potential new therapeutic strategies for devastating neurological disorders.

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Poster

235. Auditory Processing of Natural Sounds

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 235.01/Y15

Topic: D.02. Auditory

Title: Using nonstationary contrast and intensity statistics for sound category identification

Authors: *M. A. ESCABI, R. NARAYAN
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Abstract: Environmental sounds, both man-made and natural, vary on multiple time and frequency scales generating a large range of temporal, spectral and amplitude modulations that are evident in the high-order statistics of the sound spectrogram. Healthy hearing humans perceive high-order statistical regularities and use this information to categorize and discriminate

sounds. Furthermore, neural responses in the central auditory system, including inferior colliculus and auditory cortex, can be modulated by high-order sound statistics. Here we test the hypothesis that nonstationary high-order statistics derived from computational auditory model enable discrimination and identification of sound categories from a computational standpoint. A large catalogue of animal vocalizations (speech, birds, primates, frogs etc.), environmental sounds (water, wind, thunder etc.), and man-made sounds (music, machine sounds etc.) and their associated high-order statistics was developed, and the information carrying content of each statistic for sound recognition and discrimination was measured. Time-varying statistics related to the sound contrast and time-varying intensity where measured for each sound at time-scales comparable to perceptual integration of intensity and comparable to adaptation times for such features in auditory cortex. Using statistical priors as model distributions for each sound category, Bayesian classification and signal detection theory were applied to the sound database to test discrimination limits amongst sounds or categories. Using these nonstationary contrast and intensity statistics alone, we show sound categories can be recognized with near 100% accuracy given observations times in the order of ~10 sec.

Disclosures: **M.A. Escabi:** None. **R. Narayan:** None.

Poster

235. Auditory Processing of Natural Sounds

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Topic: D.02. Auditory

Support: NCRR NIH COBRE E15524

Title: Auditory evoked potentials reveal harmonic structure as a signal attribute

Authors: ***J. W. LEWIS**[1], W. J. TALKINGTON[2], B. SMITH[2], S. KHOO[1], C. FRUM[3], D. W. GRAHAM[4], M. SCHADE[5]
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Abstract: Introduction Speech and mammalian vocalizations may be differentially processed relative to other natural sounds based in part on quantifiable acoustic signal attributes, including their harmonic content (harmonics-to-noise ratio; HNR value). Our group previously used natural and artificial sound stimuli with well-defined harmonic content to reveal cortical foci that

are parametrically sensitive to HNR. Here we sought to evaluate the temporal characteristics of harmonic signal processing in cortex. We evaluated whether classical auditory evoked potential (AEP) waveforms (P1-N1-P2 complex) demonstrate parametric sensitivity to harmonic signal content. Methods We recorded cortical AEPs evoked by artificially constructed white noise stimuli (iterated ripple noise; IRNs) that differed systematically in their HNR values. Fifteen healthy right-handed participants completed two experiments, each involving the randomized presentation of six IRN harmonic profiles (-7.6, -3, +3, +9, +15, and +24 dB-HNR). During EEG recording, IRNs were delivered to the right ears of participants as they watched silent, subtitled films. For the second experiment, the six IRNs were reverse-biased in perceived loudness as a critical control. We used ERPLAB software and independent component analyses (ICA) to analyze N1/P2 AEPs and independent components with respect to different HNR conditions. Results We observed a biphasic response profile of the N1-P2 AEP complex that persisted even with reverse intensity-biased IRNs, such that the peak-to-peak N1-P2 amplitude decreased with increasing harmonicity from -7.6 to -3dB, but increased from -3 to +24dB. ICA further revealed dipole activities contributing to the differences initially seen in AEP features. Conclusions Our findings suggest that the HNR signal attribute is robustly represented in the N1 component and N1-P2 complex when an ethologically-relevant range of HNR is presented (normal speech: +6 to +15 dB-HNR). Further, our chosen IRN HNR range was broader than ranges employed in earlier studies and revealed a unique, biphasic HNR processing profile. It appears that harmonic signal processing is instantiated as a distinct intermediate cortical processing stage in the human auditory system - perhaps including two filtering functions that work in concert to simultaneously optimize harmonic signal enhancement and noise suppression. This putative signal processing principle could be tested in future models of the human auditory system, included in biomimetic prosthetic device algorithms, and used to augment hearing prosthetics that are designed specifically for the enhancement of vocalizations and speech.

Disclosures: **J.W. Lewis:** None. **W.J. Talkington:** None. **B. Smith:** None. **S. Khoo:** None. **C. Frum:** None. **D.W. Graham:** None. **M. Schade:** None.

Poster

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Support: Swiss National Science Foundation 320030B_141177

Title: Limitations in fine-grained within-category semantic auditory discrimination: Insights from spatio-temporal analyses

Authors: *R. DE MEO[1], M. M. MURRAY[1,2,3,4], R. W. THOMPSON[5], S. CLARKE[1]
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Abstract: Correct recognition of individual sound objects within a semantic category (e.g., bird songs) involves cortical regions along a left lateralized temporo-fronto-parietal network. Here, we investigated how representations of other environmental sounds, i.e. heartbeats, differ when sounds have been correctly versus incorrectly categorized. Thirteen medical students, of which 2 were excluded due to poor performance during the training session, participated in this study. EEG and behavioural data were recorded from eleven participants who completed: 1) an audio-visual training session requiring recognition of 4 categories of real patients' heartbeat sounds (the training session ended when participants reached 70% accuracy); and 2) a testing session requiring discrimination of the 4 previously learned categories on recordings of new heartbeat sounds. Accuracy data were analyzed with a one-way ANOVA with Category (A: Normal vs. B: Variable split of S2 vs. C: Both S2 are widely split vs. D: Early systolic click) as factor. There was a main effect of category ($p = 0.033$) that was due to early systolic clicks being the most difficult to recognize. EEG analyses compared correctly vs. incorrectly recognized items with a paired t-test of source estimations calculated for each participant. The results identified a distributed spatio-temporal sequence that included: i) the left fusiform and parahippocampal gyri, left cuneus and lingual gyrus and right STG at 126-155 ms; ii) the cuneus and lingual gyrus bilaterally at 182-212 ms; iii) bilateral cingulate cortex, left superior frontal gyrus and right middle frontal gyrus at 252-295 ms; and iv) the left superior frontal gyrus at 384-419 ms. Thus, correct and incorrect recognition of heartbeat sounds relied on distinct brain networks, and these networks involved areas located predominantly outside the auditory brain regions. This pattern of activation differences between correct and incorrect categorization demonstrates that errors in fine-grained discrimination of objects within a semantic category, such as heartbeat sounds, can be driven more by incorrect labeling, rather than by limitations at a lower, i.e. perceptual, processing level.

Disclosures: R. De Meo: None. **M.M. Murray:** None. **R.W. Thompson:** None. **S. Clarke:** None.

Poster

235. Auditory Processing of Natural Sounds

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Topic: D.02. Auditory

Title: Auditory network optimized for sound discrimination predicts hierarchical trends of the auditory system

Authors: *F. KHATAMI[1], M. A. ESCABI[2]
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Abstract: Sound recognition, including speech recognition, is accomplished by multiple hierarchical levels of sound processing. Starting with the cochlea, sounds are decomposed into frequency components and subsequent auditory levels such as the inferior colliculus and cortex further decompose sound into spectro-temporal components. This multi-level processing combined with highly nonlinear neural mechanisms produces a robust neural representation enabling sound recognition in acoustically challenging environments (e.g., high noise levels). Here we develop a biologically inspired computational auditory model consisting of multiple hierarchical levels of spiking neurons and test its performance in a speech recognition task. The proposed biologically inspired model contains three components. The first stage consists of a cochlear model that transforms speech into spike trains for multiple frequency channels as observed in the auditory nerve. Stage two consists of a hierarchical biologically motivated neural network of spiking neurons designed simulate the central auditory pathway. The hierarchical network consists of 6 network layers containing 100 biologically realistic spiking neurons per layer and biologically realistic architecture that contains excitatory and inhibitory connections between layers. The final stage consists of a statistical classifier that reads the output spike trains from the network to categorize and identify speech words. Upon optimizing the network to discriminate speech sounds from multiple speakers, the model achieves high performance even under conditions of low signal-to-noise ratio. Furthermore, the model predicts several features present in the mammalian auditory system hierarchy. The optimal integration time constants increase systematically across the network layers such that the most central layers are substantially slower. Furthermore, selectivity increases and spike rates decrease systematically across the network layers analogous to trends observed in the auditory system.

Disclosures: F. Khatami: None. **M.A. Escabi:** None.

Poster

235. Auditory Processing of Natural Sounds

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Topic: D.02. Auditory

Support: NIH R01-DC04290

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Hoover Fund

Title: Mapping intracranial ECoG responses onto the surface of the human superior temporal plane

Authors: *H. OYA[1], H. KAWASAKI[1], K. V. NOURSKI[1], J. D. GREENLEE[1], T. D. GRIFFITHS[2], M. A. HOWARD[1]
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Abstract: Accurate mapping of electrocorticographic (ECoG) responses recorded within the human superior temporal plane using depth electlodes (Howard et al., J Neurosurg. 84:129-32, 1996) requires careful consideration of anatomical detail. The human superior temporal plane is veiled with the overlaying frontal, parietal and temporal lobe structures, and is highly convoluted with large inter-individual variability. Traditionally used “top-down” depiction of the superior temporal plane with lines representing deep sulci (first transverse temporal sulcus and the Heschl's sulcus) is not necessarily the best way to present obtained responses. In addition to mapping the responses on individual subject basis, with data from multiple subjects, spatial patterns of responses, if stable, could be mapped onto a representative brain to facilitate the interpretation. Image processing pipeline developed in our laboratory for response mapping onto the reconstructed surface of the human superior temporal plane includes the following steps: 1) Surface reconstruction of whole hemispheres with FreeSurfer; 2) Superior temporal plane patch creation; 3) Warping of individual subjects' brains onto a template brain using spherical diffeomorphic deformation (Yeo et al., IEEE Trans Med Imaging, 29:650-68, 2010); 4) Projection of responses recorded at each recording site contact to the surface vertices is done with inverse-distance weighting; 5) Smoothing of values assigned to the surface vertices using recursive heat-kernel weighting. The Gaussian process prediction and interpolation is also evaluated. We find that manual delineation of the two major sulci and superior temporal gyrus in addition to the curvature values confined within the superior temporal plane increases reliability of the matching between the two brains. Mapped data can be visualized on both inflated and original surface models from various angles. Effects of smoothing, interpolation and parameter settings are discussed.

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Poster

235. Auditory Processing of Natural Sounds

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Support: VA-RR&D Grant C8006W

NIH Grant DC010914

Title: Human auditory cortical coding of speech in background noise as a function of age and noise type

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Abstract: Speech perception in background noise is modulated by both patient factors (e.g. age and hearing loss) and stimulus characteristics (e.g. signal-to-noise ratio (SNR) and background noise type). The purpose of this study was to improve our understanding of how signals in noise are neurally coded as a means to better diagnose and rehabilitate speech perception in noise in people with peripheral and central auditory problems. Cortical auditory evoked potentials (CAEPs) were used to determine the effects of SNR, background noise type, and age on the neural representation of speech in noise. Ten younger and ten older normal-hearing individuals participated in this study. Using a passive oddball paradigm, the syllables /ba/ and /da/ were presented in four noise conditions: quiet, continuous speech-spectrum noise (SSC), one-talker modulated noise (1TM), and four-talker babble (4TB). In addition, noise levels were varied to create three different SNRs: -3, +3 and +9 dB. Evoked response latency and amplitude, as well as rectified area measures were determined for each of the 10 conditions. A significant effect of SNR and noise type on CAEPs was found for all CAEP measures. The largest effect of SNR was seen when stimuli were presented in SSC noise followed by the 1TM and 4TB noise. Poor CAEP morphology was seen for all 4TB conditions and may be due to the spectral and temporal similarities between the signal and noise. The 1TM noise was predicted to show a stronger SNR

effect than observed. We attributed this lack of effect on the long gaps present in the 1TM noise used in this study. A significant interaction between SNR and noise type was also found: at high SNRs, the response for SSC noise was larger than for 1TM noise; however, the reverse was found at low SNRs, suggesting that speech sounds are encoded differently depending on the SNR level and noise type. There was no significant main effect of group (i.e., age) and only limited age interactions associated with SNR and noise type. These results demonstrate an important first step in understanding the link between the underlying electrophysiology associated with speech perception in noise.

Disclosures: **N. Maamor:** None. **C. Billings:** None.

Poster

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Topic: D.02. Auditory

Support: NIH Grant DC012087

Title: Vocal signal processing and social categorization during natural communication in marmoset frontal cortex neurons

Authors: ***V. JOVANOVIC**, S. H. COOP, C. T. MILLER
UC San Diego, La Jolla, CA

Abstract: Communication is an inherently interactive process. While the exchange of information is central to this process, the nature of these interactions is heavily affected by the social setting. During vocal communication, callers must distinguish between individuals before deciding how to respond. Here we examined the responses of single neurons in marmoset frontal cortex during naturally occurring vocal interactions known as antiphonal calling. In the first experiment, we recorded ~300 neurons while subjects performed this natural vocal behavior. We found that a relatively small percent of units exhibited responses to vocalizations in this context (~25%). This occurred even in populations with direct anatomical connections with auditory cortex, such as ventral prefrontal cortex. In the second experiment, we implemented a novel interactive playback design to test social category perception during marmoset antiphonal calling. In these experiments, subjects were broadcast vocalizations from one caller during bouts of antiphonal calling. During some of these bouts, the calls of a different animal were broadcast

after 2 consecutive antiphonal calls. Previously, we reported that the change in caller identity elicited significant changes in subjects' vocal behavior. Their overall response rate decreased and the latency to respond increased. To test the role of frontal cortex for social categorization in natural communication, we are currently recording the responses of single neurons in marmoset ventral prefrontal cortex while animals engage in this behavioral task. We will compare neural responses to vocalization stimuli during tonic states of antiphonal calling to unexpected changes in social categories to determine the role of frontal cortex for recognition in natural primate communication.

Disclosures: V. Jovanovic: None. **S.H. Coop:** None. **C.T. Miller:** None.

Poster

235. Auditory Processing of Natural Sounds

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Topic: D.02. Auditory

Title: Neuronal mechanisms of vocalization sound processing in the primary auditory area of common marmosets

Authors: *T. BANNO, W. SUZUKI, N. MIYAKAWA, H. ABE, N. ICHINOHE
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Abstract: Social animals including humans communicate with each other by using various types of vocalization sounds. The animal vocalization sounds contain complex acoustical features, and how the auditory nervous system processes complex sounds with various combinations of multiple acoustical features is therefore an important question to understand the neuronal mechanisms underlying our vocal communications. We carried out electrophysiological recordings from common marmosets (Callithrix jacchus), a promising model animal for vocal communications due to their highly complex vocalizing behavior, to examine neuronal activities while they are hearing conspecific vocal sounds. In this study, we acoustically analyzed three major marmoset calls (phee, trill, and twitter) and extracted sinusoidal amplitude and frequency modulations as essential spectro-temporal features of these calls. We then resynthesized sounds by simply manipulating pure tones with various combinations of these acoustical features and created a set of stimulus sounds that covered a wide range of a parametric space composed of these component feature combinations. Our recordings from the primary auditory area revealed that all of the three types of real marmoset calls activated a large proportion of cells in this region

even though these calls had distinct spectro-temporal features. We observed weaker but significant responses to the synthesized sounds whose acoustical features were matched with the real marmoset calls (simplified calls) in 55% of the real call responsive sites. Further analyses revealed that the responses to the simplified calls could be predicted by a simple multiplication of the responses to their corresponding component features in 64 % of the simplified call responsive sites. We also found that the spike timing was more precisely locked to the modulation frequency of the combination sounds with the same modulation rhythms in both of the components, compared with the rhythmic responses to their component sounds whose amplitude or frequency alone were separately modulated. In this study, we demonstrated the usefulness of our simplified calls to examine computational principles underlying the vocal communication signal processing. Our results indicate that the simplified calls contain partial but sufficient information of the real marmoset vocalizations enough to evoke a large proportion of the neurons responsive to the real calls and suggest that these neurons in the primary auditory area perform various types of neural computations to process complex acoustical features in the communication sounds.

Disclosures: **T. Banno:** None. **W. Suzuki:** None. **N. Miyakawa:** None. **H. Abe:** None. **N. Ichinohe:** None.

Poster

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Topic: D.02. Auditory

Support: NIH Grant DC012087

Title: Development of an awake-behaving fMRI preparation for investigating auditory cognition in common marmosets

Authors: *C. TOARMINO[1], C. C. YEN[2], A. C. SILVA[2], C. T. MILLER[1]
[1]UC San Diego, San Diego, CA; [2]Natl. Inst. of Neural Disorders and Stroke, Bethesda, MD

Abstract: Beyond primary auditory cortex, relatively little is known about the role that higher areas of the auditory system play in perception and cognitive functions. To address this issue, here we report on the progress of the development an awake-behaving fMRI preparation to examine the contributions of the auditory belt regions in auditory cognition. Previously, we have

developed a method for imaging auditory cortex in the awake common marmoset (Callithrix jacchus) during passive listening using fMRI. Consistent with previous neurophysiology experiments, we found: 1) robust bilateral activity in auditory cortex to stimulation consisting of tones and band-passed noise, 2) regions selective to high and low frequencies along a caudal-rostral axis throughout the auditory core, and 3) responses to noise were greatest outside of the auditory core. Our goal here is to combine our previously established imaging paradigm with a behavioral training preparation we have also established in order to investigate brain areas involved in an auditory attention task. We trained marmosets to perform a frequency sweep discrimination task where the subject must discriminate an upward-directed frequency sweep (GO stimulus) from a downward-directed frequency sweep (NO-GO stimulus) by licking or withholding licking, respectively. Currently, we are integrating the imaging and behavioral preps in order to investigate brain areas that show activity during this task. Given the attentional demands of the task, particularly when subjects perform at threshold, analyses will focus on trials with high and low discriminability to determine whether attention modulates neural responses. We hypothesize that the frontoparietal network and auditory belts regions will be active during this task, including dorsolateral prefrontal cortex during threshold performance.

Disclosures: **C. Toarmino:** None. **C.T. Miller:** None. **C.C. Yen:** None. **A.C. Silva:** None.

Poster

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Topic: D.02. Auditory

Support: NIH Grant DC012087

Title: A preparation for optogenetic photostimulation in marmoset cortex

Authors: M. MACDOUGALL[1], J. MITCHELL[2], S. SRINIVASAN[2], *C. T. MILLER[3]
[1]UC San Diego, La Jolla, CA; [2]Salk Inst., La Jolla, CA; [3]Psychology, UCSD, La Jolla, CA

Abstract: Marmosets are emerging as a powerful model for investigation of primate neurologic systems. Optogenetics offers relatively precise temporal and spatial control of neuronal activity. We employ awake, behaving head-fixed marmosets performing auditory discrimination tasks to evaluate marmoset cognition, auditory processing, and social behaviors. More could be learned about the underlying neural mechanisms of these complex primate features using optogenetic

manipulations of neurons to perturb the brain during auditory tasks. Here we sought to develop an optogenetic photostimulation preparation for marmoset cortex. This project comprised two components. The aim of the first component was to determine which viral constructs were most suitable for use in marmosets. Using various adeno-associated viral (AAV) capsids containing channel rhodopsin genes with varying promoters, we systematically examined various methods for creating a marmoset optogenetic preparation. We investigated the utility of AAV 1, 5, and 9, the promoters synapsin, CAMkII, and CAG, and we varied injection rate and volume into the marmoset cortex. The viruses were allowed to express for 6 weeks after injection and brains were harvested and examined microscopically using immunofluorescence. The channel rhodopsins also had fluorescent protein tags such as GFP, YFP or mCherry. Primary antibodies against NeuN, GABA, CD68, and GFAP were applied to fixed brain slices prior to application of secondary fluorescent antibodies. The results demonstrate excellent expression of channel rhodopsin for particular serotype/promoter combinations. Particular constructs expressed notably well in the long-tract axons of the corpus callosum and in white matter coursing through internal capsule and brainstem. The aim of the second phase of the project was to optimize photoexcitability in marmoset cortex using these constructs. We are currently refining these parameters in marmoset neocortex and will present these results. Our results here indicate that using optogenetic techniques in marmosets is a feasible and useful method of correlating neural activity with behavior in awake marmosets.

Disclosures: **M. MacDougall:** None. **J. Mitchell:** None. **C.T. Miller:** None. **S. Srinivasan:** None.

Poster

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Topic: D.02. Auditory

Support: NIH Grant DC012087

Title: Ontogeny of antiphonal calling in common marmosets

Authors: ***C. P. CHOW**, C. T. MILLER
UCSD, La Jolla, CA

Abstract: Common marmosets (Callithrix jacchus) are small, highly vocal New World primates native to the coastal rainforests of Northeastern Brazil. Many aspects of this highly voluble species' vocal behavior have been described (Bezerra and Souto, 2008), but relatively little remains known about the ontogenetic development of their vocal communication system. Nonhuman primates are commonly described as having a limited capacity for vocal control over the acoustic structure of vocalizations (Seyfarth and Cheney, 1997), but an ability to exert control over the usage of the same call types (Pistorio et al, 2006). To test whether common marmoset vocal development is consistent with this trend, we investigated the longitudinal changes in vocal development of the common marmoset from approximately 3 to 12 months during their naturally occurring antiphonal calling behavior. We recorded antiphonal calling interactions between twins as well as between those twins and their respective parents twice a month over the duration of the study period for 12 animals. Analyses focus on changes in vocal behavior and vocal signal over ontogeny. Preliminary analyses suggest significant changes in both aspects of communication over the first year of life.

Disclosures: C.P. Chow: None. **C.T. Miller:** None.

Poster

235. Auditory Processing of Natural Sounds

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Topic: D.02. Auditory

Title: Studying the neural basis of vocal communication in a naturalistic environment using wireless neural recording technology in marmoset monkeys (Callithrix jacchus)

Authors: *L. ZHAO[1], H. YI[1,2], X. WANG[1]
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Abstract: How the primate brain controls the production of speech and other species-specific vocalizations, especially when coordinating the multiple dynamic features of vocal signals, is largely unknown. Lesion studies in humans have shown that particular frontal cortical regions (such as Broca's area) are crucial for speech production, and local field potential recordings from the human brain suggest that different motor cortical regions generate specific signals associated with different phonetic elements. The generation of vocal signals involves temporally precise coordination of the larynx and other articulators and therefore likely requires organized activity

among areas within the frontal cortices. To uncover such mechanisms in the primate brain at the neuronal level, we have studied frontal cortical activities in marmoset monkeys (Callithrix jacchus), a small New-World primate species with a rich vocal repertoire, in a naturalistic environment. Using chronically implanted multi-electrode arrays with wireless neural recording technology, we examined single-unit activity from populations of neurons in premotor cortex of freely moving and vocalizing marmosets embedded in a large breeding colony. Neural dynamics of single-units showed temporal patterns related to different types of vocalizations, including correlations between neural activity and different call types or different acoustic features within a call type. These results provide evidence to help understand neural mechanisms underlying vocal control in the primate brain.

Disclosures: **L. Zhao:** None. **H. Yi:** None. **X. Wang:** None.

Poster

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Topic: D.02. Auditory

Support: FRM Grant AJE201214

Title: Norm-based neural coding of conspecific vocalization in the macaque monkey

Authors: ***S. A. LOVE**[1], M. FUKUSHIMA[2], A. DOYLE[2], R. C. SAUNDERS[2], N. FUJII[3], P. BELIN[1], M. MISHKIN[2], D. A. LEOPOLD[2]
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Abstract: Person perception is a critical aspect of social interaction; subtle differences between individuals in vocal and/or facial features are used to identify conspecifics. In humans, norm-based coding explains the encoding of both the facial and the vocal identity of individuals (Latinus et al, 2011; Leopold et al, 2001; Valentine, 1991). Facial identity is represented in reference to an internal norm in both human (Loffler, et al 2005) and monkey (Leopold et al 2006) temporal cortex. Similarly, the human temporal voice area (Belin et al 2000) represents voice identity in reference to an internal norm (Latinus, et al 2013). Recent work also shows sensitivity to conspecific vocalizations in the temporal cortex of the monkey (Petkov et al, 2008). Here we asked whether a norm-based coding strategy could explain the neural coding of vocal

identity in the monkey. An average macaque voice was created by morphing together a coo from 7 macaques. 7 voice identity continua, containing 9 stimuli (-50 to 150% in steps of 25%), were created by morphing the average with each individual coo. Stimuli contained X% of information from the average coo and 100-X% from an individual coo. To record auditory evoked potentials, we used an electrocorticographic (ECoG) array chronically implanted in a macaque monkey. This ECoG array consisted of 256 recording sites for bipolar recording at 128 locations. The electrodes were located in the medial wall, the lateral surface, and the supratemporal plane (STP) in the lateral sulcus. We found robust auditory evoked responses to the vocalization stimuli in the recording site around the primary auditory cortex (A1) in STP. Norm-based tuning for voice did appear to develop over time in some recording sites. Specifically, some electrodes showed a stimulus-selective drop in frequency power that varied systematically based on the distance of a stimulus from the center of the "voice space". Consistent with models of norm-based coding, these power drops were most pronounced for the average voice and decreased along multiple identity trajectories at higher identity strength, and particularly the extreme (caricature) voices.

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Poster

235. Auditory Processing of Natural Sounds

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Title: Oscillatory activity in amygdala represents emotion categories of naturalistic auditory stimuli

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Helsinki, Finland; [4]Lab. of Primate Neurosciences, Kunming Inst. of Zoology, Chinese Acad. of Sci., Kunming, China

Abstract: The amygdala plays a key role in the processing of emotionally salient stimuli. Changes in the gamma-band oscillatory activity in the human amygdala have been related to the discrimination of emotion categories of visual stimuli (Oya et al., 2002), but less is known about amygdala responses to auditory stimuli carrying emotional information. We recorded intracranial field potentials with chronically implanted microelectrodes bilaterally in the amygdala and the caudolateral belt of the superior temporal gyrus (STGcb), and unilaterally in a control area, the visual cortex (V1), in three Rhesus monkeys while they listened to neutral, threatening and fearful monkey calls. We analyzed the responses in two time-windows (Window 1: 0-500 ms, Window 2: 500-970 ms from stimulus onset). In Window 1, event-related potentials to the three types of auditory stimuli differed from each other in the amygdala and STGcb, but not in V1. A two-way analysis of variance (ANOVA) (factors hemisphere and stimulus) of the auditory-stimulus-related oscillatory power in the amygdala and STGcb, and a one-way ANOVA in V1, showed a significant main effect of stimulus in the amygdala ($F(1,2) = 15{,}451$, $p < 0.05$), but not in STGcb and V1. The peak power in the gamma range (30-120 Hz) in amygdala to fearful calls was greater than to neutral and threatening calls (in both, $p < 0.05$, paired t-test). In Window 2, the mean power in the beta range (15-25 Hz) in amygdala also differed between the stimuli ($F(1,2) = 18.516$, $p < 0.05$); it was greater to the threatening calls than to fearful and neutral calls (in both, $p < 0.05$, paired t-test). In Window 2, there were no significant differences between the stimuli in the oscillatory power to the three types of stimuli in STGcb and V1. These results suggest a role for the amygdala in the processing of emotionally salient, naturalistic auditory stimuli.

Disclosures: Z. Chen: None. **L. Parkkonen:** None. **J. Wei:** None. **J. Dong:** None. **Y. Ma:** None. **S. Carlson:** None.

Poster

235. Auditory Processing of Natural Sounds

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NSF CRCNS

Title: LFP's in the superior colliculus of an echolocating bat are timed with ongoing behaviors

Authors: *M. J. WOHLGEMUTH, III, C. MOSS
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Abstract: LFP SFN Abstract - 2300 characters max Our research examines local field potential (LFP) recordings in the superior colliculus (SC), a structure in the mammalian midbrain important in spatial navigation and orientation. The SC is a laminated structure, with superficial layers receiving sensory information, intermediate layers integrating sensory information into motor planning, and deeper layers directing motor actions for spatial orientation. Specifically, we are investigating how the LFP may convey circuit level sensory information into a distributed network of neurons engaged in motor planning. The echolocating bat is a particularly valuable model system, because it discretely samples its environment with sonar signals, which provide unparalleled temporal precision for correlating LFP signals with sensory processing and pre-motor commands. Chronic multichannel neural recordings were taken in the SC, while the echolocating bat tracked a moving insect from a stationary position. The recording device was a 4x4 grid of electrodes that sampled both action potentials and LFP's in a 300 square micron volume of the SC. We found a significant increase in the power of the LFP when the bat was actively tracking an object. Furthermore, the timing of sonar call production and the subsequent echo returns were tied to particular phases of the LFP. We also found that the amplitude of the LFP was highest in the medial extent of the SC, where activity is tied to sensory activation along the horizontal plane of the animal, and which corresponds with the location of the target throughout the approach vector. And lastly, we found that separate frequency bands of the LFP become phase-locked at specific times with respect to the bat's sonar behaviors. These results suggest that features of the LFP play an important role in the active sensing system of the echolocating bat, and demonstrate the utility of the bat as a model to study LFP's in the context of sensation and adaptive motor commands.

Disclosures: M.J. Wohlgemuth: None. **C. Moss:** None.

Poster

235. Auditory Processing of Natural Sounds

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Topic: D.02. Auditory

Support: NIH Grant R00DC010439

Title: Streaming of repeated embedded noise in ferret auditory cortex

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Abstract: To function in natural auditory environments, the brain must segregate different components of sound mixtures, a process known as auditory streaming. Many natural sounds contain repetitive patterns that provide a cue for auditory grouping. The present study sought first to test if ferrets can use temporal regularities to stream auditory stimuli and second to explore if a streaming effect can be measured at the level of the primary auditory cortex (A1). We trained two ferrets on a repetition embedded noise (REN) detection task. Stimuli consisted of the superposition of two broadband, spectrally overlapping signals, each composed of a continuous sequence of 300-ms noise samples with spectro-temporal correlations matched to natural sounds. The stimuli were perceived as a single stream until a target sample started to repeat in one of source signals, thereby generating a perceptually distinct foreground. We recorded extracellular responses in A1 of an awake behaving ferret during presentation of single and two simultaneous noise sequences. A simple test for streaming revealed preferential encoding of the repeated target relative to the random background stream. Specifically, the PSTH response to the repeated target in noise was more similar to the response to the target in isolation (measured by correlation coefficient) than the response to the non-repeated target in noise. Moreover, a linear classifier trained on autocorrelated responses of all cells recorded in A1 was able to robustly discriminate between the presence and absence of a repeating noise sample. This supports the idea that the information about the repeated target is present in A1 neurons, perhaps carried by an implicit temporal code. We conclude that ferrets provide a good animal model for studying the neurophysiological bases of repetition-based streaming, and representation of foreground/background segregation might be encoded at the level of A1. Ongoing studies are exploring whether the representation of the repeated foreground is more prominent in secondary areas of the auditory cortex (belt regions), known to display a more context-dependent response to behaviorally relevant sounds. We are also performing classification image (CIm) analysis on the behavioral data to shed light on the strategies used by the animal during the REN detection task.

Disclosures: D. Saderi: None. **J.H. McDermott:** None. **S.V. David:** None.

Poster

235. Auditory Processing of Natural Sounds

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Neurocure

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Berlin School of Mind & Brain

Title: Audio-haptic coordination and multisensory integration in rat auditory cortex

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Abstract: During social interactions, rats employ complex multi-modal signaling and sensing. In this study, we investigate facial interactions between conspecifics. Specifically, we look at the coordination of whisking and ultrasonic vocalization and the integration of touch and sound in the auditory cortex. To this end, we use a combination of low- and high-speed videography, extracellular neuronal data acquisition and ultrasound recordings. We find that facial touch between conspecifics is associated with increased vocalization frequency, and this is modulated by sex and estrous state of the interacting partners. Interestingly, calls are emitted at the whisking frequency (~8 Hz) and are preferentially initiated in the retraction phase of whisking. In the auditory cortex, responses to ultrasonic vocalizations are mostly excitatory and tend to be very weak in regular-spiking but stronger in fast-spiking neurons. Interestingly, neurons of female rats tend to have higher basal firing rates which is not modulated by estrous state. Facial touch on the other hand induces inhibition in auditory cortex and off-responses after termination of touch. In addition, touch resulted in increased modulation in response to ultrasonic vocalizations. Facial interactions therefore appear to involve temporally orchestrated calling-whisking patterns. While regular-spiking neurons respond weakly to ultrasonic vocalizations, their modulation is increased during facial touch.

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Poster

235. Auditory Processing of Natural Sounds

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Support: UT Neuroscience Institute (NI) Postdoctoral Research Grant

UT Center for Integrative and Translational Genomics and the UT ORNL Governor's Chair Grant

Title: Genetic dissection of infant vocalizations

Authors: *S. ROY[1], M. L. SCATTONI[2], D. H. HECK[1], L. LU[1], R. W. WILLIAMS[1]
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Abstract: Rodent pups produce series of brief ultrasonic vocalizations (USVs) when distressed or separated from their nest. As is humans, these separation-induced vocalizations are critical for parental care and infant survival. It is likely that the underlying genetic mechanisms and CNS circuitry are well conversed among many mammalian species, and we argue that the cries of a human infant and a mouse pup are true behavioral homologs. C57BL/6J (B6) and DBA/2J (D2) strains of mice differ significantly in qualitative and quantitative characteristics of separation induced USVs. We initiated a genetic dissection of USVs to discover sequence variants and brain circuitry that underlie this critical set of infant behaviors. We collected separation-induced USVs from 44 genotypes, including two common progenitor strains B6 and D2, their reciprocal F1 hybrids, and 40 BXD recombinant inbred (RI) strains on postnatal days 7, 8, and 9 (2 to 5 litters each with data on sex and weight). We scored 14 facets of vocalization and in all cases heritabilities were sufficiently high_from 20% to 50%_to warrant detailed genetic analysis. We did not detect any effects of sex or body weight. Heritabilities for ten distinct USV call types (relative numbers of upward, downward, chevrons, etc.) tended to be higher than those for quantitative traits (amplitude, duration, total number of calls, and frequency). Duration covaries positively with amplitude ($r = 0.39$, $p < .01$), but negatively with frequency ($r = -0.30$, $p < 0.05$). We mapped four primary quantitative USV traits and detected significant linkage for mean peak frequency to chromosome (Chr) 14 at 58 ± 3 Mb (LRS of ~18 and +3 kHz per *D* allele) corresponding to human Chr 13 from 19-25 Mb and Chr 14 from 22-24 Mb. This locus is

completely independent of call duration and amplitude. Duration and amplitude map jointly, but weakly to chromosomes 1, 4 ,and 9. Duration has independent linkage_but suggestive only_to proximal Chr 6 and distal Chr 8. Therefore we conclude that all of these functionally coupled vocalization traits are influenced by several genetic factors.

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Poster

235. Auditory Processing of Natural Sounds

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Topic: D.02. Auditory

Support: NIH-NIDCD R01-001641

Title: Coding of vowels in the auditory midbrain of the budgerigar and rabbit

Authors: *K. S. HENRY[1], E. G. NEILANS[3], J. M. MCDONOUGH[2], L. H. CARNEY[1]
[1]Biomed. Engin., [2]Linguistics, Univ. of Rochester, Rochester, NY; [3]Psychology, Univ. at Buffalo, Buffalo, NY

Abstract: While speech plays an integral role in human social interactions, the neural mechanisms contributing to speech coding are not well understood, especially in the lower central nervous system. Here, we compare the neural mechanisms underlying differential responses to natural English vowels in the budgerigar, a vocal learner with human-like behavioral sensitivity to complex signals, and in the Dutch-belted rabbit, a species with more limited behavioral capabilities but greater cochlear similarity to humans. Neurophysiological recordings in awake animals were made from isolated cells of the auditory midbrain using chronically-implanted electrodes. Frequency response maps, modulation transfer functions, and vowel responses were recorded in each cell. Frequency response maps define a cell's tuning to audio frequencies while modulation transfer functions describe tuning to amplitude modulation frequencies. Modulation transfer functions fell into the same basic categories in the two species. Some cells showed an increase in firing rate in response to a limited range of modulation frequencies (i.e., band-pass modulation tuning) while others exhibited decreases in firing rate (e.g., band-reject and low-pass modulation tuned cells). Frequency response maps were also broadly similar, with both species exhibiting considerable variation in the bandwidth of audio

frequency tuning across cells of similar characteristic frequency. Neural responses to vowels in both species showed substantial differences in evoked firing rate across twelve different spoken English vowels from the Hillenbrand et al. (JASA 1995, 97:3099) database. The interactions of audio frequency tuning (i.e. characteristic frequency) and modulation tuning in explaining vowel responses were explored using a computational model of the auditory system. The model included an auditory-nerve component (Zilany et al., JASA 2014, 135:283) and interactions between the time course of excitation and inhibition in the auditory brainstem and midbrain to yield modulation tuning (Nelson and Carney, JASA 2004, 116:2173). The results allow detailed comparison of auditory midbrain function between birds and mammals. Furthermore, they suggest that both audio frequency tuning and modulation tuning may shape coding of vowels in the lower central nervous system.

Disclosures: K.S. Henry: None. **L.H. Carney:** None. **J.M. McDonough:** None. **E.G. Neilans:** None.

Poster

235. Auditory Processing of Natural Sounds

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Topic: D.02. Auditory

Support: BMBF #01GQ0811

Title: Encoding of complex natural sound in auditory midbrain by small neural groups

Authors: *D. LYZWA[1,2]
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Abstract: How complex natural sounds are represented in the midbrain remains an open question. The central nucleus of inferior colliculus (ICC) is the main converging station in the auditory midbrain, and contains nearly all information about the sound. To better understand the functional organization of this nucleus, the spatial extent of the neural representation of vocalizations and their encoding by individual and groups of multi-units was explored. Spiking multi-unit activity was simultaneously recorded from 32 positions along and across isofrequency laminae of the ICC while presenting 11 species-specific vocalizations to guinea pigs. Using

neural discrimination and cross-correlation it was found that small groups of neurons reliably encode the spectrotemporally rich set of vocalizations. Combination of a few multi-units yielded improved discrimination over an individual unit, but temporal correlations between the units did not improve discrimination. Individual vocalizations with their specific spectral content are optimally encoded across large frequency regions and this should be beneficial in a behavioral context. The findings suggest a spatially broad distributed code for vocalizations in the mammalian inferior colliculus. Acknowledgement: This work was supported by the BMBF in the National Network for Computational Neuro-science, grant number #01GQ0811 to BFNT Göttingen. We would like to thank Thilo Rode, Tanja Hartmann, and Hugh H. Lim for the guinea pig recordings and vocalizations.

Disclosures: D. Lyzwa: None.

Poster

236. Extrastriate Cortex: Responses and Coding

Location: Halls A-C

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Topic: D.04. Vision

Support: NHMRC Project Grant

Title: Reorganisation of V1 and the pulvinar nucleus following early life lesion of area MT

Authors: *J. A. BOURNE[1], I. MUNDINANO[1], H.-H. YU[2], C. WARNER[1], W. KWAN[1]
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Abstract: The middle temporal (MT) area of the nonhuman primate visual cortex is a primary-like area which develops in parallel with the primary visual cortex (V1). Throughout development, area MT receives input primarily from the medial portion of the inferior pulvinar (PIm) and V1. The aim of this study was to investigate the impact of the ablation of area MT in early life on the remaining visual cortex, especially V1, and the retinopulvino-MT pathway. To analyse retinopulvino-MT connectivity in the absence of area MT early in life, we administered intraocular anterograde tracers (n=2) 12 months following the mechanical unilateral ablation of left area MT (n=5) in postnatal day 14 marmoset monkeys. Single-unit electrophysiological recordings in area V1 were also conducted in 3 animals 12 months following ablation. This was performed to observe if there were any changes to the physiological properties of V1 neurons

post-ablation. cFos immunoreactivity was also used to further quantify functional activation of neurones in V1. Pyramidal cell (neurofilament) and interneuron (calbindin and parvalbumin) markers were used to identify changes in neuronal cell populations in the visual cortex. Compared to age matched non-lesion controls, early life MT ablation resulted in an increase in retinal input into the ipsilateral PIm. Within the ipsilateral V1, a loss of cFos+ and calbindin+ cells in the lesion projection zone in layers V and VI were detected. In 2 out of 3 cases, where drifting gratings were used to characterize tuning properties of single units in V1, we observed unusually large numbers of units (>40%) with receptive fields located inside the affected part of the visual field that were not orientation selective. Other properties such as spatial frequency tuning, temporal frequency tuning, and size selectivity showed no obvious deviation from those observed in normal animals. In the third case, tuning properties were close to normal. These results suggest that the absence of MT results in a reduction of pruning within retinopulvinar connectivity. Furthermore, the absence of area MT during early life impacts on the function of V1 in adulthood.

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Poster

236. Extrastriate Cortex: Responses and Coding

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Topic: D.04. Vision

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ARC Centre of Excellence in Vision Science

Title: Spatio-temporal distribution of gamma power and coherence in primate middle temporal area of the visual cortex

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Abstract: Recurrent networks are thought to play an important role in shaping the tuning properties of motion sensitive neurons in the middle temporal (MT) area of primate visual cortex. A signature of recurrent inhibitory networks is a bump of increased power in the local field potential (LFP), occupying a narrow band of frequencies in the gamma-range (16-100 Hz). In area MT, narrow-band gamma power is elevated during presentation of moving gratings. Presentation of moving dot-fields, however, brings about a large increase in LFP power over a broad range of frequencies, without a narrow band elevation. This might imply that recurrent inhibitory networks are not activated during presentation of dot fields, but could also arise if their LFP signature is subsumed by the broadband increase in power. To address this, we analysed coherence in LFP signals measured by multi-electrode arrays, hypothesizing that coherence in gamma-band provides a more sensitive indicator of recurrent inhibitory networks. Multi-electrode recordings were made from area MT of sufentanil-anaesthetised marmoset monkeys using planar arrays (5 animals) or laminar probes inserted perpendicular to the cortical surface (3 animals; 12 recording sites). The stimulus was a grey screen or a large field of moving dots presented for 2 s. We measured the power in the induced LFP at each electrode (n = 1315), and the coherence in the induced LFP between pairs of electrodes (n = 24191). The power spectra during maintained activity showed typical 1/*f* frequency-dependence, and presentation of dot fields increased power across a broad band of gamma frequencies, as expected. Coherence during maintained activity was stronger for pairs of nearby electrodes, and stronger if the electrodes were separated normal to the cortical surface than electrodes separated tangentially. For pairs of electrodes more than 1 mm apart, presentation of dot fields reduced coherence across the entire range of gamma frequencies. However, nearby electrodes showed increased coherence across a narrow band of gamma frequencies in most recordings (3 arrays, 10 probes); the centre frequency varied between animals. This narrow band of coherence decayed with distance and did not depend on whether electrodes were separated tangential or normal to the cortical surface. These observations suggest that dot fields do recruit recurrent inhibitory networks intrinsic to area MT, which may extend across both axes of the cortical sheet.

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Poster

236. Extrastriate Cortex: Responses and Coding

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Title: Response decay kinetics in areas MT, MST, and VIP on the motion pathway in macaques

Authors: *F. VIEIRA[1], S. W. EGGER[2], K. H. BRITTEN[1]
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Abstract: It is known that visual response latencies increase in areas that are higher on the cortical processing hierarchy. While the fidelity of processing of rapidly varying stimuli will be as affected by response offset dynamics as by onset dynamics, these have received relatively little study. Here, we compared response offset dynamics of three areas on the dorsal motion pathway: the middle temporal area (MT), the medial superior temporal area (MST), and the ventral intraparietal area (VIP). We examined how the time-course of response offset was influenced by position in the cortical hierarchy and by stimulus type. Identical stimulus sequences of random-dot motion (0.5 s on, 0.5 s off, while monkeys maintained continuous fixation) were used for all three areas. Pattern motion was either linear or complex ("spiral space"). Decay dynamics were measured by fitting the post-stimulus epoch with an exponential function using maximum-likelihood methods. Decline to baseline after stimulus offset was slower for MST compared to MT by about a factor of five (81 vs. 17 ms; $p < .05$). Decay kinetics in VIP were similar to those in MST. From these observations, we conclude that decay dynamics are greatly influenced by the location of an area on the cortical hierarchy. The effect on decay dynamics is considerably larger than the effect of hierarchy on response onset latency; this averages about 5 ms between MT and MST. Therefore, it seems unlikely that the effects on decay dynamics are merely due to accumulated temporal imprecision with hierarchical processing. We consider it more likely that recurrent circuit connectivity, either within or between areas is involved in retarding response offsets. Whatever the mechanism, the slower kinetics could serve the purpose of averaging temporal noise in motion signals or be useful in predictive coding for guiding behavior.

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Poster

236. Extrastriate Cortex: Responses and Coding

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Brain Research Foundation

Title: Realistic simulations of cortical population response for the evaluation of MT decoding models

Authors: *J. LOMBARDO, B. BAYER, L. OSBORNE, S. PALMER
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Abstract: Visual sensation arises from the activity of large populations of neurons, but sampling that population activity often involves trade-offs, either in the time resolution of the recording technique or in the sampling density. One approach to the analysis of population coding is to simulate ensemble data based on real neural responses in order to fill in the sampling gaps. Here we use the pursuit system as a model to test theories of cortical population coding of visual motion. Pursuit behavior offers a valuable performance metric for such a population model, since eye movement can be well characterized, the neural circuitry is well known, and pursuit initiation is tightly coupled to responses in the middle temporal area (MT). Neurons in visual area MT are tuned to the direction and speed of local image motion, enabling the perception of motion and the initiation of pursuit eye movements. The diversity of tuning bandwidths and temporal dynamics in the responses of individual MT neurons is key for transmitting a distributed population code that is robust to noisy, correlated inputs and outputs. Including realistic heterogeneity of responses in models of MT population activity provides a better estimate of information capacity and allows for a comparison of temporal responses to the timescales of pursuit behavior. Ultimately, we seek to understand how the extra information about motion direction encoded in heterogeneous populations can be reliably and flexibly decoded and thereby drive pursuit. In order to capture and characterize the richness of MT neuronal dynamics, we modeled the distribution of temporal responses across cells in a reduced-dimensionality space, from which we can pseudo-randomly draw realistic simulated responses. These synthetic populations are in good agreement with data recorded from real MT neurons, and can be used to test the viability of different decoding strategies for the estimation of image motion, which ultimately drives pursuit behavior. We use carefully quantified direction thresholds on pursuit and perception to test our models.

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Poster

236. Extrastriate Cortex: Responses and Coding

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Title: Constraints on decoding of visual motion parameters for smooth pursuit from MT cortical population activity

Authors: *M. V. MACELLAIO, L. C. OSBORNE
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Abstract: In pursuit, visual estimates of retinal image motion are translated to motor commands to smoothly counter-rotate the eye in order to stabilize the retinal image of a moving target. The visual inputs for pursuit behavior arise in the middle temporal cortical area (MT) where neuronal responses are tuned for motion direction and speed. Motion direction discrimination in pursuit is approximately a factor of ten lower than MT neuron discrimination thresholds, suggesting that visual motion estimates are derived from cortical population responses. In previous work, we have shown that variability in the initiation of pursuit arises from variability in motion estimation, as if visual estimates are decoded from cortical inputs with errors, but are then loyally translated to movement by the oculomotor system. Here we show that while a variety of population decoding models can reproduce the accuracy of pursuit eye speed and direction, reproducing the trial-to-trial eye movement variation places strong constraints on the coordinate frame and number of free parameters in the decoding model. Using both correlated and uncorrelated Poisson model neurons coupled to experimental measurements of actual pursuit behavior, we show that the most parsimonious decoding models operate in visual coordinates (direction, speed) rather than in motor coordinates aligned with the pulling direction of the eye muscles (i.e. horizontal, vertical). We also use data-based, realistic simulations of MT populations that preserve measured spike count statistics and the natural heterogeneity of tuning

and rate dynamics to further constrain the nature of cortical population decoding for smooth pursuit.

Disclosures: M.V. Macellaio: None. **L.C. Osborne:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 236.06/Z8

Topic: D.04. Vision

Title: Motion detection in Extrastriate Area 21a of the cat cortex

Authors: *H. ASLANYAN[1], A. KHACHATRYAN[2], D. KHACHVANKYAN[1], B. KOZAK-HARUTYUNIAN[1]
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Abstract: Using extracellular recording of spike activity from single neurons in Area 21a of extrastriate cortex, we examined in detail both the spatial stationary structure of neuron receptive fields (RF) and response patterns to moving visual stimuli. We found that a small group of visually sensitive neurons revealed no responses to stationary visual stimuli, while responding vigorously to moving images. The results of our experiments show that response patterns of these neurons to moving stimuli display a high degree of diversification and elaboration of incoming visual information. It is commonly accepted that static RF structure predetermines the pattern of neuron's response to moving visual images; in our investigation, we observed only a weak correlation and, in some cases, an almost complete lack of correlation between the RF stationary structures and response patterns to moving stimuli. In this study we present observations from a small group of neurons in extrastriate area 21a which did not react to a stationary flashing spot positioned in their RFs yet responded strongly to moving visual stimuli. We found significant qualitative and quantitative differences in the intensity of evoked discharges depending on the size, contrast and direction of applied visual stimuli. Thus, a high degree of information diversification appears to be taking place. We propose that these neurons may be specialized strictly in the detection and processing of visual information required for the perception of moving images. These results allow us to suggest that a small cluster of neurons in the central visual pathways, while being unresponsive to stationary visual stimuli, most likely specialize in motion detection governed by modulatory influences of activated adjacent neurons

with RFs overlapping with the RF of the neuron under investigation. Such interactions may be temporary events taking place during the time of image motion.

Disclosures: H. Aslanyan: None. **A. Khachatryan:** None. **D. Khachvankyan:** None. **B. Kozak-Harutyunian:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Program#/Poster#: 236.07/Z9

Topic: D.04. Vision

Support: ONR N00014-11-1-0535

Title: Temporal dynamics of heading perception in the presence of moving objects

Authors: *O. W. LAYTON[1,2], B. FAJEN[3]
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Abstract: When humans navigate in the presence of an independently moving object, judgments of about the direction of self-motion (heading) are sometimes biased (Warren & Saunders 1995, Layton & Fajen 2014). Models often exploit spatial patterns in the first-order optic flow vectors to explain the heading bias. The location of the singularity known as the focus of expansion (FoE) is estimated, which specifies the observer's direction of self-motion in the absence of the moving object. Differential motion models subtract motion vectors due to the object from those of the background to recover the FoE position (Royden 2002), and motion pooling models average object and background vectors (Warren & Saunders 1995). A key implicit prediction made by many models is that only the FoE position immediately prior to when the judgment is made influences heading perception - the time history of the object's trajectory should not matter. On the other hand, the neural model of Layton, Mingolla, and Browning (2012) requires temporal integration to replicate the human heading bias. The present study tests the temporal predictions made by these competing models. Human subjects viewed simulated self-motion for a number of durations (150, 300, 500, 750, 1050, and 1500 ms) in the presence of a moving object that approached in depth. The object moved such that its final distance relative to the observer and optical size remained the same. If the predictions made by the original Warren & Saunders (1995) and Royden (2002) models are correct, then heading judgments should be

equally biased irrespective of the trial duration because the instantaneous optic flow at the end of each trial is the same. Results indicate that the time history of the object's trajectory has a large impact on heading judgments. When the trial duration matched that used in other studies (Warren & Saunders 1995, Layton & Fajen 2014), we obtained a comparable bias of 1-2°. For the shorter viewing durations, the mean variability across subjects increased and the mean bias reached ~6°. Heading judgments were not biased in trials of the same duration that did not contain a moving object. Our results indicate that heading perception in the presence of moving objects is a spatio-temporal process, which is not compatible with a number of existing models. We successfully simulated the results using the model of Layton et al. (2012), which explains the increased bias and variability at shorter viewing durations through the dynamics of neurons in primate brain area MSTd.

Disclosures: O.W. Layton: None. **B. Fajen:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

Location: Halls A-C

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Topic: D.04. Vision

Support: Centre for Integrative Neuroscience (DFG Exec 307)

Starting Independent Researcher grant from the European Research Council (project acronym: PERCEPT) awarded to S.K

Title: Processing of contrast-modulated second-order stimuli in mouse visual cortex

Authors: Z. KHASTKHODAEI, O. JURJUT, S. KATZNER, *L. BUSSE
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Abstract: In primates, visual processing in the ventral stream takes place across a hierarchy of areas, starting with representations of low-level features in primary visual cortex (V1), which are gradually transformed into high-level object representations in inferotemporal cortex (IT). In mice, extrastriate visual areas seem to form anatomically and functionally distinct groups, possibly constituting a pathway analogous to the ventral stream in other species. Whether in the mouse these areas support increasingly complex, cue-invariant representations of visual information remains an open question. To test this question we compared visual processing of

first-order gratings, characterized by changes in luminance, and second-order gratings, characterized by changes in contrast only. We recorded extracellular activity in areas V1 and LM of anesthetized mice and measured orientation tuning curves for first- and second-order gratings. We found that areas V1 and LM responded more strongly and selectively to luminance-modulated compared to contrast-modulated gratings. Among the visually responsive neurons in both areas, >90% showed significant responses to luminance gratings, while only ~70% of V1 neurons and ~30% of LM neurons were activated by contrast-modulated gratings. Even for neurons with significant responses to both types of gratings, peak firing rates for contrast-modulated gratings were lower by ~30% in V1 and by ~50% in LM. Importantly, the majority of neurons recorded in either visual area lacked clear orientation selectivity for contrast-modulated gratings. This lack of orientation-selectivity for contrast-modulated gratings in mouse visual cortex prompted us to test behaviorally whether mice can discriminate second-order orientation. We trained mice to perform a coarse orientation discrimination task. After mice reached stable orientation discrimination for luminance-modulated gratings we replaced the visual stimuli with a contrast-modulated gratings. We found a massive breakdown of performance with little generalization of orientation-discrimination across the two types of stimuli. This breakdown was not due to the lower root-mean-square (RMS) contrast of the contrast-modulated gratings: when switching back to a luminance-modulated grating with matched RMS contrast, discrimination performance returned to high. In summary, although mice can see contrast-modulated gratings their modest representation in visual cortex seems to allow only relatively poor behavioral discrimination of second-order orientation. We conclude that the mouse visual system seems to be specialized for luminance-based perception.

Disclosures: **Z. Khastkhodaei:** None. **L. Busse:** None. **O. Jurjut:** None. **S. Katzner:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Topic: D.04. Vision

Support: JST PRESTO to NT

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ARC Discovery Project to KT and NT

Title: Frequency-dependent spatiotemporal profiles of visual responses recorded with subdural electrodes in awake monkeys

Authors: *K. TAKAURA[1], N. TSUCHIYA[2,3], N. FUJII[1]
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Abstract: Electrocorticography (ECoG) constitutes a powerful and promising neural recording modality both in humans and animals. The ECoG signals are often decomposed into several frequency bands, among which so-called high-gamma band [80-250Hz] has been proposed as a reliable measure of the local cortical activity. It is typically assumed that the lower the frequency bands, the less specific the signal reflects about the local cortical activity. However, the differences across the frequency bands have not been systematically investigated. To address this issue, we obtained ECoG recordings from two awake monkeys during a retinotopic mapping task. We characterized the spatiotemporal profiles of the visual responses in the time-frequency domain. We defined the preferred spatial position, receptive field (RF) and the response latencies of each electrode in each frequency band (i.e., alpha [8-16 Hz], beta [16-30 Hz], low- [30-80 Hz] and high- gamma), and compared them across the bands. Across all the electrodes, spatial preferences were comparable across the bands. We observed that the RF of each electrode represents the contralateral visual field in all the frequency bands and that the high-gamma activity showed smaller RF than the other bands. However, a systematic difference arose in the temporal domain. The response latencies for the alpha band were always longer than the other bands. Comparison of the response profiles in both space and time within each region (V1, V4+ and TEO/TE) revealed regional idiosyncrasies. While in V1 and V4+, the latencies of the visual responses in the beta, low- and high-gamma bands were almost identical, in TEO/TE, the beta and low-gamma activity occurred 25 ms earlier than the high-gamma. Furthermore, TEO/TE exhibited a unique pattern in the spatial response profile; the high-gamma tended to prefer the foveal regions while the beta and low-gamma preferred peripheral visual fields with larger RFs. It suggests that neurons in TEO/TE first receives spatially less selective information via the beta and low-gamma, and later spatially more fine-tuned foveal information via the high-gamma. This result is consistent with a previously proposed hypothesis by Nakamura, Gattass, Desimone, Ungerleider (1993) that the visual processing in TEO/TE starts with coarse-grained information, which primes subsequent fine-grained information. In summary, our results demonstrate that the nature of computation carried out within each stage of the visual system can be learned more richly by incorporating knowledge of the spatiotemporal characteristics across all frequency bands of ECoG recordings.

Disclosures: **K. Takaura:** None. **N. Tsuchiya:** None. **N. Fujii:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Support: NWO-VICI grant

European Union Seventh Framework Program 269921

Title: Activity propagation between early visual areas determines the awareness of phosphenes

Authors: *B. V. VUGT, B. DAGNINO, P. ROELFSEMA
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Abstract: Understanding how visual awareness emerges in the brain is one of the major challenges that remain to be addressed in neuroscience. What is the difference between information that reaches awareness and information that does not? Here we directly compared information transfer along the visual hierarchy between perceived and non-perceived phosphenes, elicited by electrical microstimulation (MS) of neurons in V1. To this aim we recorded activity from neurons in area V4 with receptive fields (RFs) that overlapped with a phosphene in a task where macaque monkeys had to report the delivery of MS to area V1. MS was given in 50% of the trials and the monkeys reported the detection of a phosphene by making a saccade to the phosphene location and the absence of a phosphene by making a saccade to another stimulus. We varied the MS current to keep performance at 80%, close the threshold for phosphene awareness, so that we could compare V4 activity between trains of MS pulses of equal strength that were either perceived by the monkey (correct eye movement to location of phosphene) or missed (incorrect eye movement to the other stimulus). Our results for two monkeys show very clear differences in V4 activity between seen and missed MS trials. MS trains that reached awareness elicited activity in V4 that was much stronger than MS trains that were missed. This difference in activity occurred almost immediately after MS, but was also maintained during a later, sustained response phase. We also examined activity of V4 neurons with RFs that did not overlap with the phosphene location, but found that their activity was a poor predictor of phosphene perception. We next compared V4 activity elicited by visual stimuli (low contrast gratings) presented in the neurons' RF that were perceived and that were not, and also observed a correlation between V4 activity and perception. As the stimulus in perceived and non-perceived trials is the same, our results suggest that the state of the cortex at the moment of

the stimulus determines visibility. To examine this possibility, we investigated the power of the LFP and found that low frequency power (4-12Hz) preceding the stimulus was indeed associated with higher visibility. Together, these results show that the cortical state preceding microstimulation or visual stimuli determines the propagation of activity from lower to higher visual areas and thereby whether a phosphene or a visual stimulus will be perceived or not.

Disclosures: **B.V. Vugt:** None. **B. Dagnino:** None. **P. Roelfsema:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

Location: Halls A-C

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Topic: D.04. Vision

Support: NIH EUREKA

Title: Rapid learning impacts noise correlation in monkey V4

Authors: ***Y. WANG**[1], V. DRAGOI[2]
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Abstract: Improvement in sensory discrimination after practice is a well-documented behavioral phenomenon. It has been previously reported that neurons in early and mid-level visual cortex change their response properties after perceptual learning, including the increase in tuning curve slopes and the increase in response gain. However, whether and how the information encoded in population activity changes after learning is not fully understood. The amount of information encoded by cortical circuits depends critically on the capacity of nearby neurons to exhibit trial-to-trial (noise) correlations in their responses. Therefore, we assessed the change in neurons' correlated variability induced by learning. To accomplish this goal, we used multiple electrode recording in alert macaque V4 to record single units before, during, and after a rapid learning task. Two monkey were trained to performed a 3-stage task: in the 1st and 3rd stages (during the same session) the monkey was required to perform a color change detection task on the hemifield that is contralateral to the receptive field locations. Simultaneously, unfamiliar natural images were passively presented at the receptive field locations. In the 2nd stage, the monkey was trained to indicate whether two successive images briefly flashed for 300 ms and separated by a 1000-ms delay were presented at the same or different orientation. The images in 2nd stage

were located at the receptive field location, and were identical to those presented in stages 1 and 3. We found that the monkey's image orientation discrimination threshold decreased significantly during the time course of learning (stage 2). We examined the extent to which the noise correlations between neurons changed after learning by comparing the Pearson correlation coefficients in the 1st and 3rd stage in each session. We examined 565 pairs originating from 133 single neurons recorded in both monkeys. Despite the fact that firing rates did not change as a result of learning, we found a significant decrease in noise correlation when stages 1 and 3 were compared (35% decrease during stimulus onset and 15% decrease during the entire 1 sec stimulus period, Wilcoxon rank test, $p < 0.00001$). The decrease in noise correlations caused a 30% increase in the population signal noise ratio. These findings indicate that rapid learning (tens of minutes) impacts noise correlations in V4 such as to enhance perceptual performance.

Disclosures: Y. Wang: None. **V. Dragoi:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

Location: Halls A-C

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Topic: D.04. Vision

Support: NIMH IRP

Title: Functional MRI mapping of IT single unit responses during natural vision

Authors: *S. PARK[1,2], B. E. RUSS[1], D. B. T. MCMAHON[1,3], H. D. ELNAIEM[1], D. A. LEOPOLD[1]
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Abstract: In functional MRI, hemodynamic time courses measured throughout the brain are summarized into activity maps in a process that usually involves comparing each voxel's time series with a model. Models can be *a priori* and built into the stimulus design or can be extracted from some aspect of the stimulus or task, with an example of the latter being the mapping of particular features in natural videos (Russ et al., SFN Abstr., 2013). An alternative approach is to use neural signals themselves as models for fMRI mapping. An example of this approach is the use simultaneously measured neural and fMRI signals to map the functional distribution of

spontaneous activity at rest (Schölvinck et al., PNAS, 2010). In the present study, we combined the approaches by using stimulus-driven neural signals as models for fMRI mapping to investigate the diversity of single unit responses within a single voxel during the viewing of natural videos. We previously demonstrated that, when monkeys freely viewing 5-minute videos of complex social interaction, a population of neurons in the anterior fundus (AF) face patch within < $1mm^3$ showed a broad range of response time courses, many of which were uncorrelated, despite each being deterministically driven by the contents (Elnaiem et al., SFN Abstr., 2013). We asked whether this diversity can be explained by a differential pattern of functional interactions of individual neurons with other brain areas. To address this, we used a novel combination of fMRI and single unit recordings, where both fMRI responses from the whole brain and single unit activities from neurons in face patch AF were measured while the subjects freely and repeatedly watched 5-minute naturalistic movies. We then computed correlation coefficients between movie-driven signals recorded in these two methods. Specifically, we used spike density functions derived from each single unit as a regressor and obtained a functional picture of the whole-brain-network that is correlated to selective activity of individual neurons. In two monkeys, more than fifty single neurons resulted in correlation maps localized to visually responsive areas. We found that neurons located within a few hundred microns provided strikingly dissimilar maps, reflecting their distinct response patterns to the movie stimuli. This approach provides a means of relating the activity observed locally in single neurons to the patterns functional organization observed globally at the level of the whole brain.

Disclosures: S. Park: None. **B.E. Russ:** None. **D.B.T. McMahon:** None. **H.D. Elnaiem:** None. **D.A. Leopold:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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NIH P30 EY08098

P41RR03631

NIH 1R01EY022247

NSF 1320651

Title: Effect of image familiarity on neuronal responses in areas V2 and V4 of monkey visual cortex

Authors: *S. RAMACHANDRAN[1,2], T. S. LEE[1,2], C. R. OLSON[2]
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA

Abstract: Neurons in monkey inferotemporal cortex (IT) respond more weakly to images that are highly familiar than to novel images. Suppression is not instantaneous but rather develops at a latency of around 80 ms following response onset. This phenomenon has been assumed to have its origin in functional changes induced by visual experience at the level of IT. However, this idea has not been tested by recording at earlier stages of the ventral stream. To resolve this issue, we monitored neuronal activity, using a 32-electrode semi-chronic recording array, in a sector of areas V2 and V4 representing the lower parafoveal visual field. This approach allowed recording from 3-15 well isolated neurons per day. Over the course of twelve days, we repeatedly presented each of 25 images in superimposition on the collective receptive field of the neurons under the array. The 7.4° images, representing natural and manmade objects free of background, were of the same kind that we have used to induce the familiarization effect in IT. Although not tailored to the requirements of individual V2 or V4 neurons, the images evoked strong responses in both areas. On a subset of days (3, 8, 9, 10, 11, 12), we also presented novel images drawn from the same library as the images in the familiarization set. The novel images employed on a given day were used on that day only. On day 3, after 60 exposures to each familiar image, the strength of the population response to the familiar images was not measurably different from the strength of the population response to the novel images employed on that day. However, on day 8, after 200 exposures to each familiar image, and on every subsequent day, the trajectory of the population response differed between familiar and novel images. The familiar-image response was briefly stronger than the novel-image response but then underwent a sharp truncation so that the level of sustained activity was lower than for novel images. The truncation of the familiar-image response occurred earlier in V2 and V4 than in IT. This suggests that the effect occurred at or before level of V2 and V4 and was not simply the product of top-down feedback from IT.

Disclosures: S. Ramachandran: None. **T.S. Lee:** None. **C.R. Olson:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Topic: D.04. Vision

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Title: Maintenance of spatial information gates the processing of incoming visual information in area V4

Authors: Y. MERRIKHI[1], M. PARSA[2], E. ALBARRAN[3], *B. NOUDOOST[2]
[1]Sch. of Cognitive Sci., Inst. for Res. in Fundamental Sci., Tehran, Iran, Islamic Republic of; [2]Montana State Univ., Bozeman, MT; [3]Dept. of Neurobio., Stanford Univ., Stanford, CA

Abstract: When subjects remember a location, visual processing at that location is enhanced compared to elsewhere in space, as measured by visual discrimination performance. The feature selectivity of neurons in early visual areas makes these neurons a good candidate substrate for an enhanced visual representation underlying these changes in discrimination. However, the firing rates of neurons in extrastriate visual cortices such as V4 and V5 appear to be largely unaffected by spatial memory maintenance. An alternative hypothesis is that although the delay-period activity of these neurons is unaltered, spatial memory can still enhance their sensitivity to incoming visual information. To examine this idea, we recorded the neuronal responses of area V4 in awake rhesus monkeys while they performed a memory-guided saccade task. In this task, the target was presented for one second and the monkey had to remember the target location throughout a one second delay period while maintaining his fixation. The fixation point then disappeared, and the monkey moved his eyes to the remembered cue location to receive a reward. We measured the responses and mapped the receptive field of V4 neurons by presenting brief visual probes in a 7x7 grid of locations near one of the cue locations, both during fixation and during memory maintenance. We found that the centers of V4 receptive fields shifted towards the remembered location. This resulted in a larger number of V4 neurons responding to a given visual stimuli when remembering a nearby location (compared to the number of neurons responding to the same stimulus when remembering a location in the opposite visual hemifield). We further examined the effect of memory maintenance on noise correlations between simultaneously recorded pairs of V4 neurons, and found that remembering a location common to both V4 receptive fields decorrelates their activity. This effect of memory maintenance on visual cortical responses, which closely resembles the effect of covert spatial attention, could underlie the behavioral effects of memory maintenance on visual discrimination.

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Poster

236. Extrastriate Cortex: Responses and Coding

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China Scholarship Council

Title: Late responses of V2 neurons are enhanced by global scene context of natural movies

Authors: *J. M. SAMONDS[1], Y. LI[4], Y. ZHANG[2], T. LEE[3]
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Abstract: Cortical neurons receive inputs from other neurons within the same layer that have distinct receptive fields, as well as neurons in subsequent layers and areas with larger receptive fields. These inputs alter feed-forward input responses in interesting and complex ways that may contribute to statistical inference mechanisms. We measured the response properties of V2 neurons in awake, behaving macaques in response to natural movies and drifting sinusoidal gratings of varying aperture size to understand how spatiotemporal context of natural movies can influence the activities of V2 neurons. We found that the early stage of responses of the neurons (0-200 ms after stimulus onset) expressed surround suppression: the responses to stimuli presented in a larger aperture were smaller than to stimuli presented in a smaller aperture. This surround suppression was observed for apertures slightly larger than the receptive fields (1-2 degrees of visual angle) and saturated at 5-6 degrees of visual angle. In addition, we observed the selectivity of the tuning curve of the neurons to the spatiotemporal patterns in the movies also increased with an increase in aperture size, but the sharpness developed at a slower rate than surround suppression. Interestingly, when the aperture was increased to 9 degrees of visual angle or larger, the neurons' responses actually increased relative to the response to the same movies presented in smaller apertures. This enhancement was later than both surround suppression and sharpening of tuning and was only observed for natural scenes and not for gratings. Qualitatively, the enhancement occurs at the point at which the natural scenes could be recognized or interpreted. These findings have indirectly revealed properties of multiple distinct networks that contribute to the responses of V2 neurons. These surround inputs allow

information from other regions of the visual field as well as top-down expectations to facilitate the interpretation of the incoming visual evidence.

Disclosures: **J.M. Samonds:** None. **Y. Li:** None. **Y. Zhang:** None. **T. Lee:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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NSF 1320651

Title: Sparse and distributed representation of visual concepts in V1 and V2 neuronal populations

Authors: ***T. LEE**[1], C. MASSOT[2], G. PAPANDREOU[3], A. YUILLE[4]
[2]Ctr. for the Neural Basis of Cognition, [1]Carnegie Mellon Univ., Pittsburgh, PA; [3]Computer Sci., Toyota Technol. Inst., Chicago, IL; [4]Dept. of Statistics, Univ. of California, Los Angeles, Los Angeles, CA

Abstract: Earlier neurophysiological studies have showed that individual V2 neurons are selective for simple and complex artificial shape stimuli (Hegde and Van Essen 2003). Here, we studied whether and how neurons in early visual cortical areas are tuned to a more general set of visual concepts derived from natural images using statistical clustering techniques. In this study, we focused on 150 edge concepts, derived as cluster centers of aligned edge contour segments extracted from the human-annotated edge maps of the Berkeley segmentation database. Each of these edge concepts is associated with a set of contour patches and appearance patches of the cluster it represents. We presented the representative contour patch, the first principal component and/or prototype example of the appearance patches associated with each edge concept to the receptive fields of V1 and V2 neurons through a 3-degree smoothed aperture, recorded in awake behaving monkeys using a 32-channel recording array (Gray Matter Research), and 24-channel linear multi-electrode arrays (Alpha-Omega). In general, we found the responses of a majority of V1 and V2 neurons to the contour patterns and the appearance patterns were correlated (R =~0.4), suggesting that the neurons individually were tuned to more abstract concepts. On

average, V1 neurons exhibit stronger responses, are sparser and more selective in tunings, to contour patterns than to appearance patterns, while V2 neurons respond more strongly, with sparser and more selective tunings, to appearance patterns. These findings indicate V2 neurons prefer richer and more naturalistic stimuli than V1 neurons. While both V1 and V2 neurons exhibit some degree of sparseness in their tunings, their codes are also distributed as they responded moderately to many visual concepts. To investigate V1 and V2 codes at the population level, we have applied multidimensional scaling (MDS) and K-means clustering on V1 and V2 population response vectors. We have found that edge concepts in V1 are clustered according to their mean orientation. On the other hand V2 exhibits a clear clustering of the three types of stimuli (contour, mean appearance, example prototype). In addition, distinct clusters of visual concepts in the high dimensional space (T-Y-L junctions, lines, curves, simple and complex patterns) start to emerge in V2. These findings suggest that visual concepts that are not separable in V1 population become separable ("untangled" in the terms of DiCarlos and Cox 2007) in V2 neuronal population.

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Poster

236. Extrastriate Cortex: Responses and Coding

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Title: Relating divisive normalization to spike count correlations between and within cortical areas

Authors: *D. MONTEZ[1], D. A. RUFF[2], M. R. COHEN[2]
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Abstract: Normalization, in which a neuron's response is divisively scaled by the presence of stimuli that do not drive the cell when presented alone, is thought to be involved in a wide variety of neuronal computations. Although most normalization models are descriptive, the prevailing hypothesis is that normalization is a network phenomenon that is accomplished by scaling a neuron's response by the responses of a large pool of surrounding neurons. Even within the same brain area and under identical experimental conditions, neurons vary in the degree to which they exhibit normalization. For example, direction-selective neurons in area MT differ in the way they respond to pairs of stimuli moving in opposite directions within their receptive fields. Some neurons exhibit strong normalization, responding similarly to the average of their responses to the two stimuli alone. Others show no normalization, producing a response similar to the response to a preferred stimulus alone. We hypothesized that the extent to which a neuron exhibits normalization reflects its specific role in a cortical circuit, which might give rise to a distinctive pattern of interactions with neurons within the same cortical area and in other areas. One way to probe the interactions between a pair of neurons is by measuring the extent to which the trial-to-trial fluctuations in their responses are correlated (termed spike count correlations). We recorded simultaneously from several dozen neurons in primary visual cortex (V1) and from either a single neuron or a small group of neurons in area MT in two monkeys. We presented different combinations of moving stimuli and measured normalization and spike count correlations within and between the two areas. We also measured several different properties of each neuron we recorded, including its direction tuning, the size and location of its receptive field, the amount its responses were modulated by stimuli outside its classical receptive field and by shifts in spatial attention. We found that MT neurons that exhibited strong normalization typically had higher spike count correlations with V1 than neurons that did not show normalization. Our results suggest that neuron-to-neuron variability in normalization (and possibly other sensory and cognitive factors) can be explained by patterns of interactions between neurons across and within cortical areas. In general, our data support the idea that looking at interactions between neurons in multiple cortical areas might provide new insights into the mechanisms underlying neuronal computations.

Disclosures: D. Montez: None. **D.A. Ruff:** None. **M.R. Cohen:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 236.18/Z20

Topic: D.04. Vision

Support: NIH Grant R01 EY013802

Gatsby Charitable Foundation

Title: Spatio-temporal structure of shape-tuning in V4 receptive fields

Authors: *A. S. NANDY, J. H. REYNOLDS, J. F. MITCHELL
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Abstract: Visual shape information is processed in the ventral cortical pathway, which progresses from the primary visual cortex (V1) through intermediate visual areas including V2, and V4, and into the inferotemporal cortex (TEO and TE). Shape is initially encoded primarily in terms of local orientation in V1, but by the final stages of visual processing in IT, neurons are selective for complex objects like faces. Area V4 lies at a critical juncture in mediating this transformation. In a previous study (Nandy et al., 2013) we have shown that V4 neurons exhibit a tradeoff between curvature tuning and spatial invariance. Neurons tuned to curved shapes exhibit very limited spatial invariance, while those that prefer straight contours show spatially invariant tuning. This diversity was explained by the fine-scale structure of the receptive fields (RFs). The fine-scale RF maps for neurons preferring curved shapes had heterogeneous local variation in orientation tuning. In contrast, these were homogeneous for neurons preferring straight contours. This previous analysis presented a temporally averaged static picture of the fine-scale RF structure. Here we show that these fine-scale RFs have an underlying intricate temporal structure as well. The temporal structure for neurons that showed statistically significant temporal shifts (80 out of 96), can be qualitatively grouped into three categories: (a) progressively shifting spatial kernels, (b) distinct spatial locations (sub-units) having distinct temporal signatures, and (c) spatial kernels that dissipate over time from the center to the edges. The temporal evolution of these fine-scale RF maps is concomitant with changes in shape-tuning over time. Taken together, our results suggest the presence of a rich spatio-temporal shape code in area V4.

Disclosures: **A.S. Nandy:** None. **J.H. Reynolds:** None. **J.F. Mitchell:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Topic: D.04. Vision

Support: CIHR grant MOP-115178

FQRNT grant 149928

Title: Converging encoding strategies in dorsal and ventral visual streams

Authors: *P. J. MINEAULT[1], T. P. ZANOS[2], C. C. PACK[2]
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Abstract: At the highest levels of the visual cortex, behaviourally relevant information, including object identity, pose, and heading, can be easily read via a simple linear decoding rule. This behaviourally relevant encoding is thought to emerge as the visual signal is iteratively re-encoded to ever more abstract, invariant, and selective representations as it processed by a hierarchy of areas. While the visual encoding of motion and shape is relatively well understood in primary visual cortex, how this signal is propagated and re-encoded in extrastriate cortex is less clear. Here, we analyze the recordings from macaque areas V2 and V4 of the ventral visual stream and areas MT and MST of the dorsal visual stream to understand how the visual signal is re-encoded in extrastriate cortex. We begin by analyzing receptive field organization in area V2 (Willmore et al. 2010). We find that V2 receptive field reflect balanced excitation and suppression in the space of orientation and spatial frequency energy. Convergence of excitatory inputs leads to invariance to scale and position, while tuned suppression and the threshold nonlinearity of neurons enhance the sparseness of the representation. The interplay of excitation, suppression and thresholding creates selectivity for unique combinations of features which occur in natural images, but not in noise with matched power spectrum. We find that a similar encoding occurs in area MT (Cui et al. 2013), where excitatory and suppressive influences to the velocity and direction of inputs are finely balanced, creating a selective, invariant, and sparse representation of visual motion. We then sought to determine how downstream areas take advantage of this novel representation. Using a novel supervised learning technique, we found a parametric transformation that captures the processing in areas V2 and MT, much like half-wave rectified Gabor filterbanks can capture the processing in simple cells. We find that the computations performed by V2 and MT neurons were essential to capture the selectivity of V4 and MST neurons to complex stimuli with naturalistic structure. These results show that early extrastriate areas of both the dorsal and ventral visual streams, despite operating on different domains, perform similar computations on their input. Furthermore, the emerging representations

are forwarded, preserved and further built on in later areas, lending strong support for hierarchical models of visual processing.

Disclosures: **P.J. Mineault:** None. **T.P. Zanos:** None. **C.C. Pack:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Support: Intramural Research Program of NIMH

DFG Emmy Noether Grant to MCS

Grants by Whitehall and Alfred P. Sloan Foundation to AM

Title: Low-frequency oscillations in extrastriate cortex: Contributions of V1 and pulvinar

Authors: ***J. T. SCHMIEDT**[1], A. MAIER[2], R. C. SAUNDERS[3], D. A. LEOPOLD[3], M. C. SCHMID[1]
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Abstract: The local field potential (LFP) in visual cortex is often characterized by the following spectral pattern: before the onset of a visual stimulus, low frequency oscillations in the high alpha to low beta frequency range (13-20 Hz) typically dominate, whereas high-frequency fluctuations (>30 Hz) replace these low-frequency oscillations during stimulus presentation. However, the generative mechanisms of this alpha-beta activity *in vivo*, in particular the contributions of cortical versus thalamocortical input, remain unclear. To this end, we first longitudinally recorded LFP from chronically implanted multi-electrode arrays in extrastriate area V4 of two behaving macaques, before and after selective removal of primary visual cortex (V1). In the absence of V1 input, V4 low frequency oscillations were still present during active fixation periods indicating that V1 does not contribute to their generation. Visual stimulation with grating patterns after V1 removal however did not result in the typical ca. 30% reduction of alpha-beta power, but instead increased it by up to more than 50%. As this activity pattern was specific to stimulus locations affected by the V1 lesion, and was modulated by stimulus motion, it likely reflects unmasked input via V1-independent thalamocortical circuits. Indeed, in a second

set of recordings performed in behaving monkeys with intact V1, we found oscillatory LFP and spiking activity in the alpha-beta frequency range in the pulvinar nucleus that was enhanced by visual stimulation by ~30%. Due to the widespread and strong projections of pulvinar to visual cortex it is therefore conceivable that the pulvinar is a contributing source of the alpha-beta oscillations in extrastriate cortex.

Disclosures: J.T. Schmiedt: None. **A. Maier:** None. **R.C. Saunders:** None. **D.A. Leopold:** None. **M.C. Schmid:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Program#/Poster#: 236.21/Z23

Topic: D.04. Vision

Support: Intramural Research Program of the NIMH

DFG Emmy Noether grant to MCS

Whitehall and Alfred P. Sloan Foundation grant to AM

Title: Neural noise correlations in visual area V4 of the rhesus macaque after V1 lesion

Authors: *K. SHAPCOTT[1], J. T. SCHMIEDT[1], A. MAIER[2], R. C. SAUNDERS[3], D. A. LEOPOLD[3], M. C. SCHMID[1]
[1]Ernst Strüngmann Inst., Frankfurt Am Main, Germany; [2]Dept. of Psychology, Vanderbilt Univ., Nashville, TN; [3]NIMH, Bethesda, MD

Abstract: Neuronal co-fluctuations in visual areas of the brain unrelated to a stimulus have been extensively described and are usually termed “noise correlations”. The presence of this correlation between neurons has important implications for the ability of the brain to optimally encode sensory stimuli[1]. Traditionally, noise correlations have been thought to indicate the presence of common feed-forward input[2]. However, they have also been shown to vary according to the nature of a task or the allocation of top-down attention, which could indicate a contributing component from higher cortical areas[3,4]. We longitudinally recorded from two rhesus macaques the single- and multi-unit activities from multi-electrode “Utah”-arrays chronically implanted at the 3 degrees eccentricity representation of extra-striate area V4. Recordings took place while the monkeys performed a basic fixation task with visual

stimulation. After a two-week period an aspiration lesion was performed of primary visual cortex (V1) grey matter covering the central 7 degrees. Neuronal recordings continued after a short recovery period using the same implanted array and behavioural task. Rate correlation magnitudes were then compared between the pre and post lesion period during the fixation baselines (no stimulus present). If noise correlations arose uniquely from common feed-forward input, it would be expected that they decrease after the removal of V1, which constitutes the major V4 input. However, the experimentally measured noise correlations instead showed a significant increase (139% in monkey B and 25% in monkey F) for multi-unit activity. Despite this clear increase in noise correlation due to the lesion, the generally described relationship of decreasing noise correlation with increasing electrode distance was preserved. These results point to an origin of neural noise correlation either locally in V4 or from unmasked input from other cortical or thalamic areas. 1. Shadlen, M. N., & Newsome, W. T. (1998). The variable discharge of cortical neurons: implications for connectivity, computation, and information coding. *The Journal of neuroscience*, *18*(10), 3870-3896. 2. Cohen, M. R., & Kohn, A. (2011). Measuring and interpreting neuronal correlations. *Nature neuroscience*, *14*(7), 811-819. 3. Cohen, M. R., & Maunsell, J. H. (2009). Attention improves performance primarily by reducing interneuronal correlations. *Nature neuroscience*, *12*(12), 1594-1600. 4. Mitchell, J. F., Sundberg, K. A., & Reynolds, J. H. (2009). Spatial attention decorrelates intrinsic activity fluctuations in macaque area V4. *Neuron*, *63*(6), 879-888.

Disclosures: K. Shapcott: None. **A. Maier:** None. **R.C. Saunders:** None. **D.A. Leopold:** None. **J.T. Schmiedt:** None. **M.C. Schmid:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Whitehall and Alfred P. Sloan Foundation grant to AM

Title: Rhythmic neural activity during perceptual grouping in visual area V4 and its dependence on area V1 input

Authors: *R. KIENITZ[1], M. A. COX[2], J. T. SCHMIEDT[1], R. C. SAUNDERS[3], D. A. LEOPOLD[3], A. MAIER[2], M. C. SCHMID[1]
[1]Ernst Strüngmann Inst., Frankfurt am Main, Germany; [2]Dept. of Psychology, Vanderbilt Univ., Nashville, TN; [3]Lab. of Neuropsychology, NIMH, Bethesda, MD

Abstract: Perceptual grouping is a fundamental aspect of visual processing. However, the underlying neural mechanisms are not well understood. Here, we investigate the role of midlevel cortical area V4 neurons in perceptual grouping by tracking their responses during the presentation of the Kanizsa illusion in which perceptual grouping of inducer elements elicits the illusory percept of a visual surface. To this end, we recorded extracellular spiking activity and the local field potential (LFP) from chronically implanted multi-electrode arrays in V4 of two behaving macaque monkeys that passively viewed the Kanizsa illusion. We have previously found that neurons with their receptive field centered on the perceived surface (”surface cells”) preferably encode the Kanizsa illusion (Cox et al., PNAS, 2013). Here we extend this finding and report enhanced rhythmic spiking in the theta range (~4 - 6 Hz) of surface cells, when Kanizsa compared to control stimuli were shown. Analysis of the LFP showed the presence of gamma oscillations (30-40 Hz) in addition to those in the theta range. Interestingly, in the Kanizsa condition only, the emergence of gamma oscillations and associated spatial coherence in this frequency range was coupled in time to the phase of the theta cycle. In a second set of experiments, in which the two monkeys received a permanent V1 aspiration lesion resulting in severe perceptual deficits in the para-foveal part of the visual field, we assessed again the V4 neuronal activity in response to the Kanizsa illusion. With input from V1 largely removed, the V4 LFP showed transient activation shortly after the stimulus onset, ensuring that information reaches V4 via V1-bypassing pathways (e.g. through visual thalamus). However, the strong theta rhythm seen with intact V1 dramatically decreased after the lesion and did not differentiate between the Kanizsa and control conditions. At the same time, however, gamma oscillations did not change significantly in power, and presentation of the Kanizsa stimulus was still associated with stronger gamma power compared to control. Taken together, our results suggest an essential involvement of V1 in the generation of low-frequency rhythms in extra-striate cortex, and more generally, a role of neural rhythmicity in perceptual grouping.

Disclosures: R. Kienitz: None. **M.A. Cox:** None. **J.T. Schmiedt:** None. **R.C. Saunders:** None. **D.A. Leopold:** None. **A. Maier:** None. **M.C. Schmid:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

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Topic: D.04. Vision

Support: NIMH IRP

Title: Differential response consistency in the macaque face processing system to the viewing of social and non-social movies

Authors: *B. E. RUSS[1], D. A. LEOPOLD[2]
[1]Section on Cognitive Neurophysiol. and Imaging, Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD; [2]Section on Cognitive Neurophysiol. and Imaging, Lab. of Neuropsychology, NIH/NIMH, Bethesda, MD

Abstract: Conventional approaches to measuring the neural correlates of sensory and cognitive variables focus on isolating and controlling as many stimulus and task elements as possible. A complementary approach involves measuring the consistency of neural responses to multiple viewings of a complex, dynamic scene (Hasson et al, Science 2004). Inter-viewing correlations may be able to identify neural responses that participate in the interpretation of dynamic or contextual features of a scene that cannot be accurately modeled using a priori assumptions. Thus, this data-driven method has the potential to tap into aspects of cognition that are difficult to parameterize in more conventional paradigms. Here we use the inter-viewing method to investigate which regions of the macaque cortex are more consistently engaged by the presence of social stimuli, in this case animals, versus their absence. Five-minute movies varying in the amount of social content were shown repeatedly to two monkeys undergoing whole brain fMRI scanning. One set of movies contained clips of other animals, mostly macaques, interacting with each other or the environment, while a second set of movies contained natural dynamic scenes, such as storms, with a complete absence of animals. We produced brain-wide functional maps by computing each voxel's correlation across trials to repeated presentations of the same movies. The correlation value at each position thus reflected the extent to which a given area responded reliably to the content of a particular movie. We found that the majority of the ventral visual stream responded consistently to both the social and non-social movies. However we found that one bilateral region within the superior temporal sulcus was more consistently engaged by the presence of animals. This region overlapped the location of the middle face patch in both animals. Of the six identified face patches in each hemisphere, both the middle lateral and fundus face patches displayed a marked decrease in consistency when the subjects viewed movies that did not contain any animals. These results suggest that within the face processing system only the middle face patches were more sensitive to the presence of animals during natural viewing conditions.

Disclosures: B.E. Russ: None. **D.A. Leopold:** None.

Poster

236. Extrastriate Cortex: Responses and Coding

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 236.24/Z26

Topic: D.04. Vision

Title: A sinc wavelet describes the receptive fields of neurons in the motion cortex

Authors: *S. G. ODAIBO[1,2,3]
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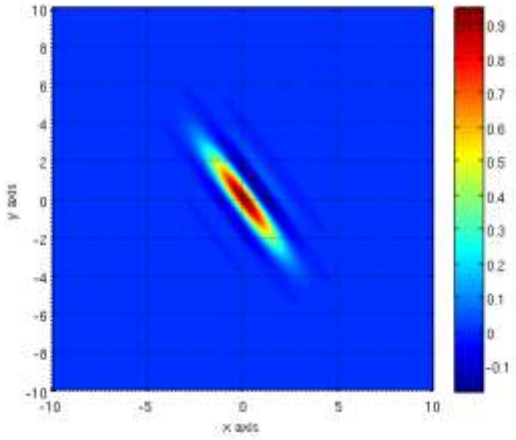
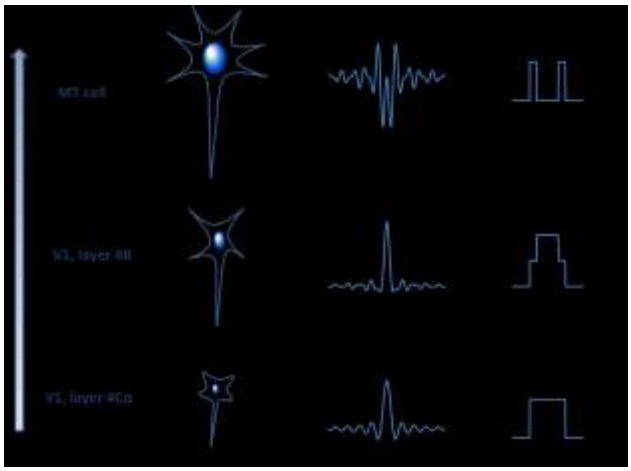
Abstract: Visual perception results from a systematic transformation of the information flowing through the visual system. In the neuronal hierarchy, the response properties of single neurons are determined by neurons located one level below, and in turn, determine the responses of neurons located one level above. Therefore in modeling receptive fields, it is essential to ensure that the response properties of neurons in a given level can be generated by combining the response models of neurons in its input levels. However, existing response models of neurons in the motion cortex do not inherently yield the temporal frequency filtering gradient (TFFG) property that is known to emerge along the V1 to MT motion processing stream. TFFG is the change from predominantly lowpass to predominantly bandpass temporal frequency filtering character along the V1 to MT pathway (Foster et al 1985; DeAngelis et al 1993; Hawken et al 1996). We devised a new model, the sinc wavelet model, which logically and efficiently generates the TFFG. The model replaces the Gabor function's sine wave carrier with a sinc (sin(x)/x) function, and has the same or fewer number of parameters as existing models. Because of its logical consistency with the emergent network property of TFFG, we conclude that the sinc wavelet is a better model for the receptive fields of motion cortex neurons. This model will provide new physiological insights into how the brain represents visual information.





Disclosures: S.G. Odaibo: None.

Poster

236. Extrastriate Cortex: Responses and Coding

Location: Halls A-C

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Program#/Poster#: 236.25/Z27

Topic: D.04. Vision

Title: Modeling attention-induced reduction of spike synchrony in the visual cortex

Authors: *N. WAGATSUMA[1,3], R. VON DER HEYDT[2], E. NIEBUR[2]
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Abstract: The mean firing rate of a border-ownership selective (BOS) neuron encodes where a foreground figure is located relative to its classical receptive field (Zhou et al, J. Neurosci 20:6594, 2000). In addition, the firing rate of these neurons is modulated by the effect of visual attention (Qiu et al., Nat. Neurosci 10:1492, 2007). Martin and von der Heydt (VSS Abstract J Vision 2013 13(9): 289) showed that top-down attention increases firing rates of BOS neurons and decreases spike synchrony between them. To understand the mechanisms of attentional modulation on mean rates and synchrony of BOS neurons, we developed a network model of spiking neurons. In the model, BOS neurons receive synaptic input from non-BOS feature-selective neurons which reflects visual input. The strength of the synaptic input is multiplicatively modulated by the activity of Grouping neurons (G) which receive their input

from BOS neurons and whose activity represents the location of visual objects in the scene (Craft et al, J. Neurophysiol. 97:4310, 2007; Mihalas et al, PNAS 18:7583, 2011). In addition, the mean firing rate of a G cell is increased when attention is directed to an object that is represented by it. Model simulations show that, in agreement with experimental findings, that attention to an object increases the mean firing rates of BOS neurons representing it while decreasing spike synchrony between pairs of such neurons. Our results thus suggest that top-down attention multiplicatively emphasizes synaptic currents due to bottom-up visual input. They furthermore suggest that attention exerts its influence on BOS cells by boosting the firing rates of G cells, rather than directly influencing the activity of BOS or other feature-selective neurons.

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Poster

236. Extrastriate Cortex: Responses and Coding

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Topic: D.04. Vision

Support: NIH Grant EY021827

Gatsby Charitable Foundation

Title: Using optogenetics to probe neural circuit mechanisms underlying biased competition and de-correlation with attention

Authors: *J. H. REYNOLDS, A. S. NANDY, J. J. NASSI
Salk Inst., LA JOLLA, CA

Abstract: A typical visual scene contains more information than our visual system can process at any moment in time. The brain utilizes attention to preferentially process behaviorally relevant stimuli. The physiological signatures of spatial attention have been well-studied in cortical area V4 of the macaque, where increases in gain, reductions in low-frequency correlated activity and a biasing of competition between nearby stimuli have all been implicated with attention. Together, these effects are thought to increase the signal-to-noise ratio of behaviorally relevant stimuli at the attended location, while largely filtering out activity associated with behaviorally irrelevant distracters. The neural circuit mechanisms underlying these effects remain poorly understood. Here, we describe experiments in which we have used optogenetic depolarization of

excitatory neurons in macaque V4 to probe attentional mechanisms. Given that optogenetic stimulation bypasses the retina, LGN and other cortical inputs, this enables us to directly perturb and test the V4 circuit in attention, without indirect effects inherited from afferent visual areas. We find evidence that optogenetic depolarization of excitatory neurons activates competitive circuits and modulates response variability. This positions us to test whether attention can bias this competition in favor of the visual stimulus and reduce optogenetic-induced correlated variability within V4.

Disclosures: J.H. Reynolds: None. **J.J. Nassi:** None. **A.S. Nandy:** None.

Poster

237. Eye Movements: Clinical and Behavioral

Location: Halls A-C

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Program#/Poster#: 237.01/Z29

Topic: D.05. Visual Sensory-motor Processing

Title: Using saccadic eye movement tasks to differentiate attention-deficit hyperactivity disorder from bipolar disorder

Authors: *S. SONCIN, D. BRIEN, A. MARIN, D. MUNOZ
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Abstract: Differentiating symptoms between Attention-Deficit Hyperactivity Disorder (ADHD) and Bipolar Disorder (BD) is difficult because the core features of ADHD are quite similar to the symptoms of BD. Executive function deficits are typical for these groups, where individuals with ADHD have problems with response inhibition and those with BD have emotion processing deficits. Response inhibition can be measured using eye movement tasks. The antisaccade task requires participants to inhibit a prepotent saccade toward a peripheral target and instead generate a voluntary response in the opposite direction. This task can be combined with emotional stimuli (faces) in order to differentiate ADHD and BD from each other and from controls. Adult subjects (18-62 years old; 22 ADHD, 20 BD, and 22 Control) performed an interleaved pro and antisaccade task (look toward vs away from peripheral target, respectively). Task instruction (pro vs anti) was conveyed by the colour of the central fixation spot. Standardized emotional faces (fear, happy, sad, neutral) were incorporated into the task along with control images (an object and no image). The images were presented behind the central fixation point shortly before (200ms) appearance of the peripheral target. In all conditions, Controls had faster mean saccadic reaction times compared to ADHD and BD groups while the

ADHD group was faster than the BD group. Controls also made less direction errors (prosaccades on antisaccade trials) than both patient groups. Emotion effects were group dependent: fear increased direction errors and saccadic reaction times for ADHD subjects while neutral had the same effect on BD subjects. The BD group had the greatest percentage of trials with multiple eye movements to reach the target while ADHD had the least, even when compared to controls. Additionally, the ADHD group produced the fewest corrective saccades, while BD made the most. Therefore, certain measures distinguish the patient groups from controls and others still show greater differences between ADHD and BD. In conclusion, these response patterns indicate that there are specific response profiles that can characterize each of these disorders. Emotions differentially affect each group implying that both response inhibition and emotion processing deficits may be valid as biomarkers for these disorders. Therefore, combining executive control and emotional processing into eye movement tasks may provide an objective measure that could be used for differential diagnosis.

Disclosures: S. Soncin: None. **D. Brien:** None. **A. Marin:** None. **D. Munoz:** None.

Poster

237. Eye Movements: Clinical and Behavioral

Location: Halls A-C

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Program#/Poster#: 237.02/Z30

Topic: D.06. Eye Movements

Support: NIH R01 NS50942

NIH R01 DC003687

Title: Saccade-related modulation of acoustic activity recorded from the external ear canal

Authors: *K. GRUTERS[1], C. A. SHERA[4], J. M. GROH[1,2,3]
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Abstract: Saccadic eye movements can quickly and substantially change the relationship between eye-centered visual space and head-centered auditory space, a relationship that must be "known" by the brain in order to coordinate a unified sense of audio-visual space. Previous research has found that saccades can evoke activity in auditory neurons located in the inferior colliculus (Porter et al., 2007; Bulkin and Groh, 2012), the lateral/medial banks of the

intraparietal sulcus (Mullette-Gillman et al., 2009), and superior colliculus (Lee and Groh, 2014). Presumably, this activity helps the auditory system calculate the hybrid reference frame that has been identified throughout the system (e.g. Mullette-Gillman et al., 2005; Porter et al., 2006; Lee and Groh, 2012). However, it is not clear at what level of the auditory system, or on what time scale, saccadic eye movement first influences auditory processes. We sought to test this question by determining whether acoustic signals recorded from ear canal display systematic peri-saccadic gain modulation. We measured sound pressure level in the external ear canal of monkeys (n=3) and humans (n=7) as they made saccades from a central fixation point to various locations along the horizontal azimuth. Sound pressure measured in this fashion reflects otoacoustic emissions generated by outer hair cells, activity of the middle-ear muscles, and possible influences from muscles near, but not necessarily specific to, the ear. Both monkeys and humans exhibited statistically significant changes in the peri-saccadic acoustic signal within individuals and at the population level (Monte Carlo simulation and ANOVA, $p<0.05$). These results indicate that eye movements influence auditory activity at the very periphery of the auditory system. Importantly, they suggest that whether or not the oculomotor system sends signals directly to the auditory system, eye movements systematically change the acoustic properties of the ear. Such changes are likely sufficient to pass eye position information throughout the entire auditory system and support a variety of interactions between vision and audition.

Disclosures: **K. Gruters:** None. **C.A. Shera:** None. **J.M. Groh:** None.

Poster

237. Eye Movements: Clinical and Behavioral

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Topic: D.06. Eye Movements

Support: NIH Grant EY10217

Title: How do patients with strabismus locate visual targets?

Authors: *J. R. ECONOMIDES, D. L. ADAMS, J. C. HORTON
Beckman Vision Ctr., UCSF, San Francisco, CA

Abstract: In subjects with strabismus either eye could potentially inform the brain about the location of a visual target, so that an accurate saccade can be made. It is unknown if the eye used

to acquire a target is also the eye that provides information regarding the target's location. Alternatively, the other eye could serve as the source for this information. To address this issue, 16 subjects with alternating exotropia and no amblyopia wore red/blue filter glasses for dichoptic stimulation while viewing stimuli on a tangent screen. Each trial began with a fixation cross, visible to either the right or left eye. After the subject fixated the cross, a peripheral stimulus appeared for 200 msec, visible to either the right or left eye. The subject's task was to look at it. Although the spot disappeared before the eye arrived, a saccade landing within a 5° window was rewarded with a tone, to motivate the subject. There were 2 main results: 1) the eye that saw the target was usually the eye that was used to fixate it; 2) when stimuli were presented in the far nasal field of the perceiving eye, subjects occasionally performed a "cross-over" saccade by placing the other eye on the target, to avoid having to make a large adducting saccade. In such cases, information about target location was obtained in one eye and used to program a saccade for the other eye. These cross-over saccades had a longer latency and were less accurate. In 10/16 subjects, purple spots were included as peripheral stimuli to test which eye was used to fixate targets that were potentially visible to either eye. First, binocular sensory maps were compiled to delineate the portions of the visual field perceived with each eye. Then the subjects' oculomotor behavior was studied by randomly interleaving red, blue, and purple peripheral stimuli. There was a close match between suppression scotoma maps and the eye used to acquire the peripheral stimulus. In other words, the target was perceived via the eye that was used to fixate it. These studies provide new information regarding the strategies used by patients with strabismus to fixate accurately visual targets, despite misalignment of the eyes.

Disclosures: J.R. Economides: None. **D.L. Adams:** None. **J.C. Horton:** None.

Poster

237. Eye Movements: Clinical and Behavioral

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 237.04/Z32

Topic: D.06. Eye Movements

Support: NIH 5R01NS037422-11

A-T Children's Project

Title: Optimal control of saccades in patients with ataxia-telangiectasia

Authors: *T. O. CRAWFORD[1], P. VASWANI[2], J. M. WRIGHT[3], H. M. LEDERMAN[3], R. SHADMEHR[4]
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Abstract: Ataxia-telangiectasia (A-T) is an autosomal recessive developmental disorder in which patients manifest neurodegeneration, ocular and cutaneous telangiectasia, and immunodeficiency. Among the prominent neurologic features, individuals with A-T have significant impairments in their oculomotor system, including difficulty with gaze holding, VOR, OKN, smooth pursuit, and saccades, though they have normal visual acuity. We assessed rapid eye movements of subjects with A-T. We asked 10 subjects with A-T and 30 control participants to make saccades to targets placed 10° to 40° apart on the horizontal axis. Recordings from control subjects demonstrated an initial saccade directly to, or near to, the target, while A-T subjects made a converging series of saccades that achieved the target with 3-4 movements. Prior work hypothesized this behavior is due to a deficit in the oculomotor system. Lewis et al. (1999) proposed that A-T individuals' saccades intend to move to the target, but are prematurely terminated, resulting in the series of saccades observed. We examined saccade dynamics and observed that peak velocity, early saccade velocity, duration, and skew were comparable to controls. Dynamics were appropriate for the saccade amplitude, but not for the target distance, suggesting that the A-T subjects' saccade hypometria is intentional. An optimal feedback controller (OFC) provides an alternate explanation for subjects' behavior. We hypothesized that A-T subjects make a series of saccades as an optimal policy given an increase in the signal dependent noise of their oculomotor system. An OFC that seeks to fixate the target accurately but also incurs a time cost makes two testable predictions. First, if there is a substantial increase in the signal dependent noise, the optimal policy is to make 3-4 saccades. Second, if visual feedback is available, these saccades will form a converging series; in the absence of visual feedback, when saccades must be planned in advance, equal amplitude saccades are predicted. Both control and A-T subjects displayed a signal dependent increase in the variance of saccade amplitudes, but the increase was 3.4 fold larger in A-T subjects. The intersaccadic interval in controls (413±12 ms) and A-T subjects (388±18 ms) was sufficient for the use of visual feedback. Given these parameters, the OFC predicts that A-T subjects should make a converging series of saccades with significantly reduced gain, as we observed. Individuals with A-T make a series of saccades that is optimal for the signal dependent noise in their oculomotor system. The "abnormality" observed in their saccades is not a primary deficit, but rather an adaptive, optimal policy.

Disclosures: T.O. Crawford: None. **P. Vaswani:** None. **J.M. Wright:** None. **H.M. Lederman:** None. **R. Shadmehr:** None.

Poster

237. Eye Movements: Clinical and Behavioral

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 237.05/Z33

Topic: D.06. Eye Movements

Title: Saccade performance in children with and without dyslexia during reading tasks

Authors: *N. Z. AL DAHHAN, D. C. BRIEN, J. R. KIRBY, D. P. MUNOZ
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Abstract: Eye movement recording is a valuable tool to investigate cognitive processes involved during reading and uncovering the cognitive and perceptual skills of average readers. It is then useful to apply these same ideas and technology of eye movement research to study readers with dyslexia. Despite dyslexia being one of the most common reading disabilities, its aetiologies are still unclear. Naming speed (NS) deficits, impaired timing mechanisms that affect reading fluency, are characteristic of reading difficulty from the early stages of reading into adulthood. NS tasks measure how quickly and accurately subjects can name sets of highly familiar stimuli (e.g., letters) randomly presented in a visual array. We used a letter NS task and three variants that were either phonologically and/or visually similar while participants' eye movements and articulations were recorded. We examined how these manipulations influenced performance and whether there were differences with increased reading acquisition from ages 6-10 and between dyslexic and average readers. Participants were in three groups (n=15/group): dyslexics (age 9-10), chronological-age controls (CA; age 9-10), and reading-level controls (RL; age 6-7). NS manipulations were associated with specific patterns of performance which were influenced by visual rather than phonological similarity. When the task was both visually and phonologically similar all groups had longer naming times and fixation durations, more naming errors, more frequent and shorter saccades, and had shorter eye-voice spans. Compared to CA controls, dyslexics performed more like RL controls and were less efficient, had longer articulation times, pause times, and fixation durations, shorter eye-voice spans, and made more errors, saccades, and regressions. Regression analyses indicated that pause time and fixation duration were the most powerful predictors of reading: longer pause times reflect more processing needed to process stimuli and prepare the correct response, and longer fixation durations reflect the greater amount of time needed to acquire visual information from stimuli. Overall, there were clear developmental changes in behavior and saccade performance in normally achieving children from ages 6-10 that appear to occur more slowly for dyslexics.

Disclosures: **N.Z. Al Dahhan:** None. **D.C. Brien:** None. **J.R. Kirby:** None. **D.P. Munoz:** None.

Poster

237. Eye Movements: Clinical and Behavioral

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Program#/Poster#: 237.06/Z34

Topic: D.06. Eye Movements

Support: DFG Fi1567/4-1

Title: Saccade planning in ambiguous situations

Authors: ***H. GERTZ**, K. FIEHLER
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Abstract: In a recent human fMRI study, we applied a pro-/anti-reach task and varied the point in time when the context rule (pro or anti) was given. The context rule had to be combined with a spatial cue in order to infer the reach goal. Thereby, we created specified conditions with the spatial cue and the context rule given before a delay period, and underspecified conditions with only the spatial cue given before the delay. We showed that movement goals (and not the physical location of the spatial cue) are represented in reach-related posterior parietal regions in both specified and underspecified conditions during the delay period. However, in underspecified conditions activation was substantially reduced. Moreover, activation in the dorsal premotor cortex observed in the specified conditions was lacking in underspecified conditions. In this study, we used the same experimental design to study saccade planning in conditions with specified and underspecified saccade goals. In the specified conditions, we presented 1 or 2 spatial cues and a context rule (pro or anti) before a variable delay period. Afterwards, participants performed pro- and anti-saccades accordingly. In underspecified conditions, only the spatial cue was given before the delay period, while the context rule was specified after the delay. A specific saccade goal could thus not be inferred before the end of the delay period. As shown in previous studies, saccade planning in specified conditions led to activation in a frontoparietal network including the frontal eye fields, the supplementary eye fields, the superior parietal lobule, and the intraparietal sulcus. Comparing the planning period of pro- and anti-saccades, we found higher activation for the planning of anti-saccades in the superior parietal lobule. Moreover, we observed activation in a similar saccade network in the underspecified condition when the context rule was unknown before the delay. In contrast to our

previous findings on reaching, the observed cortical network and the activation strength were comparable for specified and underspecified conditions. These results suggest that saccades similar to reaches are even planned *in situ*ations where the saccade goal is ambiguous.

Disclosures: H. Gertz: None. **K. Fiehler:** None.

Poster

237. Eye Movements: Clinical and Behavioral

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Program#/Poster#: 237.07/Z35

Topic: D.06. Eye Movements

Support: Leverhulme Trust Project Grant RPG329

Title: Temporal stability and training effects in "express saccade makers"

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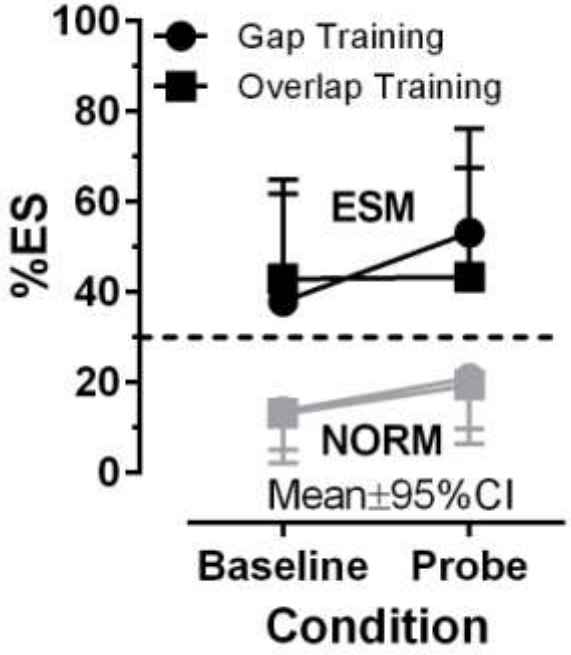
Abstract: A gap between fixation target offset and saccade target onset encourages the production of express saccades (ES; latency 80-130ms). “Express saccade makers” (ESMs) produce almost exclusively ES in gap trials, and in overlap trials (fixation target present when the saccade target appears), a large proportion (>30%) of their saccades are ES. We investigated performance stability in ESM and nonESM participants over time and the effect of repeated exposure to gap and overlap tasks. Experiment 1. 113 participants (59 ESMs) completed two blocks of 200 overlap trials in the first session (T1). Sixty provided data on a second occasion (T2; 27 ESMs; 200 trials; mean of 87 days later) and 30 provided data on a third occasion (T3; 13 ESMs; 200 trials; mean of 94 days later). Eye movements were recorded using an infrared reflectance eye tracker. For each participant, the percentage of saccades with latencies of 80ms to 130ms (%ES) was calculated for all saccades with latencies between 50ms and 500ms. Participants displayed the same relative performance both within (T1, Block 1 vs 2: ICC=0.97, $p<0.001$) and between (T1 vs T2: ICC=0.95, $p<0.001$; T1 vs T2 vs T3: ICC=0.97, $p<0.001$) sessions, thereby demonstrating high temporal stability. Experiment 2. We exposed 5 ESMs and 5 nonESMs to repeated exposure (“training”) with gap and overlap tasks (administered separately, approx. 6 weeks apart; order counterbalanced). After baseline measurement, gap training consisted of 400 gap trials completed on five consecutive days. Participants returned on

day 8 and completed 400 overlap and 400 gap trials ('probe' trials); this pattern was repeated for overlap training. While gap training produced a small but significant increase in %ES on the overlap task for both groups ($F=11.3$, $p=0.01$; mean increase: ESM=15±11%, Non-ESM=7±10%; see Figure) overlap training did not ($F=1.9$, $p=0.21$; mean increase: ESM=0.4±8%, Non-ESM=6±7%; Figure). These observations show that the performance of ESMs is stable over time and that the overproduction of ES on the overlap task in ESMs is unlikely to be a product of some environmental exposure.



Disclosures: **P.C. Knox:** None. **F.D.A. Wolohan:** None.

Poster

237. Eye Movements: Clinical and Behavioral

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 237.08/Z36

Topic: D.06. Eye Movements

Support: NWO grant VENI 451-09-016

Title: Saccadic adaptation violates Hering's law of equal innervation on multiple timescales

Authors: *T. KNAPEN[1], T. BEEMSTERBOER[2]
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Abstract: Saccades are commonly thought to be planned conjugately, a principle referred to as Hering's law of equal innervation of the two eyes. Here, we investigated the extent to which saccadic adaptation in one eye influences saccades made with the other eye. According to Hering's law, the two eyes should fully share their states of saccadic adaptation. We adopted a saccadic adaptation protocol with 6 blocks of alternating gain-up and gain-down adaptation. In two conditions, participants made saccades either with both eyes in all blocks (binocular), or with one eye patched (monocular). Importantly, the eye that was patched alternated with the blocks, ensuring that one eye would receive only gain-up adaptation and the other only gain-down adaptation. Any difference between binocular and monocular conditions in the course of adaptation across blocks would indicate a violation of Hering's law. Per-block data were fitted with both exponential and power-law adaptation functions, with the power-law fits consistently explaining more variance. This points to the existence of multiple timescales of adaptation in the single-block timecourses. Over the timecourse of the full experiment, gain-down adaptation on a long timescale decreased saccadic gain across all blocks for both conditions, pointing to a binocular adapting trace. In the monocular condition, adaptation state at the start of blocks was less influenced by the preceding adaptation in the other eye than in the binocular condition. This points to incomplete overlap of adaptation states in the two eyes. We used an extended multi-rate adaptation model to quantify this overlap, and find that fast and slow timescales show differences in binocular sharing of adaptation. Our results indicate that saccadic adaptation occurs in part at monocular levels of saccade planning and calibration, violating Hering's law.

Disclosures: **T. Knapen:** None. **T. Beemsterboer:** None.

Poster

237. Eye Movements: Clinical and Behavioral

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Topic: D.06. Eye Movements

Support: NCRR 2G12RR03060-26A1

NIMHHD 8G12MD007603-27

Title: The effect of prosaccade training on antisaccade latency and accuracy

Authors: S. MONTENEGRO, *J. A. EDELMAN
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Abstract: Prosaccades are saccadic eye movements made reflexively in response to the sudden appearance of visual stimuli; those with latencies under 120 milliseconds have been referred to as “express saccades.” Bibi and Edelman (2009) demonstrated that decreases in reaction time resulting from training express saccades along one axis (horizontal or vertical) could transfer to saccades made in perpendicular directions. . To help determine the visuomotor processes underlying this facilitation, we trained subjects to make prosaccades and probed performance on antisaccade trials, in which prosaccades are suppressed and voluntary saccades made in the direction opposite the target. Eye movements of five subjects were recorded using an Eyelink II video eyetracker interfaced with computer routines written using Experiment Builder (SR Research). Subjects trained at making prosaccades over several weeks and were probed at three phases of the training (before training, after six sessions and after twelve sessions) to examine how training affected the ability to make antisaccades. We consider three possible outcomes: 1) A finding that prosaccade training increases errors in antisaccade trials would indicate that prosaccade training facilitates visual processes, making it more difficult for reflexive visuomotor processes to be suppressed during an antisaccade trial. 2) A finding that training increases antisaccade latency would suggest that prosaccade training suppresses voluntary saccade production. 3) A finding that prosaccade training decreases antisaccade reaction time would suggest that prosaccade training has a general effect to facilitate saccade production in reaction time tasks. As in previous work (Bibi and Edelman, 2009), training resulted in the shortening of prosaccade reaction time, with a greater percentage of saccades in the express latency range. Subjects exhibited an overall decrease in reaction time in antisaccade trials after training, while the percentage of errors in the antisaccade task increased only slightly (statistically significant in only 1/5 subjects). These findings suggest prosaccade training has a generalized effect on the saccadic system, allowing it to more quickly generate saccades (pro-and anti) in reaction time tasks.

Disclosures: S. Montenegro: None. **J.A. Edelman:** None.

Poster

237. Eye Movements: Clinical and Behavioral

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 237.10/AA2

Topic: D.06. Eye Movements

Title: Visual attention and individual miniature saccadic eye movements generated during car driving

Authors: T. HIRAYAMA[1], T. OGURI[4], *K. INAGAKI[2,3], Y. HIRATA[3]
[1]Computer Sci., [2]Inst. of Sci. and Technol. Res., Chubu Univ., Kasugai, Aichi, Japan; [3]Robotic Sci. and Technol., Chubu Univ., Kasugai, Japan; [4]DENSO Corp., Nisshin, Japan

Abstract: A microsaccade (MSC) is a rapid miniature eye movement generated when we are fixating on a small visual target. It has been studied in well-controlled (or unnatural) laboratory environment, and shown to reflect location of visual attention in its statistical properties such as generation rates and direction distributions. Previously, we demonstrated that MSC-like miniature rapid eye movements were evoked in human subjects during real car driving, and the number of the evoked MSC-like eye movements increased when pedestrians and/or other vehicles were in their field of view (Miki & Hirata, 2013). Presently we repeated the same driving experiment with a new subject in addition to the same 3 subjects who participated in the previous study. The driving experiment was conducted on the campus road in Chubu University Kasugai Campus, lasting for 5 to 9 minutes with the maximum speed of 30 km/h. Binocular eye movements were measured with a video-oculography (EyeSeeCam) at the frame rate of 220 fps. First, we confirmed that the results obtained in our previous study (Miki & Hirata, 2013) were reproduced in all the 4 subjects. Then we analyzed individual MSC-like eye movements, rather than their statistical properties, in conjunction with larger saccades. It was found that most (> 87 %) of individual MSC-like eye movements preceded a gaze shift driven by a saccade in a short period of time (< 0.6 sec). When they were not followed by a saccade, they were followed by another MSC-like eye movement with a short interval (< 0.9 sec) that is shorter than the average interval (> 2.5 sec) calculated over the entire driving time. As saccades are known to accompany with spatial shift of attention, these results suggest that an individual MSC-like eye movement reflects spatial shift of subject's attention. However, the MSCs that preceded a saccade did not show clear directional correspondence with the landing position of the saccade, i.e., the location to which attention was presumably directed (53.6% in the same direction, 46.4% in the opposite direction). Further experiments and analyses are required to address the relationship between the direction of individual MSC and that of attentional shift.

Disclosures: **T. Hirayama:** None. **T. Oguri:** None. **K. Inagaki:** None. **Y. Hirata:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

Location: Halls A-C

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Program#/Poster#: 238.01/AA3

Topic: D.06. Eye Movements

Support: NSERC 43918-2013RGPIN

Title: Contribution of dorsolateral prefrontal cortex to attentional and mnemonic processes in visual search

Authors: *B. BELBECK, S. G. LOMBER, S. EVERLING, K. JOHNSTON
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Abstract: At any moment, we are faced with many more visual stimuli than can be processed at one time. Thus, a prerequisite for successful behaviour is the ability to selectively attend to relevant stimuli while ignoring irrelevant ones. A key characteristic of visual selective attention is that it may be deployed on the basis of our knowledge or the goals of the task at hand. Such may be the case when the behavioural relevance of stimuli varies frequently or when a representation of a relevant stimulus must be retained within working memory to guide visual selection. The prefrontal cortex (PFC) is thought to play a prominent role in attentional processes, as well as behavioural flexibility and working memory. Here, we used cryogenic deactivation to investigate the contribution of the dorsolateral PFC (DLPFC) to cognitive processes related to the deployment of attention using visual search tasks. Macaque monkeys performed four visual search tasks that required them to make a single saccade to a target amongst distracters, and which varied with respect to their cognitive demands. The first was a simple 'pop-out' feature search, in which the target stimulus was defined by colour. The other three tasks were variants of a conjunction search in which the target was defined by colour and shape. In these tasks, the target either remained constant throughout an experimental session, varied within-session on a trial-by-trial basis - requiring cognitive flexibility, or varied within-session with the addition of a memory delay - requiring working memory. In each experimental session, animals performed one of these tasks while the DLPFC was bilaterally deactivated. Both control and experimental behavioural data were collected within each session. A 15-minute deactivation epoch was preceded and followed by 15-minute control epochs. Deactivation resulted in minimal changes in response accuracy across tasks, but increased saccadic reaction times. These results suggest the DLPFC is involved in the deployment of attention to a target, and also contributes to the flexible and mnemonic processes needed when the search target changes or temporary retention is required. These findings are generally consistent with the notion that the PFC sends bias signals to other brain areas to guide attention during visual search.

Disclosures: **B. Belbeck:** None. **S. Everling:** None. **K. Johnston:** None. **S.G. Lomber:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

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Program#/Poster#: 238.02/AA4

Topic: D.06. Eye Movements

Support: NSF GRFP

NIH Grant EY014924

Title: Saccadic suppression of visual sensitivity in frontal eye field neurons

Authors: *R. M. KROCK[1], T. MOORE[2]
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Abstract: Saccadic suppression is a profound loss of visual sensitivity around the time of saccadic eye movements that is presumed to aid in constructing a stable visual percept by minimizing self-generated motion signals. The frontal eye field (FEF) is an oculomotor structure that plays a key role in visual sensorimotor integration, and yet its role in saccadic suppression is unknown. We investigated changes in visual sensitivity of FEF neurons before saccades using extracellular recording with 16-channel linear electrode arrays in rhesus macaques. We presented brief (16 ms), full-field visual probes during fixation or shortly (<130 ms) before saccades. The probe consisted of a field of pseudorandomly positioned, 1 dva grayscale circles with contrast varying from 2 to 32% Michelson contrast across trials. Visual responses to presaccadic probes were significantly reduced compared to probes presented during fixation across the population of visually responsive cells. Visual sensitivity, as measured by d', was also markedly reduced across the range of stimulus contrast values tested. We also measured the time course of suppression and examined presaccadic sensitivity to chromatic stimuli. Our results demonstrate that FEF neurons exhibit robust suppression of visual sensitivity around the time of saccades.

Disclosures: R.M. Krock: None. **T. Moore:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 238.03/AA5

Topic: D.06. Eye Movements

Support: CIHR

Title: Bilateral anterior cingulate cortex deactivation reduces switch costs in the macaque monkey

Authors: *J. L. CHAN, K. JOHNSTON, S. G. LOMBER, S. EVERLING
Univ. of Western Ontario, London, ON, Canada

Abstract: The ability to flexibly switch behavior in a goal-directed manner is integral to cognitive control. The anterior cingulate cortex (ACC) is thought to play a critical role in this control. Although lesion, functional imaging, and neurophysiological studies have implicated this area in a variety of cognitive functions, including conflict monitoring, performance monitoring, action selection, and decision making, ACC function remains poorly understood. In this study, the role of the ACC in top-down control was investigated by applying reversible cryogenic deactivation bilaterally to the dorsal ACC (area 24c) of a rhesus macaque monkey performing a task that required switching between two behavioral rules. The task consisted of randomly interleaved pro- and anti-saccade trials, and enabled error rates and reaction times to be examined on trials where the task rule was the same as (repeat trial) or different from (switch trial) the previous trial. Each trial began with a grey central fixation point that changed to a colored instruction stimulus to indicate the task rule. After a random interval of 500 to 700 ms, the instruction stimulus was extinguished for 200 ms prior to the onset of a peripheral stimulus (gap task). The monkey was required to make a saccade either toward or away from the stimulus on pro- and anti-saccade trials respectively. Consistent with previous studies, switch costs were observed in this interleaved saccade task. Switches to pro-saccade trials had longer saccadic reaction times than repeat pro-saccade trials, and switches to anti-saccade trials had higher error rates than repeat anti-saccade trials. These switch costs were eliminated during ACC deactivation. In addition, ACC deactivation significantly decreased error rates for both pro- and anti-saccades. Thus, the absence of ACC activity may facilitate cognitive flexibility when a task requires frequent changes in behavior. These results suggest that the ACC may play a role in tracking previously rewarded behaviors.

Disclosures: **J.L. Chan:** None. **K. Johnston:** None. **S.G. Lomber:** None. **S. Everling:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.06. Eye Movements

Support: Canadian Institutes of Health Research (CIHR)

Ontario Graduate Scholarship (OGS)

Title: Theta-band coherent activity is involved in transmission of task relevant information between frontal eye field and anterior cingulate cortex

Authors: *S. BABAPOOR-FARROKHRAN[1], T. WOMELSDORF[2], M. VINCK[3], K. JOHNSTON[4], S. EVERLING[1]
[1]Neurosci., Western Univ., London, ON, Canada; [2]Biol., York University, Ctr. for Vision Res., Toronto, ON, Canada; [3]Neurobio., Yale University, Kavli Inst. for Neurosci., New Haven, CT; [4]Physiol. and Pharmacol., Westen Univ., London, ON, Canada

Abstract: Anterior cingulate cortex (ACC) is involved in a variety of motor and cognitive functions and frontal eye field (FEF) is involved in the generation of saccades. Previous literature suggests a role for ACC in saccade control by virtue of its anatomical and functional connectivity with FEF and other saccade-related brain areas. The mechanism of information transfer between these areas has not been completely understood and involves complex interactions of different types of oscillatory and neuronal signals. It has been suggested that brain areas communicate through synchronization and this facilitates the transmission of relevant information between brain areas. In the current study, we have investigated the potential mechanisms by which information is transferred between ACC and FEF during performance of oculomotor tasks. We carried out simultaneous local field potential (LFP) recordings in the FEF and ACC of macaque monkeys (Macaca fascicularis and Macaca mulatta) while they performed memory-guided saccades and a pro/anti saccade task. In the memory-guided saccade task, the monkeys performed saccades towards the remembered target location 1000 milliseconds after a brief presentation of the target stimulus. In pro/anti saccade task, the monkeys performed saccades toward (pro-saccade) or to the opposite side of (anti-saccades) the presented stimulus. The results indicate that theta-band coherence with the frequencies between 3 to 9 Hz is modulated during the preparatory period before stimulus onset in the pro/anti saccade task. Furthermore, theta-band coherence between FEF and ACC is enhanced when visual stimuli are presented in the contralateral visual field. Our results suggest that theta-band coherent activity is

involved in the rule-specific information transmission between FEF and ACC which might facilitate task-dependent stimulus-response mappings.

Disclosures: **S. Babapoor-Farrokhran:** None. **T. Womelsdorf:** None. **M. Vinck:** None. **K. Johnston:** None. **S. Everling:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.06. Eye Movements

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RFBR (the projects № 12-04-00719)

Basic Research Program of the National Research University Higher School of Economics

Title: Electrophysiological correlates of the saccadic preparation cognitive processes in the experimental scheme with distractors at stimulation of the leading and unleading eye

Authors: *V. MOISEEVA[1,2], M. SLAVUTSKAYA[1], V. SHULGOVSKIY[1], N. FONSOVA[1]
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Abstract: To attend relevant stimuli in the visual field while ignoring distracter elements is crucial for successful goal-directed behaviour. The oculomotor system can be used as a model to study the competition between different elements in the visual space. The goal of our research was to analyse spatial-temporal parameters of saccades and presaccadic EEG-potentials at the simultaneous presentation of the target and distracting stimuli to the leading and unleading eye. 14 healthy right-handed volunteers participated in the study. Target and distracting peripheral visual stimuli were presented simultaneous monocularly on the monitor in various spatial combinations. Eye movements were recorded using the electro-oculogram. Possible combinations of visual stimuli in the same or in different visual hemifields. The complex of the positive and negative potentials was revealed in the saccade latent period. Latency of all components was shorter upon presentation of stimuli to the left, unleading eye, that may indicate the earlier saccade preparation. At the same time LP saccades were longer in this conditions

($p<0.05$). The main asymmetry in saccadic LP was revealed at the presentation of target and distracting stimuli in the left visual hemyfield at the distance 5 degrees between them. The results show that early potentials N1 and P1 were higher in amplitude and dominated in medial and controlateral parietal-occipital areas. It can be reflection of visual sensory processing and processes of motor preparation at the same time. The amplitude of the later negative potential N2 at the stimulation of the right eye increased in the case when target stimulus was at the same location than at the previous realisation. It's possible that N2 component is connected with processes of preliminary extracting of motor programme from memory together with attention processes. N2 amplitude was higher when the distance between target and distracting stimuli was 15 degrees in comparison with the minimal distance 5 degrees. It's corresponded with LP data. The findings show an active role of attention and decision-making processes in saccade programming. The work was executed at the support of RFBR (the projects № 14-04-01634 and № 12-04-00719) and it's a part of the Basic Research Program at the National Research University Higher School of Economics (HSE).

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Poster

238. Eye Movements: Cortex and Superior Colliculus

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 238.06/AA8

Topic: D.06. Eye Movements

Support: NIH Grant EY021579

Title: How do frontal eye field neurons ignore distractors while selecting target-relevant features in natural scenes?

Authors: *D. K. WOOD[1], P. RAMKUMAR[2], J. I. GLASER[2], P. N. LAWLOR[2], K. P. KORDING[2], M. A. SEGRAVES[1]
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Abstract: While searching for targets in natural scenes, two strategies may be adopted: selectively attend to locations that share features with the target (target enhancement), or selectively avoid locations that do not (distractor suppression). We want to understand how these strategies are implemented in the frontal eye fields (FEF). To this end, we trained macaques to

search for a target (e.g. a vertical Gabor) in a natural scene that also contained a distractor (e.g. a horizontal Gabor). To ensure that distractor suppression is behaviorally advantageous, we rewarded monkeys for saccading to the target, and punished them (the trial was aborted) for saccading to the distractor. We tested whether monkeys used both strategies by asking if fixation prediction could be enhanced by using both target and distractor-based priority maps. We then modeled spike trains and local field potentials (LFPs) using generalized linear models (GLMs) comprising target and distractor relevance maps, as well as bottom-up saliency and movement direction. We found neural activity that encoded target features, distractor features, both, or neither. Our results suggest that FEF may play a role in both target enhancement and distractor suppression during natural scene search.

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Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.06. Eye Movements

Support: NIH Grant EY021579

Title: The frontal eye field reflects task demands in natural scenes

Authors: *J. I. GLASER[1,2], P. N. LAWLOR[1,2], D. K. WOOD[2], P. RAMKUMAR[1,2], S. CADDIGAN[2], J. DRAPEKIN[2], B. FRICK[2], B. QIN[2], K. P. KORDING[1,2], M. A. SEGRAVES[2]
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Abstract: Primates make thousands of eye movements (saccades) per hour, but it is still an open question how the brain chooses fixation locations. Many factors are thought to influence saccadic locations, including visual "bottom-up" features like salience and goal-dependent "top-down" features. It is unknown how the visual system encodes the priority of these natural scene features, and whether they are encoded in a task-dependent manner. Studies with artificial stimuli suggested that the frontal eye field (FEF) encodes both visual salience and goal-dependent (relevant) features, but other studies using natural scenes did not replicate this effect. Here, we record eye movements, spike trains, and local field potentials (LFPs) from the macaque FEF during two tasks designed to disambiguate the effects of salience and goal-dependent

features in natural scenes: tasks with and without known search targets. We used generalized linear models (GLMs) to predict neural activity as a function of visual features and movement direction around the peri-saccadic interval. Using this method, we found that some FEF neurons differentially encoded salience and relevance between the two tasks. Our results demonstrate that the FEF encodes visual features according to their priority for the task.

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Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.06. Eye Movements

Support: NIH Grant EY014924

Title: Gating of visual input to prefrontal cortex: Orthodromic activation of FEF neurons by V4 stimulation

Authors: ***K. L. CLARK**, B. NOUDOOST
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Abstract: Visually guided behavior is achieved in part via the interaction between oculomotor structures and visual areas. To understand how these areas interact with each other we studied dynamic changes in the efficacy of inputs from visual area V4 to the frontal eye field (FEF), which project directly to one another. We electrically stimulated V4 sites while recording from FEF neurons with overlapping response fields, and identified FEF neurons that were orthodromically activated by V4 stimulation. We were particularly interested in examining the changes in the functional relationship of FEF-V4 neurons during memory encoding, memory maintenance, and saccade execution. Various computational models suggest that areas that maintain information during working memory (such as FEF) should be differentially sensitive to inputs from sensory areas (e.g. V4) during memory encoding and maintenance. Changes in functional connectivity between FEF and V4 around the time of saccades could also help explain the difference in the magnitude of presaccadic receptive field shifts between the two areas. By stimulating V4 during different epochs of the memory-guided saccade (MGS) task, we

quantified the strength of functional connectivity between V4 and FEF during these cognitive states. In the MGS task, the animal is presented with a visual cue and must remember that location, while maintaining fixation, throughout a blank delay period, then saccade to the remembered location to receive a reward. We stimulated V4 during the fixation, cue, memory, and saccade periods of the task, and quantified the stimulation efficacy, timing and reliability of evoked spikes, and synchrony between pairs of simultaneously recorded FEF neurons. We examine the relationship between the functional properties of FEF neurons and their sensitivity to V4 input, as well as how sensitivity to V4 stimulation varies as a function of LFP power and phase. These provide direct neurophysiological measures of the gating of visual information during different epochs of a cognitive task.

Disclosures: K.L. Clark: None. **B. Noudoost:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

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R01DA030750

Title: Cognitive modulation of neuronal activity in the frontal cortex of monkeys performing a simple reaction-time task with reward bias

Authors: *C. K. HAUSER, D. ZHU, M. COSTELLO, E. SALINAS, T. STANFORD
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Abstract: Reward availability is key for guiding goal-directed actions and has profound effects on sensory and motor signals throughout the central nervous system. For saccades, reward contingency can influence choice, reaction time (RT), and saccade metrics. We investigated how

these changes relate to neuronal activity in the frontal eye field (FEF), focusing on how perceptual processing and motor planning are modulated by the internal expectation of reward. We used a simple RT variant of the one-direction-rewarded (1DR) task developed by Hikosaka and colleagues. Monkeys were trained to maintain fixation at a central spot and make a saccade when an eccentric stimulus appeared at one of 4 possible locations. Crucially, on a given block of trials, only one such location (the "rewarded location") was associated with the primary reinforcer, water. At each block transition, a new rewarded location was chosen and monkeys relearned the location-reward association. Strong effects on RT were observed: relative to the mean RT in a control task (191 ms, all directions rewarded), saccades to rewarded locations were faster (161 ms) and those to unrewarded locations were markedly slower (261 ms). Moreover, the spread of RTs for unrewarded locations was much larger than for the rewarded. These differences developed quickly, within approximately 5 trials of a switch. We exploited the large spread in RT and the spatially distinct reward conditions in the 1DR task to study how FEF responses related to behavior. We found that FEF activity covaried strongly with RT and was robustly modulated by reward contingency, particularly in the case of neurons traditionally classified as visuomotor (VM); that is, units that exhibit both a visually-driven response to the onset of a stimulus in the response field and an increase in activity preceding a saccade to it. The VM neurons showed three prominent effects: (1) large changes in baseline activity (during fixation) that correlated tightly with RT and/or reward location, (2) brisk presaccadic responses that peaked early and started declining a few tens of ms before saccade onset, and (3) an extremely attenuated presaccadic activation for saccades into the response field when the rewarded location was diametrically opposite. Thus, signals that would be traditionally attributed to motor preparation (e.g., in a standard delayed-saccade task) instead reflected other cognitive processes _ possibly the locus of attention _ that are typically aligned with the saccadic vector. By comparing neuronal activity across tasks with varying attentional and perceptual demands, ongoing experiments aim to further characterize the functional role of such activity.

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Poster

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Title: MST neurons contribute to perceiving the visual world as continuous across saccades by memory remapping of moving visual stimuli

Authors: *N. INABA[1,2], K. KAWANO[1]
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Abstract: We perceive the visual world as stable and continuous despite abrupt retinal image shifts induced by eye movements. To examine how the visual system deals with the spatial localization of moving visual stimuli across saccades, we recorded activities of motion sensitive neurons at middle temporal (MT) and medial superior temporal (MST) areas in awake monkeys. The monkeys performed fixation and saccade tasks in which a spatially stable moving stimulus was presented at various visual fields. When the moving stimulus was presented throughout the periods of the saccades, the location of the receptive fields (RFs) moved with the shifts of eye position due to saccades, indicating that motion-sensitive neurons in both areas have retinotopic RFs across the saccades. When we compared the latency of the post-saccadic response from saccade-offset to the visual response latency of each neuron, most MST neurons had significantly shorter latencies from saccade-offset than the visual response latencies. In contrast, for MT neurons, the former was significantly longer than the latter, suggesting the impact of saccadic suppression. As a result, although the MT neurons' visual response latencies were significantly shorter than the MST neurons', the MST neurons' post-saccadic response latencies were significantly shorter than the MT neurons'. When the moving stimulus was presented and turned off before the saccades, most MST neurons, but no MT neurons, increased their firing rates when a saccade brought into their RFs the location of the visual stimulus, which had been visible only before the saccade. It suggests that the responses of such MST neurons after the saccades were evoked by a memory of the stimulus that had pre-existed in the post-saccadic RFs, i.e., "memory remapping". These findings suggest that the post-saccadic responses in MST evoked by the "memory remapping" might be used to fill the missing vision due to saccadic suppression in MT and contribute to the continuous and stable visual perception.

Disclosures: **N. Inaba:** None. **K. Kawano:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.06. Eye Movements

Support: Canadian Institutes of Health Research Grant

Title: The role of the superior colliculus in the coordination of the pupil orienting response

Authors: *C.-A. WANG, D. P. MUNOZ
Queens Univ., Kingston, ON, Canada

Abstract: Pupil size, as a component of orienting, changes rapidly in response to local salient events in the environment in addition to an illumination-dependent modulation. A growing body of evidence suggests that the superior colliculus (SC) encodes stimuli based upon saliency to coordinate the orienting response. Although the SC is involved causally in the initiation of saccadic eye movements and attention, its role in coordinating other components of orienting is less understood. Here, we examined how pupil dynamics are modulated by the SC and stimulus saliency. While requiring subjects to maintain central fixation, we either presented a salient visual, auditory, multisensory stimulus or delivered weak electrical microstimulation to the intermediate SC layers (saccades were not evoked). Transient pupil dilation was elicited after presentation of salient stimuli, and this dilation was qualitatively similar to that evoked by SC microstimulation. Moreover, the timing and magnitude of evoked pupil responses scaled with the level of stimulus saliency, with significantly faster and larger pupil responses observed for more salient stimuli. The same modulation of stimulus saliency was demonstrated in human subjects. Importantly, the pupil response onset latencies for salient stimuli were comparable to those produced by the pupillary light reflex and much faster than the pupillary darkness reflex, suggesting that the initial component of pupil dilation is more likely mediated by inhibition of the parasympathetic pathway. Together, the results suggest that the transient pupil orienting response is modulated by stimulus saliency, and the SC is a likely neural substrate coordinating these pupil orienting responses.

Disclosures: C. Wang: None. **D.P. Munoz:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.06. Eye Movements

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Title: Neurons in the human medial temporal lobe encode target saliency during visual search

Authors: *S. WANG[1], A. N. MAMELAK[3], R. ADOLPHS[1,2], U. RUTISHAUSER[3,2]
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[3]Dept. of Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: A key function of selective visual attention is to direct our gaze rapidly to objects of interest in the visual environment. The human medial temporal lobe plays a key role in recognizing, categorizing, and learning visual objects and is though to convey saliency and object selection signals through its dense network with other brain regions. To investigate this issue at the single-neuron level, we employed a visual search task with both social (faces and people with different postures, emotions, ages, and genders) and non-social targets (e.g., electronics, food, utensils), which participants were asked to find in an array of 24 objects. With concurrent eye tracking, we recorded from over 150 single neurons in the amygdalae and hippocampi of four neurosurgical patients (five sessions) with implanted depth electrodes. Behaviorally, we found patients rapidly oriented to, and persistently searched amongst, target-congruent objects. Trial-by-trial analysis showed that 11.7% (8.3% for amygdala and 16.7% for hippocampus) of neurons responded only when a target was found. Fixation analysis revealed neurons that responded only when a fixation fell on a target but not a distractor. By comparing the average number of spikes in a time window of the entire fixation duration between fixations on targets and distractors, we selected 24.2% of these target neurons (9.7% for amygdala and 45.8% for hippocampus; two-tailed t-test at $p<0.05$; among which 82.3% increased firing rate to targets and 17.2% decreased). Since the same objects can be either targets or distractors on

different trials, this reveals the top-down driven nature of the response, which suggests that task-relevant target saliency was encoded by a subset of neurons. We further conducted two control experiments in the same patients: one ruled out motor confounds by applying a fixed duration of search in the same task without button press, and the second ruled out working memory or object matching confounds by employing a pop-out search task in which the target was defined as one face among vehicles or one vehicle among faces (thus no predefined target). We also found 21.7% (27.8% for amygdala and 12.5% for hippocampus) of neurons that responded more to social objects than to non-social objects. Interestingly, there was a small population (4%) of target-responsive neurons that differentiated social vs. non-social objects both during single target presentation and subsequent target detection in the search array. Taken together, we found compelling evidence that neurons in the human medial temporal lobe encode object categories and saliency signals that contribute to attention.

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Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.05. Visual Sensory-motor Processing

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Conicyt to RMT

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Title: Modulation of fixational event-related potentials by low-level image features during free-viewing

Authors: *C. DEVIA[1,2], R. MAYOL-TRONCOSO[2], R. MONTEFUSCO-SIEGMUND[3], J. P. OSSANDÓN[4], A. V. HELO[5], P. E. MALDONADO[2]
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Abstract: During natural vision we explore the visual world with a fast sequence of self-initiated saccadic eye movements, which occur unconsciously about four times per second. However, most of our knowledge of visual processing originates from studies where subjects fix the gaze on a screen and are exposed to a series of visual stimuli that trigger brain activity mainly through bottom-up mechanisms. These flashed stimuli elicit an unanticipated brain activity, thus occluding ongoing and top-down processes, predictive mechanisms at work in natural vision. One way to study the features of brain ongoing mechanisms is measuring fERP, the event-related potentials locked to fixation onset. In this work, we compared fERPs at occipital sites when subjects freely explored scenes with different low-level characteristics. On seven volunteer, we recorded EEG and eye tracking when they scanned 5 categories of gray-scale images: Landscape, fractals, pink noise, gray and black images. To analyze the data we first performed an ICA decomposition to remove ocular artifacts from scalp EEG signals and then calculated the fERP. We found that a fERP was elicited at fixation onset along image exploration (landscape, fractal and pink noise), it was composed by a positive and consecutive negative deflection localized at occipital sites, and it was visible at single trial. In black images, this fERP was absent showing its cortical origin as subjects also moved their eyes in this category. Even though gray images had more luminance than the other categories, the fERP was almost absent showing a weak modulation by luminance. Finally, we verified that frequency content of the images was similar, except for gray and black categories, and that partially explained fERP amplitude modulation at different categories. We showed here in a free-viewing task that fERP at occipital sites is partially explained by low-level image features like luminance and frequency content while also high-level image features, such as familiarity, congruency and emotional content modulate fERP signals. These results suggest that both bottom-up and as well as top-down processes modulate early visual cortex activity during natural vision.

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Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.04. Vision

Support: CHIR

Title: Target selection in primate Superior Colliculus: Simultaneous recordings of single unit and local field potential

Authors: *T. IKEDA, B. J. WHITE, D. P. MUNOZ
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Abstract: The Superior Colliculus (SC) coordinates the orienting response, which converts sensory information into motor commands for the eyes and the head. The intermediate layers of the SC (SCi) integrate inputs from various brain regions to select an appropriate target. We have previously reported that single unit activity (SUA) in the SCi represents target selection processes in a color discrimination task having two separate mechanisms: one linked to the arrival time of visual signals and the other linked to the visual selection process (White and Munoz, 2011). The results showed that saccade reaction time (SRT) was determined by integrating these two mechanisms. We hypothesize that local circuitry within the SCi is playing an important role in this integrative process, and analyzed the local field potential (LFP) simultaneously recorded with SUA. The LFP is thought to represent the sum of postsynaptic potentials of neighboring neurons (Mitzdorf, 1985), and thus may reveal local processing in the SCi. Two monkeys were trained to perform a color-singleton selection task with three conditions in which luminance and color of the items were independently manipulated. 1) target and distractors were isoluminant with the background and had highly different chromaticity making the target easy to discriminate (iso-easy), 2) same target disciminability as previous condition except all items had added luminance contrast relative to the background (lum-easy), 3) target and distractors were isoluminant with the background but the chromatic difference between target and distractors was less making the target difficult to discriminate (iso-diff). SUA and LFP showed a similar phasic visual response with shorter visual onset latency for luminant (SUA: 51±11ms, LFP: 55±11ms) versus isoluminant stimuli (SUA: 68±15ms, LFP: 73±15ms). This suggests that luminance information arrives earlier than the chromatic information to the SC. However, the target discrimination time was different between SUA and LFP. SUA discriminated the target from distractors at almost the same time in condition 1 (103±38ms) and 2 (103±35ms) and much later in condition 3 (118±44ms). In contrast, LFPs showed a significant difference in discrimination time across all three conditions (condition 1: 120±37ms, 2: 104±32ms, 3: 130±32ms), which paralleled the SRTs (condition 1: 160±10ms, 2: 145±10ms, 3: 165±12ms). The results suggest that while the SUA discrimination time represents the visual selection, the LFP discrimination time might be more related to the integrated selection signals from neighboring neurons which triggers the saccadic motor command.

Disclosures: **T. Ikeda:** None. **B.J. White:** None. **D.P. Munoz:** None.

Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.05. Visual Sensory-motor Processing

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T32NS05820-07

Title: Sensory priming influences form-based perceptual decision-making and superior colliculus population activity

Authors: ***T. B. CRAPSE**, M. A. BASSO
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Abstract: Earlier studies uncovered decision-making activity in a number of sensorimotor areas including the superior colliculus (SC), a subcortical area important for eye movements and attentional allocation. Most of the studies in the monkey focus on spatial decision making tasks, eg., random dot motion discrimination with choice targets that remained fixed relative to motion direction. Here we asked whether decision-making activity in the SC extends to form-based decisions with non-spatially fixed choice target locations and whether the activity is affected by sensory priming, i.e. repetitive presentation of one stimulus. To address this, we trained a monkey on a Glass pattern (Glass, 1969) yes-no task in which the monkey had to answer whether or not it perceived structure in a briefly presented stimulus (800-1200 ms). We manipulated whether the stimulus contained structure by varying the coherence in the Glass pattern stimuli. We trained the monkey on 5 coherence levels: 100%, 51%, 26%, 13%, and 0%. If the monkey perceived no structure in the display, it made a saccade to a red peripheral target. If the monkey perceived structure, it made a saccade to a green peripheral target. The location of the choice targets varied randomly on each trial. Once trained, we introduced blocks of noise priming trials. For these sessions, we first collected ~200 trials of 'baseline' data during performance of the Yes/No task. This block was followed by a noise priming block consisting of 200 Yes/No trials in which the coherence was either 0% or 100%. Seventy-five percent of the

trials were 0% coherence. This block was followed by a final block of trials to assess the effects of the sensory priming. We found that sensory priming produced a rightward shift and a change in slope of the psychometric function indicating a change in behavioral threshold and bias. Preliminary recordings from SC neurons reveal first, that neuronal activity scales directly with difficulty level; 0% coherence exhibited the highest degree of activity, followed by 13%, 26%, etc. Second, priming with a noise stimulus amplifies this scaling. Third, the variability of neuronal responses increases following sensory priming. Finally, we find that noise correlations increase among simultaneously recorded neurons following sensory priming, suggesting heightened communication among neighboring neurons. These results suggest that form-based decision making involves mechanisms distinct from spatial-based decision making mechanisms, and points to the role of neuronal pooling mechanisms as a means of implementing sensory priming.

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Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.06. Eye Movements

Support: NIH Grant EY014885

Title: Correlates of saccade target selection in simultaneously-recorded frontal eye field and superior colliculus local field potentials

Authors: *R. M. MCPEEK[1], S. RAY[2], J. PARK[1]
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Abstract: In contrast to single-unit recordings, local field potential (LFP) recordings incorporate potentials pooled over a larger area and are sensitive to dendritic synaptic activity. Saccade target selection in the superior colliculus (SC) has been studied extensively using single-unit recordings, but few studies have investigated the LFP correlates of selection in the SC. Moreover, although the SC and frontal eye field (FEF) communicate bidirectionally, to our knowledge no previous study has analyzed simultaneously recorded LFP activity from both areas. We trained macaques to perform a target selection task in which they were rewarded for

making a saccade to a color-oddball target presented with distractors, and we recorded LFPs simultaneously in the SC and FEF during the task. In agreement with earlier reports, we found that in both the SC (e.g., Ikeda et al. 2011) and FEF (e.g., Monosov et al. 2008; Purcell et al. 2013), LFPs recorded after stimulus onset in the target selection task initially did not discriminate the target from a distractor, but over time evolved to signal the target location. Overall, the magnitude of target discrimination tended to be higher in the SC than in the FEF, perhaps due to greater clustering of neurons with similar tuning and functional properties in the SC. In the frequency domain, target selection became evident in SC and FEF LFPs earliest through modest increases in power in the beta (at 25 Hz) and gamma (typically at 40 Hz in SC and 50 Hz in FEF) bands. The SC also showed a decrease in power in the alpha band (at 10 Hz), while an increase in power at this frequency was observed for some FEF sites. We also searched for evidence of phase synchrony (coherence) between simultaneously-recorded SC and FEF sites, since coherence is thought to be an important mechanism for gating communication between areas. We found that during the time of target selection, there was an increase in beta-band coherence between some SC and FEF sites with overlapping receptive fields. We conclude that robust correlates of target selection can be observed in LFPs in both SC and FEF, and that beta-band coherence may provide a mechanism for coordinating SC and FEF activity.

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Poster

238. Eye Movements: Cortex and Superior Colliculus

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Topic: D.06. Eye Movements

Support: DFG EXC 307

Title: Layer-specific influences of microsaccades on spatial vision in the primate superior colliculus

Authors: ***C.-Y. CHEN**[1,2], Z. M. HAFED[1]
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Abstract: Saccadic suppression refers to reduced visual sensitivity around saccades/microsaccades. The sources of saccadic suppression remain elusive. One component of

suppression could occur because of retinal response properties to rapid image motion. Another component is believed to arise extra-retinally through corollary discharge. Here we studied saccadic suppression in superior colliculus, SC, an area that receives both direct and indirect retinal input. We analyzed saccadic suppression across spatial frequency (SF) channels in the most superficial layers (putatively receiving direct retinal input) and compared it to deeper layers receiving visual input only indirectly. We recorded from 87 neurons of 2 monkeys fixating a white spot over a gray background. Inside a neuron's RF, a stationary, vertical Gabor grating (0.56, 1.11, 2.22, 4.44, or 11.11 cpd) appeared. We measured peak neuronal activity 30-150 ms after grating onset. We characterized superficial-layer visual (V) and intermediate-layer visual-motor (VM) neurons. Trials when gratings appeared >100 ms before or after microsaccades served as baseline. We compared these to trials with gratings appearing <50 ms after microsaccades, a period in which maximal saccadic suppression occurs. In separate behavioral sessions, we measured reaction times (RT) when monkeys made visually-guided saccades to the gratings. During initial fixation, we detected microsaccades in real-time and then presented the gratings at specific times (0 to 200 ms) after detection. This allowed us to get a time course of saccadic suppression influences on subsequent RT. We analyzed 32 V and 55 VM neurons (covering 1-24 deg). 85/87 neurons showed significant suppression in at least 1 SF. VM neurons were twice as strongly suppressed as V neurons. Moreover, there was a SF-dependent suppression for VM neurons: it was strongest in the lower SFs and weakened for higher ones. Suppression was constant across SF for V neurons. Behaviorally, suppression mirrored the VM neurons: RT increased strongly during maximal suppression (immediately after microsaccades) and gradually returned to baseline, but this effect disappeared for high SFs. Thus, superficial layer neurons receiving direct retinal input exhibited weak suppression that was not SF dependent. Deeper layer neurons showed strong suppression that weakened with higher SFs. Deeper layer neurons were more correlated with behavioral effects, and they were also consistent with human studies for large saccades. Such human results hypothesized that the SF dependence arises from magno-/parvocellular LGN segregation, which could explain the lack of it in superficial SC layers.

Disclosures: C. Chen: None. **Z.M. Hafed:** None.

Poster

239. Pain Behavioral Models

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Title: Inactivation of the prelimbic rather than infralimbic cortex impairs acquisition and expression of formalin-induced conditioned place avoidance

Authors: *J.-Y. WANG, Z.-C. JIANG, F. LUO
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Abstract: Conditioned place avoidance (CPA) paradigm has been used to investigate the affective component of pain. Although the anterior cingulate cortex (ACC) has been demonstrated to play an important role in the affective aspect of pain, whether the other prefrontal subdivisions are involved in pain-related aversion is unknown. The present study investigated the role of the prelimbic cortex (PL) and infralimbic cortex (IL) in the acquisition and expression of formalin-induced CPA (F-CPA) in rats. GABAA receptor agonist muscimol was bilaterally microinjected into PL/IL before or after the formalin-paired training, to explore the effect of temporary inactivation of PL/IL on the acquisition and expression of F-CPA, respectively. The results showed that inactivation of PL rather than IL impaired the acquisition and expression of F-CPA. Moreover, the PL inactivation did not block the acquisition of LiCl-induced CPA, suggesting that PL is specifically implicated in the coding of pain emotion. Inactivation of PL also disrupted the expression of LiCl-CPA, indicating that PL may be involved in a general memory retrieval function.. In summary, our study demonstrated that temporary inactivation of PL rather than IL blocked the acquisition and expression of F-CPA.The results revealed the critical role of PL in the pain-related aversion, and provided new evidence for the differential roles of PL and IL in pain emotion. Future studies should be designed to investigate the role of IL in the extinction of F-CPA, so as to better understand the involvement of the prefrontal cortex in emotional pain.

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Poster

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Support: KAKENHI (26460699)

Title: Enhanced pain behavior observed in mice lacking interleukin-27

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Abstract: Interleukin (IL)-27 is a member of the IL-12 cytokine family and has been shown to have an immunosuppressive and anti-inflammatory role. The immunosuppressive effect of IL-27 is thought to depend on inhibition of the development of Th 17 cells (a newly identified inflammatory T-helper population) and on induction of IL-10 production. On the other hand, one of recent developments in pain research is the finding that cytokines, including IL-1β, IL-6, TNFα, IL-17 and IL-10, have an important role in pain regulation. For example, IL-17 induces pain behavior, while IL-10 has an anti-nociceptive effect. Because of its potent ability to negatively regulate IL-17 (which is pro-nociceptive) and to promote the production of IL-10 (anti-nociceptive), we hypothesized that IL-27 could have a major impact in the control of pain and tested pain behavior of mice which carry a null mutation in IL-27 or IL-27 receptor gene(s). IL-27 is a heterodimer of EBI3 and p28, while the IL-27 receptor consists of a specific receptor WSX-1, together with gp130, which can also be coupled with other cytokine receptors, such as the IL-6 receptor. We used 3 different mouse strains, each lacking one of these genes (apart from gp130). These mice appeared healthy and had no obvious abnormality by visual inspection. We also observed that WSX-1 knockout mice were indistinguishable from wild type mice in the open-field test, indicating that null mutation of this gene would not affect locomotor activity (total distance traveled) or anxiety level (time spend in the center area of the field). Interestingly, we found that they exhibited enhanced responses in various assessments of pain behavior, including the hotplate, von Frey and formalin tests. Furthermore intraperitoneal injection of recombinant IL-27 (rIL-27) normalized the pain threshold in EBI3 but not WSX-1 knockout mice indicating that the normalized effect of rIL-27 was WSX-1-dependent, and that phenotypes observed in knockout mice did not result from irreversible developmental changes. These results suggest that IL-27 might contribute to regulating normal sensation of pain even in healthy conditions without inflammation.

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Poster

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Title: Chronic pain-related depression of behavior: Effects of intraplantar formalin and complete freund's adjuvant on intracranial self-stimulation (ICSS) and endogenous kappa opioid biomarkers in rats

Authors: *M. LEITL[1], D. N. POTTER[2], K. CHENG[3], K. C. RICE[3], W. A. CARLEZON JR[2], S. NEGUS[1]
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Abstract: Intraplantar injection of complete Freund's Adjuvant (CFA) and formalin are two common chemical challenges for induction of chronic pain states in rodents. CFA is a heat-killed bacterial suspension that elicits an immune response at the site of its injection. Formalin is an aqueous solution of formaldehyde, a cell toxin that cross links proteins to disrupt dynamic protein interactions critical to cell viability. Both stimuli elicit hypersensitive withdrawal responses that often serve as a behavioral indicator of "pain." However, clinically relevant pain states also often include depression of behavior and mood, and preclinical evaluation of pain-related behavioral depression is an emerging area of research. This study compared effects of intraplantar CFA and formalin on the behavior of intracranial self-stimulation in rats. Male Sprague-Dawley rats were equipped with intracranial electrodes in the medial forebrain bundle and trained to lever press for pulses of electrical brain stimulation delivered across a range of 10 frequencies (56-158 Hz). Prior to intraplantar treatment, brain stimulation maintained a frequency-dependent increase in ICSS rates. Rats then received bilateral intraplantar treatment with 100 µl CFA or formalin. Pain-related depression of ICSS was evaluated for its sensitivity to reversal by the mu opioid analgesic morphine. In addition, pain-related depression of ICSS was evaluated for its relationship to central biomarkers of the endogenous kappa opioid system and its sensitivity to the kappa antagonist norbinaltorphimine (norBNI), because some other stressors

depress behavior by activating central kappa systems. There were four main findings. First, in agreement with previous studies, both CFA and formalin produced similar degrees of paw swelling and mechanical hypersensitivity. Second, CFA produced only weak and transient depression of ICSS, whereas formalin produced a robust, concentration-dependent and sustained depression of ICSS that lasted as least 14 days. Third, formalin-induced depression of ICSS was reversed by morphine doses that had no significant effect on ICSS in saline-treated rats, suggesting that formalin effects on ICSS can be interpreted as an example of pain-related and analgesic-reversible depression of behavior. Finally, formalin-induced depression of ICSS was not associated with changes in central biomarkers for activation of endogenous kappa opioid systems and was not blocked by the norBNI. These results suggest that formalin-induced depression of ICSS may serve as a useful procedure for research on neurobiology and treatment of chronic pain-related depression of behavior.

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Poster

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Support: NIH Grant RO1NS070715

Title: Effects of nicotine on pain-stimulated and pain-depressed behavior in rats

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Abstract: Agonists at nicotinic acetylcholine receptors (nAChRs) constitute one drug class being evaluated as candidate analgesics for treatment of pain. Preclinical studies to evaluate antinociceptive effects of nicotine and other nAChR agonists have relied exclusively on assays of pain-stimulated behavior, which measure behaviors that increase in frequency, rate or intensity after presentation of a noxious and putatively painful stimulus. However, drug effects in assays of pain-stimulated behavior have poor predictive validity for clinical analgesia in humans. Novel assays of pain-depressed behavior measure behaviors that decrease in frequency, rate or intensity after presentation of a pain stimulus. Pain-depressed behaviors play a key role in pain

diagnosis in both human and veterinary medicine, and procedures that measure pain-depressed behaviors may improve translational validity in tests of candidate analgesics. However, the effects of nAChR agonists have not been examined in assays of pain-depressed behavior. Accordingly, our study was designed to compare effects of nicotine in assays of pain-stimulated and pain-depressed behavior in rats. IP injection of dilute lactic acid (1.8% in 1 ml/kg) served as an acute noxious stimulus to stimulate a stretching response or depress intracranial self-stimulation (ICSS) in adult male Sprague Dawley rats. For ICSS studies, rats were implanted with electrodes targeting the medial forebrain bundle at the level of the lateral hypothalamus, and they were trained to respond under a fixed-ratio 1 schedule for a range of brain stimulation frequencies (158-56 Hz) during daily experimental sessions. Pretreatment with nicotine (0.032-1.0 mg/kg) produced a dose-dependent blockade of both acid-stimulated stretching and acid-induced depression of ICSS. Nicotine antinociception was blocked in both procedures by the nonselective nAChR antagonist mecamylamine (1.0 mg/kg). In a separate study, nicotine was also evaluated for its effects on chronic pain-related depression of ICSS induced by bilateral intraplantar injection of 5% formalin or saline (100 µl per paw). Seven days after intraplantar treatment, ICSS was depressed in the formalin-treated rats in comparison to the saline controls, and nicotine (0.01-0.32 mg/kg) produced a dose-dependent reversal of formalin-induced depression of ICSS. Doses of nicotine that blocked acid-induced depression of ICSS or reversed formalin-induced depression of ICSS produced little or no change in ICSS in the absence of the noxious stimulus. These results support a role for nicotine acting at nAChRs to alleviate pain-related depression of behavior in rats.

Disclosures: **K. Freitas:** None. **S.S. Negus:** None.

Poster

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R01 NS070715

Title: Morphine antinociception is resistant to tolerance in an assay of pain-depressed intracranial self-stimulation

Authors: *A. ALTARIFI[1], S. NEGUS[2]
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Abstract: Mu opioid receptor agonists (such as morphine) are commonly prescribed for management of acute and chronic pain. Although analgesic tolerance can be an obstacle to clinical effectiveness of mu agonists, many chronic pain patients can be maintained for long periods on stable opioid doses, suggesting that analgesic tolerance is often modest or absent. In preclinical studies, we showed previously that acute administration of morphine or other mu agonists produced antinociception in procedures in which IP injection of dilute lactic acid served as a noxious stimulus either to stimulate a stretching response or to depress intracranial self-stimulation (ICSS) in rats. The present study compared the development of tolerance to morphine antinociception for these two distinct pain-related endpoints. Test groups received daily injections of either saline (Chronic Saline) or escalating morphine doses to a terminal maintenance dose of 10 mg/kg/day (Chronic Morphine). Afterward, subjects from both groups were tested with a sequence of four treatments: (1) morphine vehicle + acid vehicle, (2) morphine vehicle + 1.8% lactic acid, (3) 1.0 mg/kg morphine + acid vehicle, or (4) 1.0 mg/kg morphine + 1.8% lactic acid. Morphine or its vehicle was administered 30 min before acid or its vehicle, and treatment order was randomized in a Latin-square design across animals. Separate groups of rats (N=6-7 per group) were used to assess morphine effects on acid-stimulated stretching and acid-induced depression of ICSS. In the stretching procedure, acid alone increased stretching, and acid effects were greater in the Chronic Morphine group. Morphine blocked acid-stimulated stretching in the Chronic Saline group but not in the Chronic Morphine group. In the assay of acid-depressed ICSS, rats equipped with electrodes targeting the medial forebrain bundle were trained to respond under a fixed-ratio 1 schedule for 0.5 sec trains of electrical stimulation (56-158 Hz in 0.05 log increments during each 30-min session). Acid alone depressed ICSS, and acid effects were again greater in the Chronic Morphine group. However, morphine blocked acid-induced depression of ICSS in both the Chronic Saline and Chronic Morphine groups. Morphine alone produced a small but significant increase in ICSS in the Chronic Morphine group only. In summary, tolerance developed to morphine antinociception in the assay of acid-stimulated stretching but not in the assay of acid-induced depression of ICSS. These data suggest that tolerance develops at different rates to different measures of morphine antinociception in rats, and morphine antinociception is resistant to tolerance in an assay of pain-depressed behavior.

Disclosures: **A. Altarifi:** None. **S. Negus:** None.

Poster

239. Pain Behavioral Models

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Topic: D.08. Pain

Title: A rat model for sciatic endometriosis

Authors: S. CHEN[1,2], W. XIE[2], J. A. STRONG[2], J. JIANG[1], *J.-M. ZHANG[2]
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Abstract: Aim of Investigation: Clinical case reports show that endometriosis of the sciatic nerve can cause mild to severe chronic leg pain, often fluctuating with the menstrual cycle. In the current study, we aimed to develop an animal model of endometriosis of the sciatic nerve for the study of its underlying mechanisms. Methods: A model of endometriosis of sciatic nerve was established in female rats by surgically implanting autologous uterine tissue adjacent to the sciatic nerve at the mid-thigh level. In some experiments the implanted tissue was removed on postoperative day (POD) 21. Estrous cycles were tested everyday for 14 days. Mechanical sensitivity of the hindpaws was assessed with von Frey filaments, and mechanical allodynia was scored by responses to a light brush stroke cross the ventral surface of the hindpaws. The ectopic tissue and adjacent ipsilateral sciatic nerve were harvested for H&E staining. Results: The sciatic nerve endometriosis model was successfully established in rats, regardless of which phase of the estrous cycle rats were in at the time of surgery. Pain behaviors were first evident on POD 5-7, and lasted approximately 10 weeks, peaking between week 3 and week 4. Pain behavior showed no correlation with the estrous cycle. In rats with the implanted tissue remove on POD 21, the mechanical hypersensitivity gradually returned to the baseline. H&E staining of implanted tissue and adjacent sciatic nerve displayed signs of robust inflammatory responses and tissue damage. Conclusions: In humans, endometriosis of sciatic nerve can cause chronic leg pain, which can be reversed by a resection of ectopic tissue. This study suggests that one possible mechanism is local inflammation at the site of lesion caused by the ectopic tissues. Further studies will focus on the ectopic activity of the affected sciatic nerve and other possible causes. This is the first report of an animal model of endometriosis of sciatic nerve.

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Poster

239. Pain Behavioral Models

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Topic: D.08. Pain

Title: Analysis of sleep disorders under pain using an optogenetic tool: possible involvement of the activation of dorsal raphe nucleus-serotonergic neurons

Authors: *H. ITO[1], M. YANASE[2], A. YAMASHITA[2], C. KITABATAKE[2], A. HAMADA[2], Y. SUHARA[2], M. NARITA[2], D. IKEGAMI[2], H. SAKAI[2], M. YAMAZAKI[1], M. NARITA[2]
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Abstract: Introduction: Several etiological reports have shown that chronic pain significantly interferes with sleep. Inadequate sleep due to chronic pain may contribute to the stressful negative consequences of living with pain. However, the neurophysiological mechanism by which chronic pain affects sleep-arousal patterns is as yet unknown. Although serotonin was proposed to be responsible for sleep regulation, whether the activity of serotonergic neurons in the dorsal raphe nucleus (DRN) is affected by chronic pain has been studied only infrequently. On the other hand, the recent development of optogenetical tools has provided a valuable opportunity to regulate the activity in genetically targeted neural populations with high spatial and temporal precision. In the present study, we investigated whether chronic pain could induce sleep disorders while changing the activity of DRN-serotonergic neurons. Furthermore, we sought to physiologically activate the DRN-serotonergic neurons with channelrhodopsin-2 to identify a causal role for the DRN-serotonin system in promoting and maintaining wakefulness using optogenetics. Methodology: We produced a sciatic nerve ligation model by tying a tight ligature around the partial sciatic nerve. In mice with nerve ligation, we investigated a sleep patterns monitored by electroencephalogram for 24 hr. To evaluate the effect of neuropathic pain on DRN-serotonergic neurons, we performed an *in vivo* microdialysis study. Using optogenetic tools, we investigated a causal relationship among DRN-serotonergic neurons firing and sleep/wake condition in freely moving mice. Results: We found an increase in wakefulness and a decrease in non-REM sleep monitored by electroencephalogram in nerve-ligated mice. In an *in vivo* microdialysis study, extracellular serotonin levels released in the prefrontal cortex by the electrical stimulation of DRN were significantly increased in nerve-ligated mice. The optogenetical activation of DRN-serotonergic neurons produced a significant increase in wakefulness and a decrease in non-REM sleep. Furthermore, the duration of non-REM sleep episode was significantly decreased during optical stimulation. Conclusions: These results suggest that neuropathic pain accelerates the activity of DRN-5-serotonergic neurons. Although further loss-of-function experiments are required, we hypothesize that this activation in DRN neurons may, at least in part, correlate with sleep disorders under a neuropathic pain-like state.

Disclosures: **H. Ito:** A. Employment/Salary (full or part-time):; Toyama University Hosipital. **M. Yanase:** None. **A. Yamashita:** None. **C. Kitabatake:** None. **A. Hamada:** None. **Y. Suhara:** None. **M. Narita:** None. **D. Ikegami:** A. Employment/Salary (full or part-time):; Hoshi University School. **H. Sakai:** A. Employment/Salary (full or part-time):; Hoshi University School. **M. Yamazaki:** A. Employment/Salary (full or part-time):; University of Toyama. **M. Narita:** A. Employment/Salary (full or part-time):; Hoshi University School.

Poster

239. Pain Behavioral Models

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Topic: D.08. Pain

Support: NIH Grant NS085510

Title: Further characterization of a rodent headache model with video analysis and EEG recording

Authors: A. MELO-CARILLO[1], C. ALEXANDRE[2], A. LOPEZ-AVILA[3], R. BURSTEIN[1], *A. M. STRASSMAN[1]
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Abstract: It has been argued that there is a need for improved behavioral models of ongoing pain, as opposed to stimulus-evoked hypersensitivity, for better modeling of clinical conditions and more effective testing of new therapies. In a new behavioral model of ongoing headache pain, Melo-Carrillo and Lopez-Avila found that application of inflammatory mediators to the cranial dura in rats produced a decrease in total exploratory behavior and a concomitant increase in resting behavior, during a 45-min. observation period, compared to saline control animals (Cephalalgia 33:1096, 2013). No difference was found in total time spent body grooming. The present study pursued these observations further by investigating possible differences in the pattern of body grooming, and by using EEG recording to characterize possible differences in behavioral state during the resting periods. Following a 7-day pre-surgery habituation period, male Wistar rats (250-300g) maintained on an inverted light-dark cycle were chronically implanted with a cannula sterotaxically positioned over a 1-mm diameter craniotomy in the frontal bone, as well as EEG electrodes. After a 2-day recovery, 2ul of a mixture of

inflammatory mediators (histamine, serotonin, bradykinin 1mM, and prostaglandin E2 0.1mM) was delivered to the dura through the cannula. Control rats received saline. Video and EEG recordings were made for 15 min. before and 45 min. after the dural infusion. Two differences were found in the stimulated rats: 1) a novel pattern of body grooming, not observed in control rats, in which episodes of face grooming were interspersed within the normal rostral-to-caudal grooming sequence (face-trunk-genitals-tail); and 2) a relative absence of slow-wave activity in the EEG during the resting periods, in contrast to the increased slow-wave activity present during resting periods in control animals. These findings indicate the induction of novel behaviors, not present in control animals, following dural stimulation. Such behaviors may serve as sensitive signs of ongoing headache pain.

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Poster

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Support: NINDS Grant NS072143

Title: Brain neuroplastic changes accompany anxiety and memory deficits in a model of complex regional pain syndrome

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Abstract: Complex regional pain syndrome (CRPS) is a painful and often chronic condition with an incidence of about 50,000 cases in the US each year. It is a major cause of work-related disability, chronic pain after limb fractures and persistent pain after extremity surgery. In addition to pain, CRPS patients often experience cognitive changes, anxiety and depression. The supraspinal mechanisms linked to these CRPS-related comorbidities are not well understood. The present study was carried out with a well characterized mouse model of tibia fracture/cast

immobilization showing the principal stigmata of CRPS observed in humans. Our central hypothesis was that fracture/cast mice manifest changes in measures of nociception, working memory and anxiety attributable to neuroplastic changes in amygdala, perirhinal cortex, and hippocampus. We demonstrate that nociceptive sensitization in these mice is accompanied by altered thigmotactic behaviors in the zero maze but not open field assay, and working memory dysfunction in novel object recognition and social memory but not in novel object location recognition. Furthermore, we found evidence of structural changes and synaptic plasticity including changes in dendritic architecture and decreased levels of synaptophysin and brain derived neurotrophic factor (BDNF) in specific brain regions. Therefore, the present findings provide novel observations regarding behavioral changes and brain plasticity in a mouse model of CRPS. In addition to elucidating some of the supraspinal correlates of the syndrome, this work supports the potential use of therapeutic interventions that not only directly target sensory input and other peripheral mechanisms, but also attempt to ameliorate the broader pain experience by modifying its associated cognitive and emotional comorbidities.

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Poster

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Title: The Coy Operant Conflict System: Adaptations allow testing of mechanical and cold sensitivity in mice with a spinal nerve ligation (Chung model)

Authors: *S. M. CARLTON, S. ZHOU
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Abstract: Pain perception involves sensory-discriminative, motivation-affective and cognitive components. To incorporate these into animal testing, complex behaviors involving supraspinal

processing are needed. We are modifying the Coy operant conflict system for rats (Coy Labs), to test mechanical and cold sensitivity in mice. The system has 3 consecutive chambers: a light (aversive), middle (stimulus) and dark (safe) chamber. Dependent measures are latency to exit (LTE) chamber 1 (emphasizing cognitive/motivation/affective components of pain) and duration (DUR) in chamber 2 (emphasizing sensory/discriminative components of pain). C57BL6 mice were made neuropathic using the Chung model which involves a tight ligation of the L5 and L6 spinal nerves (sham animals receive surgery but no nerve ligation). To test mechanical sensitivity, rough (grit 36) sandpaper was placed on the floor of chamber 2. To test cold sensitivity, an aluminum plate was cooled to 0°C and held in place under chamber 2 floor by a lab jack. This cooled chamber 2 to 17-18°C for about 10 min, but both end chambers remained at room temperature. Mice were acclimated and trained in the Coy operant conflict system for 5 days prior to nerve ligation surgery. During training to test mechanical sensitivity, the rough sandpaper was in place in chamber 2. During training to test cold sensitivity, chamber 2 was at 17-18°C. Following training, the baseline LTE and DUR were obtained for each mouse prior to surgery. Then, at 1 and 2 weeks post-surgery, mice were retested for changes in mechanical and cold sensitivity. For mechanical sensitivity, SNL mice (n = 6) had an increased LTE compared to sham (n = 5) at 1 and 2 weeks post-surgery, however, the difference was not significant. At 1 week, SNL mice spent considerably less time in the middle chamber compared to sham but this difference was not present at 2 weeks. For cold sensitivity, SNL mice (n = 10) showed no difference in LTE compared to sham (n = 9) at 1 week but showed a significant increase in LTE at 2 weeks ($p<0.05$), hesitating to leave the light chamber and enter the middle chamber when the floor was at 17-18°C. However, DUR in the middle chamber was not affected by SNL as these mice showed the same DUR as sham mice at 1 and 2 weeks post-surgery.These data demonstrate that the Coy operant conflict system can be adapted for testing mechanical and cold hypersensitivity in SNL mice. It appears that LTE is a more consistent measure than DUR in this nerve injury model, however, it is clear that an increase in Ns in the groups tested for mechanical sensitivity are also needed.

Disclosures: **S.M. Carlton:** None. **S. Zhou:** None.

Poster

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Title: Mechanisms of 16, 16 dimethyl prostaglandin (dmPGE2) in the sensitization of capsaicin induced mechanical allodynia in male Sprague Dawley rats (a model of hyperalgesic priming)

Authors: *S. M. JAFFAL
McGill Univ., Montreal, QC, Canada

Abstract: Inflammatory pain is one of the major health problems that impose burdens to society. Although acute inflammatory processes are short lived and contribute to the healing process, chronic inflammation is an unhealthy over-response that can be detected by enhanced responses to hyperalgesia and/or allodynia. Despite of the advances in pain researches, the mechanisms underlying the development of chronic pain are not fully determined. In this study, I used a model (hyperalgesic priming) for the transition from acute to chronic pain. The model involves injection of an inflammatory insult to sensitize nociceptors to a second inflammatory mediator. Specifically, I examined the effect of intraplantar injection of 5μM dmPGE2 (an analog of PGE2) on the mechanical allodynia that is induced by injection of 50μg capsaicin (TRPV1 agonist) in male Sprague Dawley rats. In all rats, the injections were intraplantar and ipsilateral and the mechanical allodynia was measured by von Frey anesthesiometer. The results show that dmPGE2 pre-injection aggravated and prolonged capsaicin induced mechanical allodynia to one week. It also enhanced the nocifensive and guarding behaviours compared to capsaicin injected animals. In addition, I found that the mechanism of dmPGE2 in the aggravation and the prolongation of capsaicin induced mechanical allodynia includes the novel PKC isoform (PKCε) and cAMP (in the initial phase of capsaicin induced mechanical allodynia), PKA (in the prolonged phase), PLC and two mitogen activated protein kinases (MAPKs): p38 and JNK pathways (in the initial and the prolonged phases). No significant effect was found for ERK-MAPK in dmPGE2-induced effect. Then, to characterize which receptor (among the 4 EP receptors of PGE2) is involved in dmPGE2 mediated responses, I injected the animals with different agonists of EP receptors followed by capsaicin injection and measurement of mechanical allodynia. I found that only EP1 agonist (17PGE2, 50μM) significantly aggravated and prolonged capsaicin effects to 5 days in a PKC dependent manner. The difference between the effect of dmPGE2 (7 days) and 17PGE2 (5 days) in the prolongation effect and the involvement of cAMP-PKA pathway in dmPGE2 mediated responses indicates that there could be a partial involvement of other subtypes of EP receptors. Also, the strong inhibition of 17PGE2 mediated effects by the general PKC inhibitor suggests the possibility of recruiting other isoforms of PKC to EP1 signalling mechanisms. Accordingly, continuing this research and identifying the exact mechanisms downstream EP receptors in TRPV1 sensitization can have therapeutic value for pain treatment in the future.

Disclosures: **S.M. Jaffal:** Other; To best of my knowledge, there is no conflict of interest between authors regarding the results of the *in vivo* work. There is for the submission issue.

Poster

239. Pain Behavioral Models

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Title: Differential components relating to pain expectation were isolated after pain-related learning in a rodent model

Authors: ***N. WANG**, S.-G. LI, J.-Y. WANG, F. LUO
Inst. of Psychology, Chinese Acad. of Sciences; Key Lab. of Mental H, Beijing, China

Abstract: Previous studies have tried to separate the human brain responses associated with expectation of pain from those associated with direct experience of pain. Although there are many human studies focus on models of pain expectation, we still need animal models to explore the neuronal mechanisms. Our aim was to establish a rodent model to assess the cue-based expectancy and its effects on nociceptive behaviors. A novel balanced crossover design that commonly used in human disciplines was utilized to address these issues. On the basis of tone-laser paired trace conditioning paradigm, a series of tasks were conducted from simple conditioning to more difficult signal discrimination task in rats. We successfully isolated four component relating to pain expectation, including expectancy-based pain, expectancy of pain, pain, and normal state. Significant voluntary withdrawal behaviors in response to pain predictive

cues were brought about after a series of pain-related conditioning task with increasing cognitive load. Moreover, the presentation of pain cue followed by sham stimuli caused even stronger responses than those by no-pain cue followed by actual stimuli. The present findings suggest that the expectation per se could produce robust nocifensive behaviors that were comparable in magnitude to the responses elicited by actual noxious stimuli in normal animals. The current study may offer a new model to explore the neurophysiological and neurochemical mechanisms at molecular level underlying the pain expectation as well as the reciprocal modulatory effects between pain and expectation.

Disclosures: **N. Wang:** None. **S. Li:** None. **J. Wang:** None. **F. Luo:** None.

Poster

239. Pain Behavioral Models

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Program#/Poster#: 239.13/BB8

Topic: D.08. Pain

Title: Effect of tapentadol on neuropathic pain in arthritis models using automated and manual methods

Authors: V. GOURA, A. VUYYURU, R. ABRAHAM, *P. JAYARAJAN, R. NIROGI
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Abstract: Tapentadol is a new class of drug that possesses analgesic action, which results from contribution of μ-opioid receptor agonism and norepinephrine reuptake inhibition. It has been approved by US FDA for moderate to severe chronic pain and neuropathic pain associated with diabetic peripheral neuropathy. Pain and disability are the primary symptoms of arthritis. There is paucity in preclinical evaluation for Tapentadol on neuropathic pain in arthritis models. Hence we evaluated the effect of Tapentadol on Sodium Mono Iodoacetate (MIA) and Complete Freunds Adjuvant (CFA) induced osteoarthritis and rheumatoid arthritis respectively. We evaluated neuropathic pain conditions like hyperalgesia and allodynia using manual (von Frey monofilaments, Randal Selitto analgesymeter) and automated (Dynamic plantar aesthesiometer) methods. We observed significant efficacy of Tapentadol in both the manual and automated method of assessment in both osteoarthritis and rheumatoid arthritis pain models. The efficacy of tapentadol is in agreement with the clinical analgesic data of tapentadol in osteoarthritis induced pain. Similarly, the analgesic effect of tapentadol in rheumatoid arthritis induced pain in rats may be a viable option for clinical studies.

Disclosures: **V. Goura:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **A. Vuyyuru:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **R. Abraham:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD.

Poster

239. Pain Behavioral Models

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Topic: D.08. Pain

Support: JSPS KAKENHI 24792247 (N.H)

JSPS KAKENHI 25861760 (S.H)

Title: Effects of 5-fluorouracil on stomatitis-induced pain in rats

Authors: K. YAMAGUCHI[1], *N. HARANO[3], S. HITOMI[2], K. ONO[2], T. SAGO[1], S. WATANABE[1], K. INENAGA[2]
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Abstract: Stomatitis is frequently developed as a side effect of anti-cancer therapy in head and neck cancer patients and induces severe pain during eating and speaking. However, there are no effective treatments for stomatitis-induced pain. In this study, to examine relationship of anti-cancer drugs with stomatitis, we investigated effects of the representative anti-cancer drug 5-fluorouracil (5-FU) on stomatitis-induced pain in rats. We intraperitoneally (i.p.) administered 5-FU (40mg/ml/kg) or saline as control at three times in male Wistar rat (7-8 weeks old). To make stomatitis, rats were treated in the oral mucosa of the mandibular vestibule with a filter paper (9 mm^2), which was soaked into 50% acetic acid, for 30 seconds under anesthesia (pentobarbital 50mg/kg, i.p.). In the stomatitis model with and without pre-administration of 5-FU, a facial rubbing behavior that is a sign of orofacial pain was measured for 3 min following direct application of capsaicin solution in the oral mucosa by using the dropping method that we have recently developed for intraoral pain assessment in conscious rats. 5-FU administration decreased remarkably food consumption and body weight, compared with control group. Stomatitis with ulcer was observed in both groups on day 2 after acetic acid treatment. Until day

5, although stomatitis was visibly disappeared in the control group, it was still remained in the 5-FU group. Facial rubbing behavior following capsaicin application showed same level in both groups before making stomatitis and on day 2. However, the behavior was significantly increased in 5-FU group on day 5, compared with control group. From these results, 5-FU administration leads slow healing process of stomatitis and consequently induces long-lasting stomatitis-induced pain.

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Poster

239. Pain Behavioral Models

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Topic: D.08. Pain

Title: Electrolytic lesion of the infralimbic cortex attenuates distraction analgesia in rats

Authors: *C. T. MCNABB[1], M. M. WHITE[1], C. A. SALCIDO[1], P. N. FUCHS[2]
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Abstract: Distraction has been shown to reduce pain in clinical and experimental settings, but the underlying neurobiology of distraction's analgesic effect is not fully understood. In humans, the presentation of competing attentional stimuli (i.e. pain and cognitive tasks) has been associated with activation of the orbitofrontal cortex (OFC). There is evidence that the human OFC is functionally homologous to the infralimbic (IL) region of the medial prefrontal cortex in rats; however, the IL has never been selectively investigated in animal research of distraction analgesia. Therefore, this study utilized stereotaxic electrolytic lesions of the IL to elucidate the region's role in a rat model of distraction analgesia. One hundred eleven male Sprague Dawley rats between 7-9 months old were randomly assigned to receive either a bilateral electrolytic lesion to the IL or a sham surgical procedure. Rats were allowed one week to recover and were then habituated to an empty testing chamber for 10 minutes once a day for 7 days. On the eighth day, rats underwent a formalin test in the same chamber. Each rat was randomly assigned to receive a subcutaneous injection of .01 ml of either 1% or .5% formalin into the plantar surface of the left hindpaw. Each rat was immediately placed in the testing chamber, which was randomly assigned to either remain empty or to contain an upside down falcon tube to serve as a distracting object during the test. An observer quantified the amount of time spent with the paw

down, up, and licking/biting the paw in order to generate formalin pain scores organized into 5-minute time bins. Animal movement patterns were also recorded with behavioral tracking software. Repeated measures ANOVA of the formalin pain scores unexpectedly showed a main effect for lesion, $F(1, 103) = 5.27$, $p < .05$, as well as a lesion by distractor interaction, $F(1, 103) = 4.39$, $p < .05$. Posthoc comparisons revealed that the IL lesion was associated with significantly lower pain scores than the sham condition in the empty chamber, $p < .01$, indicating that the IL lesion reduced formalin pain. Additionally, there was a significant difference in formalin pain scores between empty and distractor conditions in the sham animals, $p < .05$, but not in the IL animals, $p = .45$, ns. These results demonstrate that the distractor had an analgesic effect on the sham animals, but that the distraction effect was absent in the IL animals. We conclude that the bilateral electrolytic IL lesion attenuated distraction analgesia and that the infralimbic cortex is a critical mediator of distraction analgesia in rats.

Disclosures: C.T. McNabb: None. **M.M. White:** None. **C.A. Salcido:** None. **P.N. Fuchs:** None.

Poster

239. Pain Behavioral Models

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Topic: D.08. Pain

Title: Assessing the aversive nature of pain with an operant approach/avoidance paradigm

Authors: *C. A. SALCIDO, A. L. HARRIS, C. T. MCNABB, M. M. WHITE, P. N. FUCHS
Psychology, Univ. of Texas at Arlington, Arlington, TX

Abstract: Preclinical assessments of pain have been criticized for failing to adequately characterize the complexity of the clinical pain experience in humans. Recently developed preclinical pain assessments such as the Place Escape/Avoidance Paradigm (PEAP) have improved upon this shortcoming by quantifying the aversive component of pain more directly. Nevertheless, the PEAP is not designed to evaluate the impact of competing homeostatic drives, such as hunger, which may influence pain-related behavior. Therefore, we developed an approach-avoidance paradigm that measures the aversiveness of pain by allowing a rat to either satisfy hunger or avoid noxious stimulation. Twenty-six male Sprague Dawley rats were placed on a food-controlled diet and trained to lever-press for appetitive rewards in an operant chamber. On test day, animals were randomly assigned a subcutaneous injection into the plantar surface of

the hindpaw of either 1% carrageenan or normal saline. Animals were then placed in a modified operant chamber sitting atop a mesh platform, and a lever was presented for 10 seconds at 30-second intervals for a total of 30 minutes. During the test, lever presses were immediately followed by stimulation of the injected paw with a suprathreshold Von Frey filament. This presented the rat with an approach-avoidance conflict in which it had the opportunity to receive noxious mechanical stimulation to the injected paw in order to obtain appetitive reward or to avoid noxious stimulation and forego appetitive reward. Two separate one-way ANOVAs showed significant differences between carrageenan and saline groups in mean latency to lever-press, $F(1, 24) = 10.10$, $p < .01$, as well as the percentage of trials yielding lever-presses, $F(1, 24) = 7.02$, $p < .05$, respectively. The results of this study indicated that the noxious stimulation of carrageenan-induced inflammation resulted in the suppression of reward-seeking behavior and suggested that the motivation to avoid pain superseded the motivation to alleviate hunger. Future research should be conducted to further validate the paradigm and to investigate the neural mechanisms underlying approach-avoidance conflicts that are often present in pain patients.

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Poster

239. Pain Behavioral Models

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Topic: D.08. Pain

Title: Clinically relevant endpoints in a recurrent nitroglycerin migraine model in rats

Authors: *K. J. SUFKA[1], S. M. STASZKO[2], A. P. JOHNSON[2], M. E. DAVIS[2], R. E. DAVIS[2], T. A. SMITHERMAN[2]
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Abstract: Diagnostic criteria of migraine include a minimum of five recurrent episodes of severe unilateral head pain with sensitivity to light and sound, exacerbation by activity, and nausea/vomiting, among other signs; chronic migraineurs often show co-morbid stress-related disorders. Rodent models have induced migraine via nitroglycerin (NTG) and subsequently measured allodynia and/or hyperalgesia, but neither of these endpoints are diagnostic criteria. In a series of studies, we show in male Sprague-Dawley rats that, in contrast to a single NTG (10 mg/kg/2ml IP) administration, both 3 and 5 NTG injections over a 2 week period produce

decreased time in the light portion of and decreased photo-beam breaks (hypoactivity) in a light-dark (L/D) box compared to vehicle controls 2 hrs post final NTG administration. Patterns of weight change over this period were indicative of nausea and appetite loss. Interestingly, 1, 3, and 5 NTG injections did show modest changes in the rat grimace scale but did not affect responses on thermal allodynia measures; 3 and 5 NTG injections did not alter measures of anxiety (EPM) or depression (FST) 24 hrs post final NTG episode. Finally, we show that 0.3 and 1.0 mg/kg sumatriptan given IP 30 min after NTG produces a dose-dependent attenuation of both migraine hypo-activity and loss of body weight; time in light portion of L/D box under sumatriptan was in predicted direction but not significant. Collectively, the 3 or 5 NTG dosing migraine model with light-dark box measures of light sensitivity and activity and body weight changes may reflect a more clinically relevant episodic migraine simulation than existing rodent models.

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Poster

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Title: RGS4 modulates sensory and affective components of chronic pain states

Authors: *F. CARR[1], S. GASPARI[1], M. STRATINAKI[2], V. MITSI[1], V. ZACHARIOU[1]
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Abstract: Regulator of G protein signalling 4 (RGS4), an intracellular modulator of several monoamine and opioid receptors, is expressed at high levels in pain-associated CNS regions, including the dorsal horn and medial prefrontal cortex (mPFC). We have previously demonstrated a role for RGS4 in modulating analgesic properties of opiates in acute pain states, and efficacy of tricyclic antidepressants in chronic neuropathic pain. In the present study we investigated the contribution of RGS4 to both sensory and affective components of chronic inflammatory and neuropathic pain states. We used global RGS4 knockout mice and targeted

depletion using viral mediated gene transfer to examine the contribution of RGS4 to several pain models. The formalin test was used as a measure of sub-chronic pain, intraplantar injection of Complete Freund's adjuvant was used as a model of chronic inflammatory pain, and spared nerve injury (SNI) was used as a model of neuropathic pain. Several behavioural paradigms were then used to assess sensory symptoms (nociceptive licking behaviour in the formalin test, and mechanical allodynia and thermal hyperalgesia in the CFA and SNI models) and affective components (anxiety- and depression-like behaviours) in these pain models. Global RGS4 knockout mice displayed reduced nociceptive behaviours in the second, but not the first, phase of the formalin test. Although thermal nociception in the CFA model was unaffected by genotype, mechanical allodynia was significantly attenuated in RGS4 KO mice. RGS4 KO mice spent less time immobile in the forced swim test, and consumed more sucrose in the sucrose preference test at 3 weeks post CFA injection, indicating a reduction in depression-like behaviours associated with the inflammatory pain state. Analgesic efficacy of the delta opiate agonist SNC80 (5mg/kg, s.c.) was also increased in the RGS4 KO group, at 24h post CFA. In contrast to the pro-nociceptive role of RSG4 identified in inflammatory pain models, targeted depletion of RGS4 in the medial prefrontal cortex (mPFC) did not alter mechanical allodynia in neuropathic pain. In addition, mPFC specific depletion increased depression-like behaviours in the sucrose preference and social interaction tests, at 6 and 10 weeks post SNI. In conclusion, we have identified a complex role for RGS4 in modulating the sensory and affective dimensions of inflammatory and neuropathic pain.

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Poster

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JSPS KAKENHI 25861760 (S.H.)

Title: Analgesic effect of Hangeshashinto, a traditional Japanese medicine, on oral ulcer-induced intraoral pain in rats

Authors: *S. HITOMI[1], K. ONO[1], Y. OOMIYA[2], K. TERAWAKI[2], A. KANEKO[2], Y. UEZONO[3], K. INENAGA[1]
[1]Kyushu Dent. Univ., Fukuoka, Japan; [2]Tsumura & Co., Ibaraki, Japan; [3]Natl. Cancer Ctr. Res. Inst., Tokyo, Japan

Abstract: It is well known that oral mucosal pain in head and neck cancer patients treated with chemo-radiotherapy is persistent and intractable, resulting under-nutrition and low quality of life. Recently, it has clinically reported that Japanese herbal medicine Hangeshashinto is effective on the oral mucosal pain. However, mechanism of the analgesic effect has not well known. In this study, we investigated the oral ulcer-induced mucosal pain and efficacy of Hangeshashinto to the pain in rats using new behavioral technique to keep mouth open for direct stimulations. Treatment with acetic acid in the labial fornix region of the inferior incisors developed obvious oral ulcer, showing severe infiltration of inflammatory cells and epidermolysis in histology. Application of Hangeshashinto to oral ulcer region was not any changed the pain-related grooming behavior, suggesting Hangeshashinto does not have pungent effects on intraoral region. Head withdrawal threshold to mechanical stimulation to the oral mucosa was significantly decreased in oral ulcer compared to sham. The decrement of mechanical threshold was recovered to naive level from 30 minutes to 3 hours after topical application of Hangeshashinto to the oral ulcer region. From these results, Hangeshashinto recovered oral ulcer-induced mechanical allodynia long-lastingly.

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Poster

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Topic: D.08. Pain

Support: UAB Comprehensive Neuroscience Center

Title: Chronic, mild stress prior to spinal cord injury increases post-injury allodynia in male Sprague Dawley rats

Authors: *A. MOHAIMANY-APONTE[1], M. T. ROBBINS[2], C. L. FLOYD[3]
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Abstract: Spinal cord injuries (SCI) affect 12,000 persons annually in the United States, and over 320,000 persons are currently living with chronic SCI. In addition to loss of motor and sensory function, SCI induces neuropathic pain in up to 66% of SCI patients. Neuropathic pain can be constant or episodic, and is a main contributor to lower quality of life. Although the underlying mechanisms and modulators of neuropathic pain are poorly understood, inflammation is hypothesized to contribute to the development and maintenance of neuropathic pain. Moreover, stressors have been shown to cause an inflammatory response and increase hypothalamic pituitary axis sensitivity; however, no studies have investigated the effects of chronic, mild stress (CMS) on subsequent development of neuropathic pain after SCI. Thus, the goal of this study was to evaluate the hypothesis that CMS prior to SCI may increase the incidence or severity of neuropathic pain in a clinically-relevant model of cervical SCI in adult male Sprague Dawley rats. Rats were divided into the following groups: Uninjured control (received laminectomy only; LAM) + CMS exposure or SCI + CMS exposure. Prior to the induction of SCI, rats in the CMS group were exposed to 30 intermittent foot shocks (1mA) for 15 minutes for 7 consecutive days. Rats in the no stress group were placed in the chambers without foot shocks. SCI groups received a hemicontusion at the fifth cervical region of the spinal cord. After SCI, assessments of pain and functional recovery occurred weekly on separate days for 30 days. Evaluations of neuropathic pain included thermal hyperalgesia (Hargreaves test), mechanical (Von Frey) and cold allodynia (acetone test). Motor function was assessed via dominant paw usage (cylinder test). We found that rats in the SCI group exhibited significantly decreased motor function in the ipsilateral forepaw compared to control groups; however, there was no stress effect on motor function. Rats that received CMS prior to SCI showed increased cold allodynic responses, but no stress effect in thermal hyperalgesia or mechanical allodynia was observed. These findings indicated that mild stress prior to SCI increases subsequent neuropathic pain. Future investigations will be conducted to elucidate potential mechanisms and therapeutics.

Disclosures: A. Mohaimany-Aponte: None. **M.T. Robbins:** None. **C.L. Floyd:** None.

Poster

239. Pain Behavioral Models

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Topic: D.08. Pain

Support: CEBRID

AFIP

FAPESP

Title: Sleep deprivation potentiates neuropathic pain induced by chronic constriction injury of sciatic nerve in mice

Authors: *G. R. MOLSKA[1], L. I. G. PAULA FREIRE[2], C. E. N. GIRARDI[2], D. SUCHEKI[2]
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Abstract: Previous studies have shown that sleep restriction or deprivation increases pain sensitivity in healthy volunteers and patients with painful diseases. In addition, sleep deprivation potentiates acute, inflammatory and chronic pain in animal models. The aim of this study was to investigate whether paradoxical sleep deprivation (PSD) enhances thermal and mechanical hypernociception in mice subjected to chronic constriction injury of the sciatic nerve (CCI). Male C57BL/6J mice (3 month-old, n = 20, 5/group) were submitted to CCI and after recovery (2 to 3 days), were subjected to sleep deprivation for 72 hours. Pain sensitivity was assessed by von Frey and hot plate tests before surgery (baseline measurement), immediately after PSD, and 1, 2 and 7 days after PSD. Analysis was done with the following groups: control naïve (CTL), CCI, PSD and CCI+PSD. Mice subjected to CCI, PSD and CCI+PSD showed a pain threshold lower than CTL. Sleep deprivation potentiated peripheral nerve injury-induced pain, since the pain threshold in CCI+PSD group was significantly lower compared to CCI and PSD groups, in both tests. These results demonstrate that animals with peripheral nerve injury submitted to paradoxical sleep deprivation have higher pain sensitivity. Based on these results, further studies will be conducted to elucidate the mechanisms involved in paradoxical sleep deprivation potentiation of hypernociception.

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Poster

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Support: NIH/NIDCR DE021849

Title: Non-invasive, *in vivo* assessment of the modulating effect of persistent pain on the output of the mouse central pattern generator for mastication

Authors: *C. G. WIDMER[1], J. MORRIS-WIMAN[2]
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Abstract: The masticatory central pattern generator (CPG) controls the activation rhythm and excitatory drive to masticatory motoneurons during incising and chewing with refinement of activity by peripheral sensory afferent input. Previous animal studies have evaluated the CPG after implantation of EMG electrodes or placement of a skull-mounted detector for jaw motion analysis. These studies evaluated a limited number of chewing/incising cycles and did not measure the forces generated during these activities. We have developed a non-invasive technique that can be used in the mouse home cage environment that evaluates three-dimensional incising forces over a 24 hour period. These data can be analyzed to determine the incising frequency, incising peak force and loading and unloading time, thus evaluating rhythm and excitatory drive of the CPG. We have also developed a masticatory muscle persistent pain model that causes a mild/moderate pain without muscle tissue destruction for a period of 4-5 weeks. The purpose of this study was to evaluate correlations of force output parameters in the absence and presence of persistent pain to determine the effect of pain on the CPG for mastication in the natural environment. *Methods:* CD-1 mice (n=4 females) were evaluated for incising force over a 24 hours period for two baseline and two pain time points (T7 and T14). Two injections (separated by five days) of acidic saline into the left masseter muscle created a persistent pain condition. Interspike intervals (ISI), peak amplitudes and temporal parameters (loading and unloading times) were assessed. Correlation coefficients were calculated using multiple linear regressions (Statistica 12, StatSoft, Inc.) with interspike interval as a dependent variable and peak forces and temporal parameters as independent variables. Partial correlation coefficients were calculated to determine the relative contributions by the independent variables. *Results:* During baseline conditions, variation in interspike intervals was significantly associated with the independent variables (peak force, temporal parameters) with correlation coefficients ranging from 0.13-0.87 (median = 0.53). ISI most commonly co-varied with peak 1 force amplitude and peak 2 load time duration. Pain was associated with modulation of the CPG output by slowing the rhythm of incising (mean reduction = 0.31 Hz) and was associated with higher correlations among the ISI and peak force and loading times (median = 0.61). *Conclusions:* Persistent

masticatory muscle pain affects the masticatory CPG by slowing incising rhythm and inhibiting afferent input to reduce variability of CPG output parameters.

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Poster

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Topic: D.08. Pain

Title: Behavioral characterization of a tamoxifen-inducible Nav1.7 KO mouse

Authors: *T. WU, L. DENG, K. SCEARCE-LEVIE, D. HACKOS
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Abstract: Selective inhibition of the voltage-gated sodium channel Nav1.7 has been proposed as a potentially effective novel therapeutic strategy for the treatment of pain. Strong support for Nav1.7 as a pain target comes from loss-of-function mutations in humans that result in congenital insensitivity to pain (CIP), as well as gain-of-function mutations that cause chronic painful disorders such as inherited erythromelalgia (IEM) and paroxysmal extreme pain disorder (PEPD). While total deletion of Nav1.7 in mice causes a lethal perinatal phenotype (possibly due to anosmia that also occurs in human CIP patients), nociceptor-specific knockout mice are viable and show increased pain thresholds. Here, we examined whether the absence-of-pain and anosmia phenotypes observed in human CIP patients can be replicated via an acute (as opposed to life-long) loss of the Nav1.7 channel from dorsal root ganglion neurons in adulthood by generating a tamoxifen-inducible Nav1.7-floxed mouse model. This conditional knockout model may mimic some aspects of therapeutic pharmacological inhibition of Nav1.7 in humans. Mice bearing a floxed version of the Nav1.7 gene were crossed with mice expressing CreERT under the control of the pCAAG promoter. A cohort of bigenic mice and Nav1.7-floxed single transgenic controls were dosed with tamoxifen at age 13 weeks to induce Cre-Lox recombination and Nav1.7 deletion. Pilot experiments using qPCR confirmed that this dosing regimen successfully knocks out Nav1.7 mRNA in DRG neurons in less than 1 week. Mice were behaviorally characterized before and after Nav1.7 deletion in assays of nociception (hot plate test) and olfactory function (hidden food test), as well as measures of general behavior, weight, locomotor activity, and grooming behavior. Nav1.7 cKO mice showed a reduced response to acute thermal stimuli on the hot plate test by 12 days post-dose, and a complete lack of response

by 26 days post-dose, confirming an inducible CIP-like phenotype. General health, activity, and behavior were not different between WT and cKO mice and remained unchanged with Nav1.7 deletion. Olfactory function also appeared to remain intact. We conclude that this model of Nav1.7 deletion in adulthood is sufficient to reduce pain responsiveness, providing proof-of-concept that pharmacological inhibition of this channel may reduce pain perception in humans.

Disclosures: **T. Wu:** A. Employment/Salary (full or part-time):; Genentech. **L. Deng:** A. Employment/Salary (full or part-time):; Genentech. **K. Scearce-Levie:** A. Employment/Salary (full or part-time):; Genentech. **D. Hackos:** A. Employment/Salary (full or part-time):; Genentech.

Poster

239. Pain Behavioral Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 239.24/BB19

Topic: D.08. Pain

Support: Johns Hopkins Neurosurgery Pain Institute

Title: Pain response in a rat model of intravertebral breast cancer

Authors: *R. SARABIA ESTRADA[1], P. ZADNIK[2], I. JMENEZ-ESTRADA[3], L. YANG[2], Z. GOKASLAN[2], D. SCIUBBA[2]
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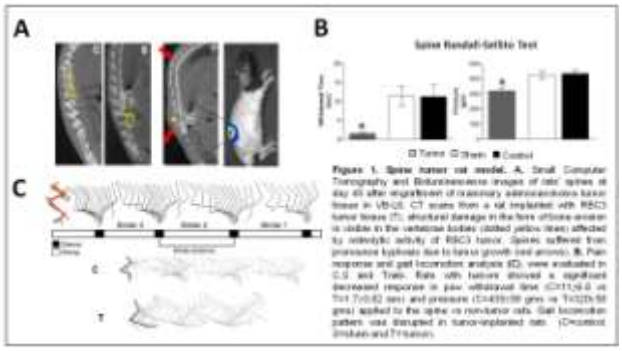
Abstract: Bone is the third most common site for metastasis representing a poor prognosis in cancer patients. Metastases to the spine are common source of pain; fractures; hypercalcemia; and neurological deficits reducing patients' quality of life. Microfractures occur in metastatic regions of cancerous bone that can no longer handle ordinary loading, causing spine deformation and nerve impingement. As the disease progresses, pain increases and it becomes more difficult to treat. A key obstacle in the study of this disease is the lack of reliable, and reproducible animal models to study cancer pain. Behavioral evaluation was conducted in a group of 20 athymic rats: 6 spine tumor-implanted (human mammary adenocarcinoma); 7 sham and 7 control rats. Randal-Sellito test was used to evaluate the nociceptive response in rats. Tumor growth was monitored by BLI and CT. Pain response was significantly reduced (Day 45) in time (C= 11±6.8, S=11±8.2 and T=1.7±0.82 sec) and pressure (C=435±59, S=428±71 and T=320±58 grams) applied in

tumor rats compared with control or sham; a slight non-significant reduction was observed in the paw withdrawal in tumor implanted-rats. BLI imaging revealed severe tumor invasion in the lower spine that was qualitatively related with lytic lesions, characterized by a decrease in the density of the bone in lumbar spine consistent with tumor location on bioluminescence. There was near-complete ablation of the posterior elements of the spine. Locomotion gait analysis showed a disruption in the normal pattern of the rat's gait. Our intraspinal metastatic tumor model animals showed locomotor and sensory signs that are in accordance with some of the clinical manifestations in humans. These signs included a locomotor deficit and an increase in noxius sensation. Our model offers a reliable method to evaluate alternative approaches to treat pain in patients with metastatic spine disease.



Disclosures: **R. Sarabia Estrada:** None. **P. Zadnik:** None. **L. Yang:** None. **Z. Gokaslan:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Deputy SPine, AO Spine NOrth America, Medtronic, NREF, INtegra Life Sciences and K2M.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Spinal Kinetics and US Spine. **D. Sciubba:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Depuy Spine, Research Grant Recipient. F. Consulting Fees (e.g., advisory boards); Medtronic, Nuvasiv, Globus and Depuy. **I. Jmenez-Estrada:** None.

Poster

239. Pain Behavioral Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 239.25/BB20

Topic: D.08. Pain

Support: Veteran's Administration

Dr. Miriam and Sheldon Adelson Biomedical Research Foundation

NIH-NINDS R01NS057456

Title: Assessment of pain outcomes in rodent models of severe spinal cord injury

Authors: C. A. G. LEE-KUBLI[1], M. INGVES[2], K. W. HENRY[2], M. TUSZYNSKI[1,3], *W. M. CAMPANA[2]
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Abstract: Several candidate therapies for promoting regeneration after spinal cord injury are advancing toward clinical testing. It is essential that candidate therapies are evaluated for effects on pain outcomes, particularly because chronic neuropathic pain is a common problem after SCI and results at least in part from aberrant sprouting. Accordingly, regenerative strategies, by promoting axonal growth, could improve or worsen pain outcomes. Moreover, pain outcome testing should be performed in models of severe SCI which more accurately reflect the severity of most human injuries. We characterized pain-related behaviors and their associated cellular mechanisms in adult rats that underwent severe, T3 complete transection. This severe injury was associated with emergence of above-level neuropathic pain behaviors, including tactile allodynia and spontaneous forepaw lifting. This was associated with significantly greater expression of the activated microglial marker Iba1 ($P<0.05$) in the C8 spinal segment. GFAP expression also increased at C8 ($P<0.05$), suggesting that astrocytes may be involved in the maintenance of chronic pain. Complete T3 transection also resulted in enhanced withdrawal reflex to cold cutaneous stimuli in the hindpaws and prolongation of heat withdrawal latency, changes that likely reflect perturbations of local reflex circuits below the injury. Lumbar spinal cord segments exhibited significantly reduced GAD67 ($P<0.05$) and increased Iba1 ($P<0.05$) and CGRP ($P<0.05$) expression. While enhanced withdrawal reflexes below the level of the lesion may not impact the perception of pain in rats with a T3 full transection, they may be of concern if neural regenerative or neuroprotective therapies re-establish connectivity with the brain. These data begin to establish the pattern of changes that occur above and below the level of a severe spinal cord lesion site, and will be valuable for evaluating candidate therapies for SCI, including neural stem cell implants (Lu et al., Cell 2013). Studies underway are being extended to perhaps the most clinically relevant model of spinal cord injury, severe contusive injury.

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Poster

239. Pain Behavioral Models

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Program#/Poster#: 239.26/BB21

Topic: D.08. Pain

Support: NIH grant NS077882

Title: Closed head injury promotes a selective trigeminal hyper-nociception: implications for the acute emergence of posttraumatic headache

Authors: T. BENROMANO[1], R. DEFRIN[2], A. H. AHN[3], J. ZHAO[4], C. G. PICK[1], *D. LEVY[4]
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Abstract: Headache is one of the most common symptoms following traumatic head injury, but the mechanisms underlying the emergence of such posttraumatic headache (PTH) remain unknown. These mechanisms may be related to local injury to deep cranial tissues leading to enhancement in cranial nociception, or could involve damage to supra-spinal pain processing pathways, as a result of brain injury. Here we tested the hypothesis that following head trauma there is an acute preferential enhancement of nociception from deep cranial tissues. We show that at 48 hours after an experimental model of closed head injury in mice, there was an acute enhancement in localized nociceptive responses to a noxious stimulus evoked by injection of dilute formalin into a deep cranial tissue, the calvarial periosteum. In parallel studies, we show that closed head injury did not lead to facilitation of the nociceptive responses following injection of formalin into an extracranial tissue, the hindpaw. Using a histological analysis of the calvarial periosteum 48 hours following closed head injury, we further demonstrate an increase in the number of activated mast cells, indicating a local inflammatory response. Our data provides evidence that mild head injury gives rise to enhanced processing of nociceptive information emanating from deep cranial tissues but does not affect nociceptive responses at non-cranial tissues. We therefore propose that in this head trauma model, there is peripheral sensitization of primary afferents innervating deep cranial tissues, such as the periosteum, rather than the enhancement of supra-spinal pain processing. Trauma evoked acute inflammatory responses within the calvarial periosteum could play a role in promoting cranial nociception and the emergence of PTH.

Disclosures: T. Benromano: None. **R. Defrin:** None. **A.H. Ahn:** None. **J. Zhao:** None. **C.G. Pick:** None. **D. Levy:** A. Employment/Salary (full or part-time):; Beth Israel Deaconess Medical Cneter. B. Contracted Research/Research Grant (principal investigator for a drug study,

collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH/NINDS.

Poster

239. Pain Behavioral Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 239.27/BB22

Topic: C.13. Sensory Disorders

Support: NIDCR Grant 1R03DE023153-01A1

Title: Characterization of two animal models of orofacial pain

Authors: *H. DE BRITO GARIEPY, A. RAMSEY, E. PODBORITS, C. LEE, J. GIBBS
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Abstract: Background: Orofacial pain is a common complaint that affects the lives of millions of people around the world. It impacts important physiological functions, such as chewing, swallowing, talking and laughing. Neuropathic pain in the trigeminal system is frequently observed clinically, but the mechanisms involved are still largely unknown. Microglia and astrocyte activation is believed to play a role, however this process is poorly understood, particularly in the trigeminal system. The present study was undertaken to characterize two murine models of orofacial pain. Methods: Under ketamine/xylezine anesthesia, mice received either bilateral ligation of the mental nerve (MNI) or a dental pulp injury (DPI) in the first maxillary molar. To measure mechanical pain, von Frey filaments were used with the up-down method. c-Fos expression was induced using a cold air burst either in the first maxillary molar (DPI) or directly onto the lower lip (MNI) in order to measure cold-evoked nociceptive input in the central nervous system. Quantitative PCR was performed to evaluate transcript levels of two nerve injury-associated genes, NPY and ATF-3, 21 days following either injury. Results: MNI and DPI mice showed persistent (>21 days) mechanical allodynia in the orofacial region, which was reversed by the anticonvulsant drug gabapentin (10mg/kg, i.p.). ATF-3 protein and mRNA and NPY mRNA expression was confirmed in the mandibular (MNI) and maxillary (DPI) division of the trigeminal ganglion by immunohistochemistry and PCR in injury vs control animals. GFAP and IBA-1, glial markers were increased in both models of orofacial pain in comparison to sham operated-mice. Repeated cold stimulation caused a significant increase of Fos expression in different areas of the trigeminal nucleus in both mice models. Conclusion and implication: Our results provide behavior and molecular data characterizing two murine models

of orofacial pain. Furthermore, the activation of glial cells in the trigeminal system are involved in the maintenance of orofacial mechanical allodynia. Characterizing differences between two animal models of orofacial pain will help develop a novel therapeutic target for neuropathic pain.

Disclosures: H. De Brito Gariepy: None. **J. Gibbs:** None. **A. Ramsey:** None. **E. Podborits:** None. **C. Lee:** None.

Poster

239. Pain Behavioral Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 239.28/BB23

Topic: C.13. Sensory Disorders

Title: NeurotropinR) ameliorates depression-like behavior in chronic pain via induction of brain-derived neurotrophic factor

Authors: *T. ISHIKAWA[1], S. YASUDA[1], H. FUJIMURA[1], K. FUKUHARA[1], K. ISHIKAWA[1], Y. IDA[1], Y. IWANAGA[1], T. IBUKI[2]
[1]Div. of Neurosciences, Yamaguchi Univ. Grad. Sch. Med., Yamaguchi, Japan; [2]Dept. of Anesthesilogy, Grad. Sch. of Med. Science, Kyoto Prefectural, Kyoto, Japan

Abstract: Background and Aims: Neurotropin (NTP) is a non-protein extract isolated from inflamed skin of rabbits inoculated with vaccinia virus, and used clinically for treatment of neuropathic pain. Moreover, NTP may activate the descending pain inhibitory system. Depression-like behavior is often complicated by chronic pain. However, little is known about NTP mediated preventing effect on mood disorder in chronic pain and its molecular mechanisms. We aimed to investigate the effects of NTP based on brain-derived neurotrophic factor (BDNF)-mediated signaling and gene expression in chronic pain. In addition, these effects of NTP were compared with other, pregabalin which is an anticonvulsant, anxiolytic analgesic used to treat neuropathic pain and fibromyalgia. Material and Methods: A chronic constriction injury (CCI) model was constructed in Sprague-Dawley rats. The pain response was assessed using the paw withdrawal latency (PWL) and depression was assessed by the immobility time in a forced swim test (FST). NTP was orally administered in dose of 50 NU (Neurotropin Unit) and 100 NU/kg for 7 days from day 7 after CCI. To ensure analgesic and anti-depressant effects of NTP, either anti-BDNF antibody, K252a (tyrosine kinase inhibitor) or 5,7-dihydroxy tryptamine (5,7-DHT, selective toxin of 5-HTergic neuron) was injected icv. Changes in pERK1/2 (immunohistochemistry), 5-HT and BDNF (ELISA), and BDNF mRNA (RT-PCR) were

measured in the anterior cingulate cortex (ACC) and rostral ventromedial medulla (RVM) 14 days after CCI. Results: After CCI, the rats showed a decrease in PWL associated with extension of the time of immobility. NTP reduced the decrease in PWL and the increased FST in a dose-dependent manner while pregabalin did not affect any of FST. These effects of NTP were reversed by anti-BDNF antibody, K252a, and 5,7-DHT, but analgesic effects of pregabalin were not reversed by K252a. NTP normalized the CCI-induced excessive activation of pERK1/2 associated with decreased pCREB and BDNF mRNA in ACC and RVM, and these changes were reversed by 5,7-DHT. In contrast, pregabalin did not affect them in chronic pain Conclusion: NTP ameliorated chronic pain and pain-related negative emotion by normalizing the induction of BDNF associated with 5-HTergic system. Pregabalin showed the analgesic effects and no anti-depressant effects without mediating BDNF pathway. These results suggest that NTP may represent the additional drug strategies against chronic pain associated with depression.

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Poster

239. Pain Behavioral Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 239.29/BB24

Topic: D.08. Pain

Title: Effects of chronic pain on activation of inflammatory brain mechanisms and development of depressive-like behaviors

Authors: M. CARDER, M. LEONG, M. LENDE, K. WATSON, R. FIRKINS, L. YUAN, *V. DURIC
Physiol. and Pharmacol., Des Moines Univ., Des Moines, IA

Abstract: Clinical reports indicate that many chronic pain patients also develop symptoms of mood disorders, especially major depressive disorder (MDD); however, the underlying neural mechanisms linking chronic pain conditions and depressive behaviors are still poorly understood. Our previous studies have demonstrated that rodent models of chronic pain mimic some of the stress-like alterations in intracellular signaling and cellular architecture (e.g., decreased MAPK signaling and reduced rate of neurogenesis) within the hippocampus, a limbic brain region involved in regulation of mood. Furthermore, recent reports suggest that stress-induced activation of interlukin-1-beta (IL-1ß)-mediated inflammatory mechanisms suppress

neurogenesis in the adult rat hippocampus and, therefore, may present novel factors contributing to the depressive-like effects observed in chronic stress models of depression. Thus, in this study, we examined the effects of persistent pain on activation of immune-inflammation processes in the limbic brain regions. Male rats were initially exposed to either injection of complete Freund's adjuvant (CFA; model of chronic inflammatory pain) or spared nerve injury (SNI; model of chronic neuropathic pain). Both pain models produced robust mechanical hypersensitivity over the 21 day period, accompanied by depressive-like phenotype. In parallel with the behavioral effects, exposure to pain also induced changes in expression of proteins involved in microglial pro-inflammatory signaling pathways that resemble previously observed responses to stress and depression. Preliminary results indicate that pain evoked upregulation of specific members of Nod-like receptor (NLR) family of inflammasome multiprotein complex within the hippocampus. The results of this study may ultimately contribute towards the identification of new treatment targets and the development of novel clinical strategies to diminish the mental health consequences of chronic pain.

Disclosures: M. Carder: None. **M. Leong:** None. **M. Lende:** None. **K. Watson:** None. **R. Firkins:** None. **L. Yuan:** None. **V. Duric:** None.

Poster

239. Pain Behavioral Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 239.30/BB25

Topic: D.08. Pain

Title: Acute ketamine attenuates long-lasting neuropathic pain-induced social avoidance in mice

Authors: *Z.-C. YANG, P. ZHANG, D.-J. GE
Huai'An First People's Hosp., Jiangsu, China

Abstract: Previous studies reported that chronic neuropathic pain induced depressive behaviors like anhedonia and despair which could be reversed by acute ketamine treatment both in animal and patients. Here, we found that long-lasting neuropathic pain also induce social avoidance, another core syndrome of major depressive disorder. In a chronic constriction injury (CCI) model of sciatic nerve in mice, we found that CCI surgery induced a long-lasting stable thermal hyperalgesia in the affected hindpaws when compared to unaffected hindpaws, or naïve control and sham animals. Twenty-eight days, but not 7 days following CCI surgery, mice developed significant social avoidance shown as decreasing interaction durations with a strange retired

aggressor, and increasing corner durations in social interaction test. And no significant changes were observed in the distances animals travelled and the mean velocity. A single low dose of ketamine (40mg/Kg or 20mg/Kg, intraperitoneal injection) administrated 24 hours before social interaction test significantly reversed CCI-induced decrement in interaction durations and increment in corner durations, but failed to affect the paw withdrawal latencies. In the future, we will investigate if CCI could induce social avoidance in mice to their littermate, and the underlying molecular mechanisms of neuropathic pain-induced social avoidance in the brain. This study indicates that long lasting neuropathic pain induces social avoidance behavior, and single low dose of ketamine maybe a potential rapid way to alleviate this behavior.

Disclosures: Z. Yang: None. **P. Zhang:** None. **D. Ge:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.01/BB26

Topic: D.08. Pain

Support: RO1 DE17794

DE22743

NS67686

Title: Toll-like receptor 4 regulates central sensitization, touch-evoked itch, and inflammatory pain

Authors: *Q. HAN
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Abstract: Toll-like receptors (TLRs) are typically expressed in immune cells and play a critical role in pathogen recognition and innate immunity activation. Our previous studies have shown that TLR3 and TLR7 are expressed by nociceptor neurons in the dorsal root ganglia to mediate itch sensation. TLR4 is the most investigated TLR family member and has been implicated in the pathogenesis of chronic pain via regulating glial activation in the spinal cord. However, the role of TLR4 in itch regulation has not been investigated. We examined histamine dependent and independent itch in wild-type and TLR4 knockout (KO) mice. We found compound 48/80-induced itch (histamine-dependent) is normal in TLR4-KO mice. Furthermore, chloroquine-

induced itch (histamine-independent) is also intact in TLR4-KO mice. We then investigated compound 48/80-induced alloknesis (touch-evoked itch), which is mediated by central sensitization. Strikingly, compound 48/80-induced alloknesis is abrogated in TLR4-KO mice. Consistently, formalin-induced second phase spontaneous pain, which is known to be mediated by central sensitization, is also abolished in TLR4-KO mice. Finally, spinal cord long-term potentiation (LTP) in intact mice following tetanic stimulation of the sciatic nerve is also impaired in TLR4-KO mice. Our data suggest that TLR4 contributes to central sensitization and the pathogenesis of inflammatory pain. Our data also suggest that TLR4 is not required for acute itch but essential for inducing central sensitization-mediated alloknesis. This study is supported by NIH grants RO1 DE17794, DE22743, and NS67686 to R.R.J.

Disclosures: Q. Han: None.

Poster

240. Inflammatory Pain

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NIH Grant R01 DE22743 RRJ

NIH Grant R01 NS67686 RRJ

NIH Grant R21 NS82895 ZZX

Title: Extracellular and secreted microRNAs are novel pain mediators: Involvement of TLR7 and TRPA1

Authors: *T. BERTA[1], Z.-Z. XU[1], C.-K. PARK[1], Q. HAN[1], X.-J. LIU[1], R.-R. JI[1,2]
[1]Anesthesiol., [2]Neurobio., Duke Univ. Med. Ctr., Durham, NC

Abstract: MicroRNAs (miRNAs) are small non-coding RNAs and play critical roles in regulating gene expression. Although miRNAs are typically considered intracellular entities, miRNAs can also been detected in various body fluids including CSF as biomarkers for diseases. However, the functional role of extracellular miRNAs is still elusive. The miRNA let-7b is one of the most abundant miRNAs and contains immune stimulatory GUUGUGU motif. It is highly

enriched in dorsal root ganglion (DRG). Using quantitative RT-PCR we detected let-7b in the culture medium of DRG neurons and let-7b secretion is increased following neuronal depolarization (KCl) and nociceptor activation (capsaicin), indicating an activity-dependent release of let-7b. Interestingly, intraplantar injection of let-7b inhibitor reduced formalin (0.5%) induced spontaneous pain, suggesting a possible role of secreted let-7b in mediating inflammatory pain. Intraplantar injection of let-7b, but not the mutated oligonucleotides also elicited dose-dependent spontaneous pain. In particular, let-7b-induced spontaneous pain is abolished in mice lacking toll-like receptor 7 (TLR7), which is known to be activated by single-strand RNAs and expressed in nociceptor neurons of DRG. let-7b-induced spontaneous pain is also reduced in TRPA1 knockout mice and suppressed by TRPA1 antagonist. Intraplantar let-7b also induced persistent mechanical allodynia, which is abrogated in mice lacking TLR7, TRPA1, and MyD88. Patch clamp recordings revealed that let-7b induced rapid (within a minute) inward currents and action potentials in small-sized DRG neurons that co-express TLR7 and TRPA1, and these actions of let-7b are all abolished in TLR7 and TRPA1 knockout mice. Finally, low concentration formalin (0.01%)-induced TRPA1 currents is reduced by extracellular application let-7b inhibitor. Collectively, our findings support a functional role of extracellular and secreted miRNA for rapid nociceptor excitation and pain induction via TLR7 and TRPA1. Extracellular miRNAs represent a new class of pain mediators/neuromodulators and potential targets for developing novel pain treatments.

Disclosures: **T. Berta:** None. **Z. Xu:** None. **C. Park:** None. **Q. Han:** None. **X. Liu:** None. **R. Ji:** None.

Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Support: IMI Europain

NIH NS067459

UCSD Anesthesiology Lab Research

Title: Anti-nociceptive actions of Botulinum toxin B on spinal sensory processing and AMPA receptor trafficking

Authors: *S. SIKANDAR[1], M. MAARDH[2], A. H. DICKENSON[1], L. SORKIN[2], T. L. YAKSH[2]
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Abstract: Aim We explored the effects of intraplantar (i.pl.) Botulinum toxin B (BoNTB), which blocks neurotransmitter release from primary afferent terminals, on spinal nociceptive processing and AMPA receptor trafficking in an inflammatory model of pain. We also investigated transsynaptic effects of i.pl. BoNTB by activating the system post-synaptically using i.pl. BoNTB pretreatment in conjunction with i.t. NMDA. Methods Adult male mice (C57Bl6; 20-30g) were used for all experiments. Drugs were administered i.pl. (30 μL BoNTB, 30 μL saline or 20μL 2% carrageenan) and i.t. (5 μL NMDA or saline). Mechanical withdrawal thresholds were assessed with von Frey filaments. For c-Fos staining, carageenan mice were perfused and spinal cord tissue cut at 30 μm. For Western blots, following i.pl. carrageenan (2 h) or i.t. NMDA (5 min), spinal cords were hydroextruded and lumbar quadrants and L3-L6 DRGs were snapfrozen. Western membranes were probed for relevant antibodies and incubated with horseradish peroxidase-linked secondary antibodies. All data was normalized to a loading control. Results We found that i.pl. carrageenan induces mechanical allodynia, as well as phosphorylation of spinal dorsal horn AMPA receptor subunit, GluA1, at the PKA site and phosphorylation of protein kinase B (AKT). Our data shows that a 48-hour pretreatment with i.pl. BoNTB blocked evoked allodynia, the increase in p-GluA1ser845 and p-AKTser473 and the increase in carrageenan-induced Fos expression. BoNTB also elicited a decrease in vesicle-associated membrane protein products (VAMP) in DRGs. All of these data can be explained by BoNTB inhibition of primary afferent release. Administration of i.t. NMDA increased dorsal horn levels of p-GluA1ser845 and p-AKTser473. Surprisingly, these postsynaptic effects were also blocked by ipsilateral i.pl. BoNT. Effects of i.pl. BoNTB on NMDA-evoked GluA1 trafficking to the plasma membrane are currently being investigated. Conclusions We report novel findings of BoNTB actions on spinal nociceptive processing. Pretreatment with i.pl. BoNTB reduces pain behavior in a carrageenan model of acute inflammation. Furthermore, BoNTB pretreatment in carrageenan mice reduces neural activation and phosphorylation of Akt and GluA1. By using spinal administration of NMDA, we bypass the primary afferent and show that i.pl. BoNTB has postsynaptic actions on pAkt and pGluA1. Given these results it is likely that we will observe modulation of GluA1 trafficking into the plasma membrane. These results provide crucial insight into the mechanisms of BoNT in modulating central nociceptive processing, and importantly its potential to exert transynaptic actions.

Disclosures: S. Sikandar: None. **M. Maardh:** None. **L. Sorkin:** None. **T.L. Yaksh:** None. **A.H. Dickenson:** None.

Poster

240. Inflammatory Pain

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Program#/Poster#: 240.04/BB29

Topic: D.08. Pain

Support: NSERC Discovery grant to Weiya Ma (RFN.356021)

Title: Signaling transduction events involved in agonist-induced PGE2 EP4 receptor externalization in cultured dorsal root ganglion neurons

Authors: B. ST-JACQUES[1], *W. MA[2]
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Abstract: Prostaglandin E2 (PGE2), a well-known pain mediator enriched in inflamed tissues, plays an essential role in the development of chronic pain conditions such as inflammatory and neuropathic pain. PGE2 EP4 receptor, which couples to Gs and activates cAMP/protein kinase A signaling, is known to play a major role in those chronic pain conditions. Both peripheral and central sensitizations underlie the genesis of chronic pain state. Although PGE2 is known to sensitize nociceptive dorsal root ganglion (DRG) neurons (nociceptors), the underlying mechanisms remain incompletely understood. We recently reported that PGE2 and EP4 agonists stimulated cell surface trafficking of EP4 in cultured DRG neurons and that dual stimulations of EP4 agonist induced greater levels of intracellular cAMP in cultured DRG explants than a single stimulation (St-Jacques & Ma, Pain, 154:313-323, 2013), suggesting that facilitating EP4 cell surface trafficking likely underlies PGE2-induced nociceptor sensitization. However, the signaling transduction pathways mediating PGE2-facilitated EP4 externalization remains largely unknown. In the present study, using antibody feeding based EP4 externalization assay, we attempted to dissect out those signaling transduction events invovled in EP4 agonist-induced EP4 externalization in cultured DRG neurons. We found that EP4 agonist, and EP3 agonist to a lesser extent, but not EP1 and EP2, induced EP4 externalization. The CaMKII inhibitor, extracellular and intracellular calcium chelators blocked EP4 agonist 1-OH-PGE1-induced EP4 externalization. The inhibitors of cAMP, PKA, PKC, PKCε, MAPK, PI3K and Akt, but not PLC, suppressed 1-OH-PGE1-induced EP4 externalization. The activators of adenylate cyclase, two PKA specific cAMP analogs and an Epac specific cAMP analog induced EP4 externalization. We also observed that capsaicin and potassium chloride enabled to induce EP4 externalization to a lesser degree than EP4 agonist. A neutralizing IL-6 antiserum or a NGF sequester partially suppressed agonist-induced EP4 externalization. Taken together, these data suggest that by activating cAMP/PKA, cAMP/Epac, PKC/PKCε, MAPK and PI3K-Akt signaling transduction

pathways, EP4 agonist induced EP4 externalization. Agonist-induced EP4 externalization requires extracellular and intracellular calcium and CaMKII. IL-6 and NGF are partially involved in agonist-induced EP4 externalization while capsaicin and KCL enable to stimulate EP4 externalization, suggesting that cross-stimulation of cell surface trafficking of pain-facilitating receptors likely underlies cross-sensitization of nociceptors among pain mediators.

Disclosures: B. St-Jacques: None. **W. Ma:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.05/BB30

Topic: D.08. Pain

Support: NIH NS082746

NIH NS027910

Title: Akap150 mediates transition to chronic pain

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Abstract: Chronic pain affects over 100 million Americans every year, and remains one of the most under-treated conditions in the world. Despite this, little is know about the mechanisms that control the transition from acute to chronic pain. Although many central, synaptic, and peripheral mechanisms contribute to this transition, we have discovered a unique signaling pathway that engenders chronic sensitivity to peripheral sensory neurons. In animals challenged with an acute prostaglandin E2 (PGE2) injection, we waited 5 days and determined sensitivities to thermal and mechanical stimuli. Rodent behavioral models indicate that AKAP150 is required for the transition from acute to chronic pain. Our hypothesis was that a feed-forward mechanism is created by acute injury (PGE2) that stimulates glutamate receptor-mediated AKAP150 association with and sensitization of TRPV1 in sensory neurons. Indeed, depolarization of peptidergic sensory neurons elicits the release of neuromodulators, including calcitonin gene-related peptide (CGRP) and glutamate, which can act on either adjacent terminals or the same terminal. We found that sensory neuron depolarization increases extracellular glutamate, and that activation of mGluR1 increased AKAP150 association with TRPV1, and sensitized naïve

responses to an agonist stimulus. Further, recording from nociceptors using an *in vitro* skin-nerve preparation confirms that AKAP150-dependent TRPV1 sensitization is occurring at the level of terminal innervation. Together, our findings identify AKAP150 scaffolding as an important event in the transition from acute to chronic pain.

Disclosures: **N.A. Jeske:** None. **M.P. Rowan:** None. **R. Gomez:** None. **J. Du:** None. **S.M. Carlton:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.06/BB31

Topic: D.08. Pain

Support: Fapesp - SP Brazil

Title: The activation of neuronal cannabinoid CB1 receptor inhibits inflammatory hyperalgesia via inactivation of P2X3 receptor in rats

Authors: *C. A. PARADA, M. G. OLIVEIRA-FUSARO, D. ARALDI, I. J. M. BONET, C. H. TAMBELI
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Abstract: Aim of investigation: The present study was addressed to investigate the mechanism by which activation of cannabinoid CB1 receptor in the primary afferent neuron reduces the inflammatory hyperalgesia. Methods: Experiments were conducted in accordance to the Committee on Animal Research of the State University of Campinas- Unicamp, São Paulo - Brazil. Drugs or vehicle were subcutaneously injected in the rat's hind paw. The functional blockade of CB1 receptors expression on peripheral sensory neurons was performed by the intrathecal injection of ODN antisense: 5'- TGA ATC ATG CGG ACC GCG T -3' or ODN mismatch: 5'- TTA CTC AGG CTG GCC GAG T -3', one time a day (30 μg/10 μL per day) for 4 days. Intrathecal injection was performed in anesthetized rats with halothane. A 26-gauge needle was inserted in the subarachnoid space on the midline between L4 and L5 vertebrae. ODN was injected at 1μl/s. Western Blot was performed using specific primary antibodies to CB1. Data was performed with G-BOX, and normalized to the ponceau control. The mechanical nociceptive threshold was measured using electronic von Frey. The hyperalgesia was presented as Δ withdraw threshold. ELISA was used to determine the concentration of TNF-α, IL-1β and

CINC-1 in subcutaneous tissue. The neutrophil migration was evaluated by myeloperoxidase kinetic-colorimetric assay. One-way ANOVA followed by Tukey test was used for statistical analysis as appropriated ($P<0.05$). Rats per group = 6 to 8. Results: We confirmed that anandamide or ACEA, the non-selective or the selective cannabinoid CB1 receptor agonist, respectively, administrated in the peripheral tissue of rat hind paw reduces the carrageenan-induced mechanical hyperalgesia in a dose-response manner. This response is not reversed by naloxone, but was reversed by the selective cannabinoid CB1 receptor antagonist AM251 locally administrated in the subcutaneous tissue, as well as by the intrathecal treatment with oligodeoxynucleotide (ODN) antisense against CB1 receptor. ACEA also reduced the hyperalgesia induced by bradykinin or by the selective P2X3 and P2X2/3 receptors agonist αβmeATP, but not by TNF-α, IL-1β, CINC-1 or PGE2. Although local peripheral administration of ACEA prevented carrageenan-induced hyperalgesia, it did not affect neither the release of TNF-α, IL-1β, CINC-1 or the neutrophil migration induced by carrageenan. Conclusion: Take together the data of this study suggest that the mechanism underlying the anti-hyperalgesic effect mediated by CB1 receptor activation in peripheral afferent nociceptor involves inactivation of neuronal P2X3 receptor. Research **Support:** FAPESP.

Disclosures: **C.A. Parada:** None. **M.G. Oliveira-Fusaro:** None. **D. Araldi:** None. **I.J.M. Bonet:** None. **C.H. Tambeli:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.07/BB32

Topic: D.08. Pain

Title: Effects of morphine on formalin-evoked somatic and affective components of nociception in male and female rats

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Abstract: There is increasing evidence for the presence of sex differences to pain and nociception in humans and animals. Occurrence of certain chronic pain conditions is more prevalent in women than in men. Similar to the findings in humans, female rats have a greater nociceptive sensitivity in behavioral models of mononeuropathy, inflammatory, and tonic pain

conditions as compared to male rats. Sex differences in models of tonic nociception is of particular interest to us, because i) tonic nociceptive transmission is a critical component in development of wind-up and the early stages of central sensitization; and ii) animal models of tonic nociception (notably the formalin model) are easily reproducible and well characterized. Therefore, we sought to determine the somatic and affective components of formalin-evoked nociception in male and female rats. Moreover, the effects of morphine in the two components were also examined. The somatic component of formalin-evoked nociception was measured by assessing paw flinches across a 60min testing session using an automated formalin nociceptive analyzer. The affective component of formalin-evoked nociception was measured using a conditioned place aversion (CPA) paradigm. In this model, formalin was paired with the preferred compartment of a CPA apparatus in a 2-day CPA conditioning procedure. Our preliminary findings suggest that male and female rats exhibited similar levels of formalin-evoked flinching behavior across phases 1 and 2. Likewise, male and female rats exhibited similar levels of aversion to a formalin paired environment. Morphine treatment during conditioning in the CPA study resulted in diminished place aversion in male and female rats. Although, morphine exhibited a trend for a stronger blockade of formalin-evoked CPA in males as compared to females. If these results persist, they will suggest that morphine produces a sexually dimorphic response in somatic and affective responses to tonic spontaneous pain.

Disclosures: **L. Harton:** None. **A. Nazarian:** None. **J. Richardson:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

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Program#/Poster#: 240.08/BB33

Topic: D.08. Pain

Support: CIHR Grant 10007021

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CFI Grant 10005585

Title: Role of microglia and P2X7 receptors in arthritis pain

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[1]Neurosci., [2]Vet. Med., Univ. of Calgary, Calgary, AB, Canada; [3]Univ. of Calgary, Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Arthritis pain is one of the most common and debilitating chronic pain conditions, and its negative impact will continue to grow in scope as the population ages and arthritis becomes more prevalent. The sequelae of arthritis pain are commonly attributed to peripheral joint pathology. Although joint pathology is an underlying factor, some patients with severe joint degeneration report little to no pain, while others with minor joint pathology experience debilitating and unremitting pain. The discordance between joint pathology and pain severity suggests joint damage is not necessarily a direct predictor of ensuing chronic pain. Rather, new lines of evidence implicate pathological changes within the central nervous system as being an important component of arthritis pain. In the present study, we examined the importance of microglia and ATP-gated P2X7 receptors (P2X7R) within the spinal cord in arthritis pain. The principal goal was to evaluate the cellular changes in spinal microglia and the concomitant progression of joint pathology in a monosodium iodoacetate (MIA) rat model of osteoarthritis (OA). Adult male Sprague Dawley rats were given an intra-articular injection of MIA. Changes in mechanical and thermal nociceptive threshold following MIA-injection were assessed using the Von Frey and thermal Hargreaves tests, respectively. We found that the onset of mechanical allodynia and thermal hyperalgesia occurred as early as day 3 post-MIA injection. On day 7 post-MIA injection, we detected a significant increase in iba-1 expression, a microglia marker, on the ipsilateral spinal dorsal horn. Given that ATP-gated P2X7Rs are critically involved in the pathophysiology of osteoarthritis, and the emergent role of these receptors in microglia mediated chronic pain signaling, we next asked whether spinal P2X7Rs are causally involved in MIA-induced arthritic pain behaviours. We found that repeated intrathecal injection of the P2X7R antagonist, Brilliant Blue G (BBG), over 7 days markedly attenuated the development of mechanical allodynia and thermal hyperalgesia in MIA-injected rats. In addition, we discovered that a single acute injection of BBG in animals with established MIA-induced arthritic pain transiently reversed both the mechanical and thermal pain hypersensitivity. Collectively our results suggest that spinal P2X7Rs are causally involved in the development and tonic ongoing expression of arthritis pain.

Disclosures: **M.J. Mousseau:** None. **A. Pilapil:** None. **J. Matyas:** None. **T. Trang:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.09/CC1

Topic: D.08. Pain

Support: FAPESP

Title: Sex differences in the antihyperalgesic and anti-inflammatory effect of P2X7 receptor blockade on carrageenan-induced arthritis in the rats knee joint model

Authors: *C. H. TAMBELI, J. M. TEIXEIRA, C. A. PARADA, E. V. DIAS
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Abstract: Aim: The aim of this study was to investigate whether the anti-hyperalgesic and anti-inflammatory effects induced by the blockade of P2X7 receptors in the knee joint differs between male and female rats. Methods: Male and females Wistar rats (2 months old, 200-250g) were used in this study. The experimental procedures were approved by the Ethics Committee in Animal Research at the UNICAMP (2049-1). The hyperalgesic responses (gait disturbance, seconds) were quantified using the Knee-joint Incapacitation Test (Tonussi, Pain 48; 421, 1992), the local concentrations of TNF-α, IL-1β, IL-6 and CINC-1 were measured by Enzyme-linked Immunosorbent Assay (ELISA) and neutrophil migration was quantified in the rats knee joint by the method of quantification of myeloperoxidase enzyme activity (MPO), 3 hours after the administration of carrageenan (300µg/knee). One-way ANOVA followed by Tukey test was used for statistical analysis as appropriated ($P<0.05$). Rats per group = 6 to 8. Results: Intra-articular administration of carrageenan (300µg/knee) induced articular hyperalgesia (Mean±EPM, males: 54.3±1.3s; females: 49.6±1.5s), release of TNF-α (males: 1139.17±90.47pg/mL; females: 947.1±130.47pg/mL), IL-1β (males: 12670.1±1271pg/mL; females: 12251.4±1089.5pg/mL), IL-6 (males: 8720.94±335.2pg/mL; females: 8044.3±1013.5pg/mL), CINC-1 (males: 3967.7±521. 2pg/mL; females: 3887.2±162.3pg/mL) and neutrophil migration (males: 23645.6±2145.13 neutrophils/joint; females: 21858.1±1253.1 neutrophils/joint). Co-administration of the selective P2X7 receptor antagonist A-740003 (568µg/Knee in males and 142µg/knee in females) significantly reduced the articular hyperalgesia (males: 14.5±1.3s; females: 17.6±1.4s; $P<0.05$, Tukey test), the release of TNF-α (males: 305.4±43.3pg/mL; females: 304.9±59.8pg/mL; $P<0.05$, Tukey test), IL-1β (males: 6013.7±832.9pg/mL; females: 9190.7±832.9pg/mL; $P<0.05$, Tukey test), IL-6 (males: 1285.5±218.6pg/mL; females: 1127.8±336.3pg/mL; $P<0.05$, Tukey test), CINC-1 (males: 250.5±21.6pg/mL; females: 1829.3±323.9pg/mL; $P<0.05$, Tukey test) and the neutrophil migration (males: 5174.3±704.68 neutrophils/joint; females: 9795.1±1179.1 neutrophils/joint; $P<0.05$, Tukey test) induced by carrageenan. Although, the lowest dose of A-740003 (142µg/knee) significantly reduced ($P<0.05$, Tukey test) carrageenan-induced articular hyperalgesia, cytokines release and neutrophil migration in females it did not in males.

Conclusion: Females are more responsive than males to the anti-hyperalgesic and anti-inflammatory effects induced by the blockade of P2X7 receptors in the rat knee joint.

Disclosures: C.H. Tambeli: None. **J.M. Teixeira:** None. **C.A. Parada:** None. **E.V. Dias:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.10/CC2

Topic: D.08. Pain

Support: NIH R01 NS56122

Nih R01 GM102346

Title: A novel phenotypic marker reveals resident macrophage monitoring of neuronal activity within sensory ganglia

Authors: *D. C. MOLLIVER[1], E. S. SCHWARTZ[2]
[1]Biomed. Sci., Univ. New England, Biddeford, ME; [2]Anesthesiol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: There is extensive evidence for interactions between peripheral sensory axons and immune cells at sites of peripheral injury. However, there has been limited investigation of resident immune cell function within peripheral sensory ganglia, and whether their actions have an impact on functional properties of nociceptors. We report the identification of a novel marker of resident macrophages whose expression is rapidly suppressed within dorsal root ganglia (DRG) in response to a noxious insult to a distant peripheral tissue. The initial project asked whether one-dimensional SDS-PAGE provided sufficient resolution to identify changes in protein levels occurring in sensory ganglia in response to peripheral inflammation. The protein banding profile from lumbar L2-5 DRG was visualized by silver stain, and compared between mice with and without inflammation of the hindpaw induced by injection of complete Freund's adjuvant (CFA) 24 hours before sample collection. A striking difference was the loss of a high molecular weight band (250 kD) in samples from inflamed mice. This band was excised and analyzed by mass spectroscopy. A high confidence target ($p<0.01$) was identified as myosin-4. Validation by Western blot revealed that the myosin-4 band matched the molecular weight of the

band identified in silver-stained gels and was almost completely lost 24 hours after hindpaw injection of CFA. Immunohistochemistry for myosin-4 in combination with F4/80, a macrophage marker, revealed a network of F4/80+ macrophages in naïve DRG and extensive colocalization with myosin-4, indicating expression of myosin-4 in resident macrophages. Staining was also seen in macrophage-like cells cultured from bone marrow, but not in neuronal cultures. Analysis of mouse and human peripheral tissues revealed labeled macrophage-like cells, suggesting that myosin-4 is a marker of resident macrophages in human as well as mouse. To confirm that this phenotypic switch was not due to a systemic inflammatory response to CFA, the TRPV1 agonist capsaicin was injected into the hindpaw to directly excite nociceptors. 24 hours later, Western blot analysis indicated a loss of myosin-4 protein in L2-5 DRG. We propose that resident immune cells within DRG monitor nociceptor activity and respond to noxious insult with a change in phenotype. The significance of ganglionic resident macrophage responsiveness to neuronal activity and the possibility of a role in the regulation of nociceptor function at the soma are under investigation.

Disclosures: D.C. Molliver: None. **E.S. Schwartz:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.11/CC3

Topic: D.08. Pain

Title: The systemically active NAAA inhibitor, ARN726, alleviate inflammation in an animal model of rheumatoid arthritis

Authors: F. BONEZZI, O. SASSO, S. PONTIS, *D. PIOMELLI
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Abstract: Fatty acid ethanolamides (FAEs) are a family of lipid messengers that participate in the control of multiple physiological functions including pain and inflammation. They include agonists of cannabinoid receptors, such as anandamide, and agonists of type-α peroxisome proliferator-activated receptors (PPAR-α), such as oleoylethanolamide and palmitoylethanolamide. The FAEs are degraded by two intracellular lipid amidases: N-acylethanolamine acid amidase (NAAA) and fatty acid amide hydrolase (FAAH). Although NAAA and FAAH share the ability to cleave lipid amide bonds, they differ markedly in primary structure, substrate selectivity, and cellular localization. NAAA, primarily localized to the

lysosomal compartment of macrophages, is an N-terminal nucleophile cysteine amidase that displays a strong preference for saturated FAEs such as PEA. FAAH, found on the outer face of mitochondria and endoplasmic reticulum of most mammalian cells, is a member of the amidase signature family of serine hydrolases and displays broader substrate selectivity, but preferentially hydrolyzes monounsaturated and polyunsaturated FAEs such as OEA and the endocannabinoid anandamide. These enzymes have attracted growing attention as potential targets for drug therapy. In the present study we tested the effects of the first systemically active NAAA inhibitor, ARN726, in a model of rheumatoid arthritis, and compared them to those of the peripherally restricted FAAH inhibitor URB937. Arthritis was developed in Sprague-Dawley rats by hind-paw injection of Complete Freund's Adjuvant (CFA). The disease developed within 24 h of injection and caused paw oedema and thermal hyperalgesia. Drugs were administered 7 and 14 days after induction. Immunohistochemistry for NAAA was performed on tissue sections from paw, spinal cord and brain, with focus on the areas involved in pain sensation and modulation. Spinal cord sections were stained with Iba-1 as a marker of microglia. Behavioral tests demonstrated that ARN726 reduces both paw oedema and heat hyperalgesia, while URB937 is active on hyperalgesia, but as no effect on oedema. NAAA immunoreactivity was found in paw and in the rostral ventrolateral medulla (RVM) of CFA-treated animals. Low NAAA signal was detected in paw tissue, but not in RVM of naïve rats. Robust microglial activation in spinal cord, especially at the lumbar level, was observed, but NAAA immunoreactivity was not evident in this structure. The results suggest that pharmacological blockade of intracellular NAAA activity causes significant anti-inflammatory effects in the CFA model of arthritis.

Disclosures: F. Bonezzi: None. **D. Piomelli:** None. **O. Sasso:** None. **S. Pontis:** None.

Poster

240. Inflammatory Pain

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Program#/Poster#: 240.12/CC4

Topic: D.08. Pain

Title: Participation of peripheral P2Y1and P2Y6 receptors in formalin-induced inflammatory pain in rats

Authors: *V. SOLANO-OLIVARES[1], P. BARRAGAN-IGLESIAS[2], J. PINEDA-FARIAS[2], F. FLORES-MURRIETA[1,3], V. GRANADOS-SOTO[2], J. RODRÍGUEZ-SILVERIO[3], H. ROCHA-

GONZALEZ[3]
[1]Inst. Nacional De Enfermedades Respiratorias, Mexico, D.F., Mexico; [2]Cinvestav sede sur, México, D.F., Mexico; [3]Escuela Superior de Medicina-IPN, Mexico, D.F., Mexico

Abstract: Metabotropic P2Y receptors subfamily consists of eight functional mammalian receptors. P2Y1 and P2Y6 receptors have been described in the sensory nervous system, but their participation in behavioral pain models at peripheral levels has been scarcely studied. This study assessed the role of peripheral P2Y1 and P2Y6 receptors in formalin-induced inflammatory pain, and determined their localization and distribution in dorsal root ganglia (DRG) by western blot and immunostaining. Ipsilateral, but not contralateral, local peripheral pre-treatment with the endogenous non-selective P2Y1 (ADP, 100-1000 nmol/paw) and P2Y6 (UDP, 180-300 nmol/paw), or selective P2Y1 (MRS2365, 0.1-10 nmol/paw) and P2Y6 (PSB0474, 0.1-0.10 pmol/paw) receptor agonists increased 0.5% formalin-induced flinching behavior. Concordantly, local peripheral pre-treatment with the selective P2Y1 (MRS2500, 0.01-10 pmol/paw), and P2Y6 (MRS2578, 3-30 nmol/paw) receptor antagonists significantly decreased 1% formalin-induced flinching behavior. Furthermore, the pronociceptive effect of ADP (100 nmol/paw) or MRS2365 (10 nmol/paw) and UDP (300 nmol/paw) or PSB0474 (10 pmol/paw) was blocked by the selective P2Y1 (MRS2500, 0.01 nmol/paw) and P2Y6 (MRS2578, 3 nmol/paw) receptor antagonists, respectively. Western blot analysis confirmed the presence of P2Y1 (66 KDa) and P2Y6 (36 KDa) receptors in DRG and sciatic nerve, and double immunostaining showed that 62.2% and 81.2% of DRG neurons express P2Y1 and P2Y6 receptors, respectively. Results suggest that peripheral activation of P2Y1 and P2Y6 receptors plays a pronociceptive role in formalin-induced pain. The use of antagonists of these receptors may be a useful strategy to treat local inflammatory pain.

Disclosures: **V. Solano-Olivares:** None. **F. Flores-Murrieta:** None. **J. Rodríguez-Silverio:** None. **H. Rocha-Gonzalez:** None. **V. Granados-Soto:** None. **P. Barragan-Iglesias:** None. **J. Pineda-Farias:** None.

Poster

240. Inflammatory Pain

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Program#/Poster#: 240.13/CC5

Topic: D.08. Pain

Support: Pediatric Rheumatic Disease Laboratory, University of Saskatchewan

Royal University Hospital Foundation

Children's Hospital Foundation of Saskatchewan

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Title: Protein levels of the resolvin D1 receptors GPR32 (G-protein coupled receptor 32) and FPR2 (formyl peptide receptor 2) are higher in juvenile rats with arthritis compared to young adult rats with arthritis

Authors: *T. D. WILSON-GERWING, A. M. ROSENBERG
Dept. of Pediatrics, Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: The pathophysiologic networks that mediate inflammatory pain in the peripheral and central nervous systems differ depending on whether inflammation is acute or, as in arthritis, progresses to become chronic. We have shown that age influences inflammatory pain responses and that juvenile rats with experimental arthritis (collagen-induced arthritis; CIA) have different outcomes than young adults; juvenile rats exhibit more rapid resolution of inflammation while young adults have more destructive and persistent inflammatory disease. Our preliminary results suggest that age-related differences in joint inflammation and pain may be explained by an imbalance between endogenous pro- and anti-inflammatory mediators. We hypothesized that the more favourable outcomes observed in juvenile rats with arthritis were due to a greater availability of receptors for the potent anti-inflammatory resolvin D1. Juvenile (5 week old) and young adult (13 week old) rats were injected intradermally with bovine type II collagen resulting in monophasic polyarthritis in the hind paws. Naïve age-matched rats were included as controls. Two weeks after the onset of CIA, animals were euthanized and dorsal root ganglia (DRG) were sectioned and processed by immunohistochemistry to detect levels of the resolvin D1 receptors GPR32 (G-protein coupled receptor 32) and FPR2 (formyl peptide receptor 2). In the naïve state, juvenile rats have higher levels of both GPR32 and FPR2 in the DRG compared to naïve young adult rats. Two weeks following CIA, there were increased levels of both GPR32 and FPR2 that was most noticeable in the juvenile CIA rats. Young adult rats with CIA had a slight increase in levels of GRP32 and FRP2 compared to the naïve state. This suggests young adult rats are less able to resolve acute inflammatory insult at the onset of CIA, and thus predisposed to experiencing higher levels of inflammation and pain following CIA than juvenile rats. A better understanding of interrelationships involved in the balance between resolution of acute inflammation and development of chronic inflammation/inflammatory pain will guide development of new approaches for arthritis patient care.

Disclosures: T.D. Wilson-Gerwing: None. **A.M. Rosenberg:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.14/CC6

Topic: D.08. Pain

Title: Autoantibody profiling in complex regional pain syndrome using luciferase immunoprecipitation system (lips) assay

Authors: A. BAYAT[1], *M. J. IADAROLA[1], A. BUVANENDRAN[2], A. J. MANNES[1], J. S. KROIN[3]
[1]Clin. Center, NIH, Dept. of Perioperative Med., BETHESDA, MD; [2]Dept. of Anesthesia & Critical Care, Univ. of Chicago, Chicago, IL; [3]Rush Med. Col., Chicago, IL

Abstract: Background: Complex regional pain syndrome (CRPS) represents a chronic pain condition that arises subsequent to peripheral nerve injury (CRPS-II) or in the absence of an apparent precipitating nerve injury (CRPS-I). It is usually occurs in the extremities and is characterized by severe pain, swelling, autonomic disturbances, skin changes and functional and movement impairments. The age range at presentation for CRPS is typically between 36 and 42 years, but it can occur in the extreme ages of children as well as the elderly. The presentation of CRPS occurs mainly in women who predominate over men in the range of 60 to 80%. The pathogenesis of CRPS is unclear and some evidence of immune system involvement has been elaborated. To test this possibility further we directly examined serum from CRPS and nerve injury patients for the presence of autoantibodies to a wide variety of potential autoantigens as well as antibody levels to some neurotropic viruses. **Methods:** Antibody levels against 50 different antigens were determined in serum samples of 103 patients [70 patients with CRPS (M=27,F=33); 28 patients with neuropathic pain (NP) (M=F=14); 4 patients with low back pain (LBP) (M=4) and one patient with post herpetic neuralgia (PHN) (F=1)] and 37 healthy volunteers to evaluate the role of the adaptive immune system in pathology of CRPS disease by using Renilla luciferase-antigen recombinant proteins in an immunoprecipitation (LIPS) assay. Antibody levels were measured as light units with a luminometer. Selection of target antigens was made based on previous studies in CRPS patients as well as antigens identified using a high density protein array probed with patient sera. Some known auto-antibodies in common auto-immune disorders and as well as antibodies against neurotropic viruses were measured in these patients. **Results:** Eleven patients in the CRPS group (16%) had high antibody titer against IL1-α. High titers to IL1-α were also observed in two NP patients and one healthy volunteer. Although we saw some sporadic high levels of antibodies to several other antigens; we did not

observe enrichment of specific autoantibodies in the CRPS patients for eighteen other cytokines or for antigens from known autoimmune disorders such Sjogren 's syndrome, Stiff Person Syndrome or Lupus, nor was there a clear association with antibody titers to neurotrophic viruses such as HSV. **Discussion:** These data identify a subset of patients with IL1-a autoantibodies. While a broad-spectrum sensitivity to auto-antigens does not appear to be a component of CRPS, further investigation of additional proteins and peptides may reveal a CRPS autoimmune signature.

Disclosures: A. Bayat: None. **M.J. Iadarola:** None. **A. Buvanendran:** None. **A.J. Mannes:** None. **J.S. Kroin:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.15/CC7

Topic: D.08. Pain

Support: NIH NS082746

Title: Endothelin and bradykinin signaling divergence in sensory neurons

Authors: *K. SZTEYN, R. GOMEZ, N. A. JESKE
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Abstract: Endothelin (ET) and bradykinin (BK) are two endogenous peptides that signal through Gαq-protein coupled receptors (GPCRs) to cause nociceptor sensitization and pain. Both peptides activate phospholipase C to stimulate Ca^{2+} accumulation, diacylglycerol production and protein kinase C activation, and are desensitized by a G-protein receptor kinase 2-dependent mechanism. However, ET produces a greater and longer-lasting nocifensive response than BK in multiple models, indicating a divergent signaling mechanism in primary afferent neurons. Using sensory neurons cultured from rat Trigeminal Ganglia, we have identified a pathway by which ET activates secondary Ca^{+2} channels to maintain neuronal depolarization. Inhibitors to ligand-gated ion channels and calcium release activated calcium (CRAC) channels abrogated ET effects on sensory neuronal Ca^{+2} accumulation measured in real time. Indeed, under these circumstances, the previous significant differences in pharmacological desensitization of BK and ET responses became identical. Furthermore, the withdrawal of Ca^{+2} from extracellular buffer also stunted any prolonged response to ET, indicating that plasma membrane channels contribute

to ET effects. Taken together, these ongoing studies highlight an important mechanistic difference between ET and BK signaling pathways in sensory neurons that may support why ET nocifensive behavior is significantly greater. Research support provided by NIH NS082746.

Disclosures: K. Szteyn: None. **R. Gomez:** None. **N.A. Jeske:** None.

Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Support: NS072432

Title: Complement C5a contributes to inflammatory heat hyperalgesia via TRPV1 and macrophage-dependent mobilization of NGF in mouse

Authors: L. P. SHUTOV[1], C. A. WARWICK[1], R. P. GUPTE[1], A. J. SHEPHERD[1], D. J. CLARK[2], T. M. WOODRUFF[3], D. P. MOHAPATRA[1], *Y. M. USACHEV[1]
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Abstract: The complement system is a principal component of innate immunity. It consists of more than 30 proteins that are rapidly recruited through a cascade of enzymatic reactions to contribute to host defenses through diverse mechanisms. In spite of growing evidence implicating the complement system in the development of pain hypersensitivity, the underlying mechanisms are not understood. We found that heat hyperalgesia induced by intraplantar injection of complete Freund's adjuvant (CFA) was markedly reduced in C5a receptor (C5aR) knockout (KO) mice and by administering the C5aR antagonist PMX53. Our data suggest the CFA-induced mechanical sensitization is also diminished in C5aR KO mice. Injection of 500 ng C5a into a mouse hind paw produced strong thermal and mechanical sensitization that lasted for at least 3 hrs, and fully recovered to pre-injection levels within 24 hrs. Heat hyperalgesia produced by C5a was blocked by the TRPV1 antagonist AMG9810 and was strongly reduced in TRPV1 KO mice. Somewhat surprisingly, C5a-induced mechanical sensitization was also inhibited by AMG9810 or by TRPV1 deletion. Examination of inflammatory mediators in the skin induced by intraplantar C5a injection showed a rapid increase in the levels of nerve growth

factor (NGF). Moreover, pre-injection of NGF-neutralizing antibody blocked the C5a-induced heat hyperalgesia. Immunohistochemical analysis of plantar skin sections showed prominent expression of C5aR in macrophages. Drug-induced macrophage depletion in transgenic MAFIA (macrophage Fas-induced apoptosis) mice abolished the C5a-induced heat hyperalgesia. Notably, intraplantar injection of NGF was still able to produce heat hyperalgesia in the macrophage-depleted mice. We propose that the complement fragment C5a contributes to inflammatory heat hyperalgesia by inducing macrophage-dependent production/release of NGF and consequent sensitization of TRPV1 to heat. Our findings highlight the importance of macrophage-to-neuron signaling in the pathogenesis of inflammatory pain and identify C5a and NGF as key mediators in this cross-cellular communication.

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Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Support: Université de Strasbourg

CNRS

INSERM

GLORIA, FP7 Grant n°: 602919

Title: Microglia specific Delta-Opioid Receptor deletion mouse model characterization

Authors: *H. MAURIN[1,2], D. REISS[3], L.-A. ROECKEL[3], B. L. KIEFFER[3], C. GAVÉRIAUX-RUFF[2,4]
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Abstract: Aim of investigation: Recently, some evidence for activated microglia being key-players in chronic pain persistence were described. Moreover both microglia and astrocytes have been proposed as critical actors in opiate-induced hyperalgesia and tolerance. The opioid receptors which are G protein-coupled receptors can be found under three different types, mu, delta and kappa, all of them being involved in analgesia. Delta opioid receptors (DOR) are of prime interest as potential therapeutic targets for chronic pain, besides the mu-opioid receptor that represents the main target of the clinically used opiates. Methods: In the present study, we focused on the role of microglial DOR, by generating a new conditional knockout mouse line with a specific deletion of specific DOR in microglia. We crossed the previously described DOR-floxed mice (Gaveriaux-Ruff et al. 2011) with microglia deleter LysM-Cre mice (Clausen et al. 1999). Results: We characterized this new mouse model by molecular and cellular approaches in both brain and spinal cord, followed by behavioral pain tests. In addition, we study the impact of the DOR deletion in microglia on inflammatory pain, following Complete Freund's Adjuvant (CFA) injection into hindpaw, as a model for inflammation-induced pain, and neuropathic pain induced by partial sciatic nerve ligation. Conclusion: Taken together, these results provide information of microglial DOR implication in pain perception, and suggest that microglial DOR are of prime interest in studying neuropathic pain.

Disclosures: H. Maurin: None. **D. Reiss:** None. **L. Roeckel:** None. **B.L. Kieffer:** None. **C. Gavériaux-Ruff:** None.

Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Support: PRF-BSR12130708

BMU20120310

Title: B vitamins suppress serine protease and protease-activated receptor 2 as well as behaviorally expressed hyperalgesia in Achilles tendonitis rats

Authors: W. B. SONG[1], Z.-J. HUANG[1], *X.-J. SONG[1,2]
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Abstract: We have recently shown that activation of Protease-activated receptor 2 (PAR2) is critical to development of hyperexcitability of dorsal root ganglion (DRG) neurons after nerve injury and the serine protease proteolytic activity and PAR2 activation may play an important role in development of inflammation and pain after Achilles tendonitis. Here we report that i.p. B vitamins can relieve tendon injury-induced hyperalgesia and its accompanied inflammation as well as suppress serine proteases activity and PAR2 activation. Achilles tendonitis was induced by a subcutaneous injection of collagenase in the hind limbs (500 units per rat). Collagenase-induced tendonitis resulted in a rapid-onset (within a day) and long-lasting (at least two weeks) mechanical allodynia and thermal hyperalgesia, in addition to the morphological manifestations of inflammation and tissue damage, e.g., the lesion site was largely filled with loose fibrous tissue, granulation tissue and connective tissues and intensive inflammatory cells accumulation. During the tendonitis, the serine protease proteolytic activity was increased and level of expression of trypsin and tryptase, the two major serine proteases, as well as the PAR2 protein are increased. The proinflammatory cytokines IL-1β and TNF-α were also greatly increased in the inflamed tendon. Repeated intraperitoneal injection of vitamin B1 (100 mg/kg), B6 (100 mg/kg), B12 (2 mg/kg), B vitamin complex (VBC) containing vitamin B1, B6 and B12 (33/33/0.5 mg/kg) (for consecutive 7 days), respectively, significantly reduced the thermal hyperalgesia and the histological signs of inflammation. ELISA assay showed that B6 and VBC greatly suppressed the increased levels of IL-1β and TNFα. Repetitive treatment of B6 and VBC (7 consecutive days) inhibited the tendon injury-induced increase of levels of serine protease proteolytic activity and upregulation of expression of PAR2, trypsin and tryptase. This study indicates that B vitamins can relieve tendon injury-induced thermal hyperalgesia and its accompanied inflammation probably through inhibiting serine proteases activity and PAR2 activation.

Disclosures: **W.B. Song:** None. **Z. Huang:** None. **X. Song:** None.

Poster

240. Inflammatory Pain

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Program#/Poster#: 240.19/CC11

Topic: D.08. Pain

Support: R01 NS069915 (MRV)

Title: The small GTPase Ras mediates Epac-induced sensitization of rat sensory neurons

Authors: *B. SHARIATI[1,2], E. L. THOMPSON[2], R. WANG[2], G. D. NICOL[2,3], M. R. VASKO[2,3]
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Abstract: Activation of a class of guanine nucleotide exchange factors (GEFs) known as exchange proteins directly activated by cAMP (Epacs) elicits hypernociception in animal models of pain presumably through a sensitizing effect on sensory neurons. However, the question remains as to the downstream signaling pathways in sensory neurons that mediate sensitization secondary to activation of Epacs. To address this question, we employed as endpoints of excitability the evoked release of calcitonin-gene related peptide (CGRP) and the number of action potentials (APs) generated in response to a ramp of depolarizing current. Pretreating sensory neurons with 3 μM of the acetoxymethyl-conjugated Epac agonist, 8-CPT-2'-O-Me-cAMP-AM (8CPT-AM) prior to and during a 30 nM capsaicin or 30 mM KCl stimulus augments the release of CGRP without affecting resting release. Relative to 3 μM of a PO_4AM_3 control, the 8CPT-AM compound increases capsaicin- and potassium-stimulated release of CGRP from 182 ± 9 to 284 ± 23 and 108 ± 7 to 240 ± 9 fmol/well/10 min, respectively. In current-clamp recordings where small-diameter sensory neurons are injected with a ramp of depolarizing current sufficient to elicit 2-4 APs under control conditions, superfusion with 3 μM 8CPT-AM over a 10 minute period increases the number of APs evoked by the ramp from 2.7 ± 0.4 to 9.5 ± 0.9. Internal perfusion with a pipette solution, wherein 3 mM of GDP-βS substitutes for 0.3 mM GTP, completely blocks sensitization produced by 3 μM 8CPT-AM. In contrast, the ability of 3 μM of the PKA agonist, N^6-Benzoyladenosine-3′,5′-cAMP-AM (6BNZ-AM) to augment AP number was unaffected by internal perfusion with GDP-βS. These data suggest that Epac-induced sensitization is a G-protein mediated phenomenon. Stable infection of isolated sensory neurons with a lentiviral (LV) construct containing the sequences to dominant negative Ras (DN-Ras), an internal ribosome entry site (IRES), and green fluorescent protein (GFP) also blocked the increase in AP firing produced by 3 μM 8CPT-AM, but did not block the increase in AP firing produced by 3 μM 6BNZ-AM**.** When small-diameter sensory neurons are internally perfused with 30 μg/mL of a Ras-neutralizing antibody over a 20 minute period, exposure to the Epac agonist does not augment the number of APs evoked by the ramp of current. Altogether, these data demonstrate that Epac-induced sensitization of sensory neurons is mediated by activation of the small GTPase, Ras.

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Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Support: ERC grant agreement n° 260914

Title: Tumor Necrosis Factor alfa rapidly modulates gating properties of tetrodotoxin-resistant sodium channels, leading to acute nociceptive hyperexcitability

Authors: S. GUDES[1,2], B. KATZ[1,2], S. LEV[1,2], *A. BINSHTOK[1,2]
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Abstract: Sodium channels are key players in determining the input-output properties of peripheral nociceptive neurons. Changes in gating kinetics or in expression levels of sodium channels by proinflammatory mediators, lead to increased neuronal gain and to direct activation of nociceptors. These effects could account for the pain hypersensitivity observed during inflammation. Proinflammatory mediator tumor necrosis factor α (TNFα) is secreted during inflammation and is strongly associated with the rapid onset, as well as long lasting, inflammation-mediated increase in nociceptive excitability via its effect on sodium channels. However, the underlying mechanism of the rapid component of TNFα-mediated nociceptive hyperexcitability and acute pain hypersensitivity is still not fully unraveled. Here we show, that TNFα rapidly increases excitability of peripheral nociceptive neurons and leads to acute pain hypersensitivity in adult rats, via a complex modulation of nociceptive sodium channels. TNFα primarily affects the slow and persistent tetrodotoxin-resistant (TTX-r) fractions of the sodium current, but also moderately increases TTX-sensitive (TTX-s) currents. TNFα leads to increase in availability of TTX-r sodium channels by partial relief of voltage dependence of slow inactivation, via p38 mitogen-activated protein kinase (p38 MAPK), thereby increasing nociceptive gain. Furthermore, TNFα in a p38 MAPK-dependent manner, increases current density and shifts the activation of the persistent fraction of the TTX-r current in a hyperpolarized direction, producing a substantial inward current around the threshold potential. Thus, during acute inflammation, TNFα-mediated p38MAPK-dependent increase in the availability of TTX-r sodium channels modifies the excitability of peripheral nociceptive neurons.

Disclosures: **S. Gudes:** None. **A. Binshtok:** None. **B. Katz:** None. **S. Lev:** None.

Poster

240. Inflammatory Pain

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 240.21/CC13

Topic: D.08. Pain

Title: Tnfα is involved in paclitaxel-induced chemotherapy-induced painful peripheral neuropathy

Authors: *Z. WU[1], M. MATA[2], D. FINK[2]
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Abstract: Treatment with the chemotherapeutic drug paclitaxel causes an early pain syndrome beginning shortly after the start of therapy and characterized by severe mechanical and cold allodynia followed later in the course with the development of neuropathy characterized by sensory loss. We have previously reported that paclitaxel administration to rats induces expression and release of soluble TNFα (sTNFα) in satellite cells of the dorsal root ganglion (DRG) through activation of the TLR4, and that activation of TNFR in DRG neurons by sTNFα results in increased expression of the pain-related ion channels TRPA1 and TRPV4. In the current study, we found that continued administration of paclitaxel (16mg/kg*)* for 5 weeks resulted in an increase in TNFα expression in the nerve that coincided with the emergence of electrophysiological abnormalities (decreased sensory nerve amplitude and reduced conduction velocity) and with increased in calpain activity in peripheral nerve. Expression of the soluble p55 TNF receptor in nerve *in vivo* using a herpes simplex virus-based vector blocked the increase in expression of TNFα and activation of calpain in sciatic nerve in paclitaxel animals, and prevented the reduction in sensory amplitude and conduction velocity. Taken together with our previous observation, the current results suggest that early entry of paclitaxel into DRG causes neuropathic pain by inducing expression of TRPA1 and TRPV4, while prolonged treatment results in nerve damage through induction of calpain activity. Both processes are triggered by paclitaxel activation of TLR4 in glial cells to trigger expression of TNFα.

Disclosures: **Z. Wu:** A. Employment/Salary (full or part-time):; Department of Neurology, University of Michigan. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH NS038850 and NS069378. **M. Mata:** None. **D. Fink:** None.

Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Support: US Fulbright Foundation

Title: Effects of alpha-7 nicotinic positive allosteric modulator on mechanical allodynia and TNF-alpha following LPS-induced neuroinflammatory pain in mice

Authors: *M. ABBAS, S. RAHMAN
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Abstract: Evidence indicates that inflammatory immune activation contributes to pathophysiology and maintenance of neuroinflammatory pain involving central nervous system alpha7 nicotinic acetylcholine receptors (nAChRs). Here, we examined the effects of TQS, an alpha7 nAChR positive allosteric modulator, on mechanical allodynia, hyperalgesia, tumor necrosis factor-α (TNFα) and norepinephrine (NE) following lipopolysaccharide (LPS)-induced neuroinflammation in mice. Pretreatment of TQS (1 or 4 mg/kg, i.p.) reduced LPS-induced increased mechanical allodynia in a dose-dependent manner. The effect TQS (4 mg/kg) was significantly ($p<0.05$) different from control. Similarly, pretreatment of TQS (1 or 4 mg/kg) significantly ($p<0.05$) reduced LPS-induced increased hyperalgesia in a dose-dependent manner. Furthermore, pretreatment of TQS (1 or 4 mg/kg) reduced LPS-induced increased TNFα level in hippocampus. In addition, pretreatment of TQS (1 or 4 mg/kg.) reversed the LPS-induced reduction of NE level in hippocampal tissue. Taken together, these results suggest that TQS decreases LPS-induced neuroinflammatory pain by modulating hippocampal TNFα and NE levels. Therefore, alpha-7 nicotinic positive allosteric modulatory site could be a potential target for the treatment of neuroinflammatory pain.

Disclosures: M. Abbas: None. **S. Rahman:** None.

Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Support: CNPq

Title: Time-course of local release of inflammatory cytokines in a plantar incision model in rats

Authors: *M. A. MEDEIROS[1], N. M. SOUZA[2], L. L. CASTRO[2], F. A. VANDERLINDE[2]
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Abstract: Postoperative pain is a common and unique form of acute pain and despite advances in their study, it remains undertreated. Tissue adjacent surgical incisions undergoes inflammation and nociceptive sensitization. Recent studies demonstrate that several cytokines may participate in the enhancement of nociception near these wounds. Thus, before analyze the time-course of local release of inflammatory cytokines in the plantar incision model in rats, we analyzed the pattern of thermal and mechanical hyperalgesia using the nociceptive tests of von Frey test and Hargreaves, respectively. Plantar incision surgery produced a reduction of the withdrawal thresholds in both tests from 1 hour to 96 hours after surgery. Therefore, we analyzed the local release of pro-inflammatory (IL-1β, IL-6 and TNF-α) and anti-inflammatory cytokines (IL-10 and IL-4) by chemiluminescence at 2, 6, 8, 24, 48, 72 and 96 hours after the plantar incision. Plantar incision surgery produced a significant increase in the concentrations of IL-1β and IL-6 six and 8 hours after incision. The concentrations of TNF-α were not statistically different from controls at any time-points studied. The concentrations of IL-10 significantly increased only 2 hours after incision. IL-4 was reduced 24 and 48 h after surgery. The maintenance of hyperalgesia does not appear to be associated with a sustained increase in the local concentrations of these cytokines. Different mechanisms and mediators must contribute to the maintenance of the hyperalgesia in this model of postoperative pain.

Disclosures: M.A. Medeiros: None. **N.M. Souza:** None. **L.L. Castro:** None. **F.A. Vanderlinde:** None.

Poster

240. Inflammatory Pain

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Support: Z01DE000664-16

Fondecyt 11110136

PAI 79100009

Title: TNF-alpha overexpression in the tooth pulp and root results in painful pulpitis

Authors: B. HALL[1], *E. UTRERAS PURATICH[2], M. PROCHAZKOVA[1], A. TERSE[1], J. DOLAN[3], A. KULKARNI[1]
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Abstract: TNF-alpha is a proalgesic cytokine that is commonly expressed following tissue injury and inflammation, leading to pain hypersensitivity in nociceptors. Chronic inflammation in tooth pulp can cause painful pulpitis, and in bone an imbalance in bone formation and resorption that often results in bone loss. Considering the known link between TNF-alpha and inflammatory pain, we utilized a transgenic mouse to conditionally overexpress TNF-alpha in both odontoblasts and osteocytes in order to study oral pain that would result from subsequent development of pulpitis and bone loss. A transgenic mouse line was engineered that requires Cre-mediated recombination to overexpress TNF-alpha. These mice were bred with a Dentin Matrix Protein 1 (DMP1) Cre line to overexpress TNF-alpha in both tooth pulp and bone. The resulting mice show inflammation in tooth pulp that resembles pulpitis, as well as periodontal bone loss. We detected increased TNF-alpha in the mandible of these mice and active TNF-alpha signaling in both tooth and bone. In the tooth pulp and alveolar bone, inflammatory infiltrates were seen that were comprised of CD3+ lymphocytes and Mac2+ macrophages. Lastly, we were able to demonstrate masticatory dysfunction in these mice, which can indicate oral pain. Using this mouse model displaying these inflammatory disorders, we can dissect out the molecular changes within the primary afferent nociceptors that innervate teeth and bone that occur due to inflammatory pain signaling.

Disclosures: B. Hall: None. **E. Utreras Puratich:** None. **A. Terse:** None. **M. Prochazkova:** None. **A. Kulkarni:** None. **J. Dolan:** None.

Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Title: Pharmacologic blockade of the cannabinoid enzyme monoacyglycerol lipase attenuates hyperalgesia induced by inflammatory arthritis

Authors: *S. R. NASS, S. G. KINSEY
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Abstract: Rheumatoid arthritis (RA) is the most prevalent chronic inflammatory joint disease characterized by pain, stiffness, swelling, and breakdown of cartilage in synovial joints. Current RA analgesic treatments are ineffective or induce negative side effects. Cannabinoids have antihyperalgesic and anti-inflammatory properties; however, the challenge remains to harness the medical potential of cannabinoids without inducing negative psychomimetic effects. An alternative approach focuses on manipulating endogenously produced cannabinoids (i.e., endocannabinoids). The most prevalent endocannabinoid, 2-arachidonoyl glycerol (2-AG), is catabolized by the enzyme monoacylglycerol lipase (MAGL). Pharmacological inhibition of MAGL increases brain levels of 2-AG and significantly decreases acute inflammatory pain. The present study tested the hypothesis that MAGL inhibition decreases hyperalgesia, and pain suppressed behavior, caused by collagen-induced arthritis (CIA), a well-established animal model of inflammatory arthritis. To induce CIA, male mice were immunized with an emulsion of collagen and complete Freund's adjuvant. CIA causes arthritic symptoms (i.e. paw redness and swelling), and significantly decreases spontaneous locomotor activity. We investigated the antihyperalgesic and decreased pain suppressed behavioral effects of the selective MAGL inhibitor JZL184 on CIA-induced thermal hyperalgesia in the hotplate and tail immersion assays, and spontaneous locomotor activity, respectively. Thermal hyperalgesia was significantly attenuated by JZL184 (8 or 40 mg/kg, ip) on both the hotplate and tail immersion assays. The pain suppressed spontaneous locomotor activity was reversed by JZL184 (8 mg/kg, ip). These results suggest that MAGL inhibition may be a promising strategy for the treatment of pain caused by inflammatory arthritis.

Disclosures: S.R. Nass: None. **S.G. Kinsey:** None.

Poster

240. Inflammatory Pain

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Topic: D.08. Pain

Support: OCRID Grant P20GM103648

Title: Effect of glutamate on carrageenan-induced acute pleurisy in rat

Authors: S. DAS[1], Z. ZHANG[1], M. B. ANDERSON[1], *K. S. CURTIS[2], K. E. MILLER[1]
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Abstract: Carrageenan-induced pleurisy is a well-established model of acute inflammation yet the pleural innervation and its role in inflammations is not well studied. In this project we are interested to understand the pleural innervation and its modulation of acute pleurisy by the neurotransmitter glutamate pathway. Rat models of glutamate and carrageenan-induced acute pleurisy were investigated to study the effect of glutamate. Our preliminary results suggested an increase in total cell count (almost 10 fold) as well as total exudate volume (up to 3 ml) in carrageenan-induced pleurisy. The transcript levels of glutamatergic genes in dorsal root ganglia (DRG) T1-4, e.g., glutaminase (GLS), is down-regulated in carrageenan-induced pleurisy while in glutamate-induced pleurisy, there is no effect on GLS message levels. Vesicular marker, VGLUT2, is down-regulated in both glutamate-induced as well as carrageenan-induced pleurisy models. Aspartate aminotransferase (AST) message levels did not show any significant changes in either treatment. Exitatory amino acid transporter EAAT3 is upregulated in glutamate-induced pleurisy while in carrageenan-induced pleurisy, it is downregulated. We further investigated to characterize the effect of glutamate on carrageenan-induced pleurisy in rat models. In these rats, glutamate pretreatment increased the carrageenan-induced total cell count while glucocorticoid (GC) pretreatment reduced the total cell count. At the same time, the visceral pleural membrane was extracted by shaving the frozen-lung very superficially and total RNA as well as total protein was isolated from these samples. Preliminary data indicates that carrageenan-induced pleurisy rescues the glutamate- and GC-induced down-regulation of AST and GLS message levels in visceral pleura. Contrary to message levels, carrageenan attenuated the expression of AST and VGLUT proteins in both glutamate- and GC-induced pleurisy models. Glutamate treatment increased the expression of all glutamatergic marker proteins, VGLUT2, AST as well as PGP9.5. Visceral pleural extraction was also isolated from different locations of lung namely, apical, basal and interlobar. VGLUT2 protein expression did not show site specific expression except in glutamate treated animals; VGLUT2 expression was increased on inter-lobar sections. The same was true for neuronal marker PGP 9.5 and Peripherin. Our future directions aim at further characterizing the glutamate dose response and their respective inhibitors on these proteins and their respective pathways which guide the neuroimmunomodulation of innervation in acute pleurisy in rat models.

Disclosures: **S. Das:** None. **K.S. Curtis:** None. **Z. Zhang:** None. **M.B. Anderson:** None. **K.E. Miller:** None.

Poster

241. Pain: Ion Channels and Physiology

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 241.01/CC19

Topic: D.08. Pain

Support: CIHR

AIHS

Title: Activity dependent upregulation of Cav3.2 channels by USP5 - implications for pain

Authors: ***P. STEMKOWSKI**, A. GARCÍA-CABALLERO, V. M. GADOTTI, L. CHEN, A. CHEN, N. D. BERGER, T. K. LAPOINTE, C. ALTIER, P. PODGORNY, G. ZAMPONI
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Abstract: BACKGROUND: Cav3.2 T-type calcium channels are essential contributors to the transmission of nociceptive signals in the primary afferent pain pathway. In various pathological conditions linked to pain, including diabetic neuropathy, intestinal inflammation and nerve injury, Cav3.2 channel expression (and activity) in nociceptive neurons is upregulated. Our laboratory has shown that this aberrant T-type channel expression involved an interaction with USP5, a deubiquitinating enzyme highly expressed in dorsal root ganglion (DRG) neurons and whose action is to decrease ubiquitination of Cav3.2, thereby leading to greater T-type channel stability in the cell membrane. Since the enhanced association between USP5 and Cav3.2 channels occurs in response to peripheral inflammation or nerve ligation, we hypothesized that the increase in neuronal activity, common to both injuries, is critical to this process. To test this possibility, we have adopted a new optogenetic method that allows for the transcutaneous activation of nociceptors with light without physical injury (Daou et al., 2013; J. Neurosci. 33: 18631-18640). METHODS: Mice homozygous for the Rosa-CAG-LSL-ChR2(H134R)-EYFP-WPRE conditional allele (*loxP*-flanked STOP cassette) were crossed with mice that express cre recombinase in TRPV1 cells. Thermal thresholds to radiant heat were determined in F1 males (8 - 12 weeks old) before and 40-60 min following prolonged (10 min) transcutaneous stimulation of the right hindpaw with suprathreshold (10 mW/mm^2) blue light (473 nm) under 2.5% isoflurane-induced anaesthesia. The laser was pulsed at 10 Hz with light delivered through a 200

μm fibre optic cannula at a distance of 0.5 cm from the plantar surface. The non-stimulated left hindpaw served as an internal control. Mice were then euthanized and L5 DRG harvested for a co-immunopreciptiation assay where Cav3.2 immunoprecipitates were probed for USP5 binding. RESULTS: We observed an ipsilateral 70% decrease hindpaw withdrawal latency to radiant heat (n=3), along with a 2.5 fold increase in binding of USP5 to Cav3.2 channel protein in L5 DRG (n=2). CONCLUSION: Neuronal activity in the absence of peripheral injury is sufficient to activate USP5 dependent enhancement of Cav3.2 channel activity. This in turn may reduce the firing threshold of afferent fibers, leading to a prolonged increase in neuronal excitability and thus a self-propagating sensitization. Although this scenario may involve the release of mediators, these experiments have the propensity to identify a novel form of activity-dependent plasticity in the primary afferent pain pathway.

Disclosures: P. Stemkowski: None. **A. García-Caballero:** None. **V.M. Gadotti:** None. **L. Chen:** None. **A. Chen:** None. **N.D. Berger:** None. **T.K. Lapointe:** None. **C. Altier:** None. **P. Podgorny:** None. **G. Zamponi:** None.

Poster

241. Pain: Ion Channels and Physiology

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Title: Hyperphosphorylation of Kv3.4 channels in DRG neurons as a putative mechanism of peripheral pain sensitization induced by spinal cord injury

Authors: *B. ZEMEL, T. HALA, A. LEPORE, M. COVARRUBIAS
Thomas Jefferson Univ., Philadelphia, PA

Abstract: The high-voltage activated A-type Kv3.4 channels are robustly expressed in dorsal root ganglion (DRG) neurons, where they regulate spiking and the repolarization of the action

potential (AP) in putative nociceptors (Ritter et al., J Physiol, 2012). The effects on AP repolarization depend on direct slowing/elimination of Kv3.4 fast inactivation following protein kinase C (PKC) activation. In addition, persistent PKC activation induces slow downregulation of the Kv3.4 current in heterologous cells and DRG neurons (unpublished). Calcineurin inhibition accelerates this downregulation. These modulations phenocopy changes induced by spinal cord injury (SCI) in putative rat DRG nociceptors exhibiting increased proportions of small (<16 pA) and slow inactivating currents. It is therefore possible that these changes are in part responsible for the SCI-induced hyperexcitability of the nociceptors and the associated pain sensitization (Ritter et al., submitted). Suggesting a peripheral mechanism of pain sensitization in SCI, we hypothesize that hyperphosphorylation of Kv3.4 channels might play a significant role in the SCI-induced hyperexcitable state of DRG neurons. The SCI-induced downregulation does not result from changes in gating, transcription, splicing and translation (Ritter et al. submitted). Consistent with Kv3.4 internalization, anti-Kv3.4 immunostaining demonstrated reduced surface expression in DRG neurons from SCI rats. Interactions between Kv3.4, PKC and related signaling molecules are further supported by the membrane-delimited nature of the elimination of Kv3.4 fast inactivation upon activation of G-protein coupled receptors in DRG neurons suggesting the presence of a Kv3.4 signaling microdomain (Ritter et al., J Physiol, 2012). Accordingly, immunostaining and confocal imaging demonstrated colocalization of Kv3.4 with the calcineurin α-subunit and PKCγ. To further probe the signaling microdomain hypothesis, we will present AKAP79/Kv3.4/PKC/calcineurin colocalization studies and high-resolution stimulated emission depletion (STED) imaging. We will also report validation of these results by co-immunoprecipitations and electrophysiology. Based on these studies, the current working hypothesis proposes that SCI promotes PKC activation and/or calcineurin inhibition, which renders Kv3.4 channels hyperphosphorylated and primed for internalization.

Disclosures: **B. Zemel:** None. **T. Hala:** None. **A. Lepore:** None. **M. Covarrubias:** None.

Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Support: National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology 2012M3A7B4035199

Title: Facilitation of hyperpolarization-activated cyclic nucleotide-gated channel by Prostaglandin E1 in dorsal root ganglion neurons in mice

Authors: *S. LEE, P. CHO, I. AN, S. JUNG
physiology of department department medicine of college Hanyang university, Physiol. of Dept. Dept. Med. of Col. Hanyang Univ., Seoul, Korea, Republic of

Abstract: Facilitation of hyperpolarization-activated cyclic nucleotide-gated channel by Prostaglandin E1 in dorsal root ganglion neurons in mice Sang Hoon Lee, Pyung Sun Cho, In Kyung An, Sung Jun Jung Department of Biomedical Science, Graduate School of Biomedical Science & Engineering, Hanyang University Hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels and their current, I_h have been suggested to play an important role in neuropathic pain by involvement in spontaneous ectopic discharges after peripheral nerve injury. Prostaglandin E1 (PGE1) has been well known as a therapeutic agent for lumbar spinal stenosis in clinical fields. The present study investigated the cellular action of PGE1 on HCN channel in primary dissociated neurons of dorsal root ganglion (DRG) in mice and pain behavioral responses. Amplitude of I_h increased about 130% by PGE1 in a dose-dependent manner in the medium-sized (30 - 40 μm) DRG neurons. Adenylyl cyclase inhibitor and 8-Br-cAMP inhibited the facilitation of I_h current by PGE1. EP2 receptor antagonist inhibited the facilitation of I_h induced by PGE1. Exposure of HCN-expressing DRG neurons to PGE1 increase in action potential frequency. In behavioral test, hind paw injection of PGE1 to hindpaw reduced the threshold to mechanical stimuli, indicating allodynia. In addition, PGE1-induced allodynia was blocked by CsCl and EP2 antagonist. In conclusion, PGE1 facilitated I_h current in medium-sized DRG neurons in mice, which was mediated by EP2 receptors that transmit a signal to adenylyl cyclase and increase the intracellular concentration of cAMP. Exposure to PGE1 would control action potential firing by enhancing their excitability in HCN-expressing DRG neurons. This molecular mechanism might be associated with PGE1-induced allodynia. Acknowledgements: This research was supported by Nano-Material Technology Development Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012M3A7B4035199).

Disclosures: S. Lee: None. **P. cho:** None. **I. An:** None. **S. jung:** None.

Poster

241. Pain: Ion Channels and Physiology

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 241.04/CC22

Topic: D.08. Pain

Title: Participation of cation channels in the peripheral pronociceptive effect induced by hydrogen sulfide in hyperglycemic rats

Authors: *J. E. ROA-CORIA[1], F. FLORES-MURRIETA[1,2], V. GRANADOS-SOTO[3], H. ROCHA-GONZÁLEZ[1]
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Abstract: Hydrogen sulfide (H2S) is considered a gasotransmitter that produces a pronociceptive effect in several pain models, but little is known about the molecular targets involved in such effect. The aim of this study was to establish the participation of TRPV1, TRPA1, ASIC, T-type calcium and L-type calcium channels in the nociception induced by H2S in peripheral neuropathy associated with diabetes. Hyperglycemia was induced by streptozotocin (50 mg/kg, ip) in Wistar rats, which had values of glucose >350 mg/dL within 3 weeks. Nociception was assessed by injection of 0.5 % formalin into the right hind paw of the rat. Local peripheral ipsilateral, but not contralateral, injection of H2S (3-100 µg/paw) increased nociceptive behavior in a dose-dependent manner. Moreover, pronociceptive effect of H2S was prevented by local administration of capsazepine (TRPV1 receptor antagonist; 3,10µg/pata), HC-030031 (TRPA1 receptor antagonist; 316,1000 µg/paw), mibefradil (T-type calcium channel blocker; 0.3,10 µg/paw), amiloride (ASIC blocker; 0.001,0.1 µg/paw) or bezamil (ASIC blocker; 0.0001,0.01 µg/paw), but not amlodipine (L-type calcium channel blocker; 1,30 µg/paw). These data suggest that the local pronociceptive effect induced by H2S in rats with peripheral neuropathy could be mediated by activation of TRPV1,TRPA1, ASIC and T-type calcium channels, so the use of blockers of these channels may be a useful strategy to treat painful peripheral neuropathy associated with diabetes.

Disclosures: J.E. Roa-Coria: None. **F. Flores-Murrieta:** None. **V. Granados-Soto:** None. **H. Rocha-González:** None.

Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Support: NIH GM078244

Title: Increased expression of Nav1.7, TRPV1 and Peizo2 channels contribute to the diabetes-induced hyperexcitability of dorsal root ganglion neurons

Authors: *M. E. O'LEARY[1], C. HO[2]
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Abstract: The hindpaws of STZ-treated diabetic rats display increased sensitivity to thermal and mechanical stimulation consistent with the development of neuropathic pain. To further characterize the underlying mechanisms we used a combination of electrophysiology and single-cell RT-PCR to investigate changes in ion channel function and expression in small-diameter (<25 μm) sensory neurons isolated from dorsal root ganglia (DRG). In response to supra-threshold stimulation, the neurons of diabetic rats displayed a parallel increase in the frequency of action potential firing and TTX-S Na^+ current amplitude. Single-cell analysis of potential TTX-S Na^+ channel isoforms revealed a selective increase in Nav1.7 mRNA in the neurons of diabetic rats that correlated with the observed increased Na^+ current. Auxiliary β subunits are known to regulate voltage-gated Na^+ channels and altered expression could also contribute to changes in Na^+ currents. Although β_1 and β_4 were found to predominate in small-diameter neurons their expression was not altered by diabetes. A piezoelectric probe was used to directly apply mechanical stimulation to the neuronal cell bodies. The peak mechanical currents measured in diabetic neurons were 2-fold larger than those of controls. Transcript analysis of this population revealed a significant increase in Piezo2 expression but no change in TRPA1. Screening revealed that TRPV1 was the predominant thermosensitive TRPV channel present in small-diameter neurons and its mRNA was upregulated in neurons of diabetic rats. Increased expression of TRPV1 is further supported by an increase in the capsaicin-activated currents in this population of neurons. These findings suggest that the upregulation of TTX-S Nav1.7, mechanotransducing Peizo2 and thermosensitive TRPV1 channels in small-diameter sensory neurons contribute to the increased mechanical and thermal sensitivity of diabetic rats.

Disclosures: M.E. O'Leary: None. **C. Ho:** None.

Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Title: Expression of alpha-2-delta-1 subunit at the lumbar spinal cord in spinal cord injury rats

Authors: K. KUSUYAMA[1], *T. TACHIBANA[1], H. YAMANAKA[2], K. NOGUCHI[2]
[1]Orthopaedic Surgery, [2]Anat. and Neurosci., Hyogo Col. of Med., Nishinomiya, Japan

Abstract: Spinal cord injury (SCI) commonly results not only in motor paralysis but also in the emergence of neuropathic pain, both of which can impair the quality of life of SCI patients. In clinical fields, it is well known that pregabalin, which binds to the voltage-gated calcium channel alpha-2-delta-1 subunit (Cava2d-1) has therapeutic effect on neuropathic pain after SCI. Previous study has demonstrated that SCI increased Cava2d-1 in L4-6 dorsal spinal cord of SCI rats demonstrated by Western blot and that the increase of Cava2d-1 was correlated with tactile allodynia of the hind paw. However, the mechanisms of effects of pregabalin on SCI-induced neuropathic pain are poorly understood. In this study we examined the cellular distribution of Cava2d-1 expression in L4-6 spinal cord of SCI rats using immunohistochemistry. Spinal cord contusion injury was produced in male Sprague- Dawley rats (180-200 g). Rats were anesthetized by intraperitoneal pentobarbital administration and the lamina of the T10 vertebrate was then removed. A contusion injury was generated using the IH impactor devise that emulates a 100-kilodyne weight-drop onto the dura mater from a distance of 3-4 mm(N=5). Sham control rats received laminectomy without the contusion injury (N=5). During pentobarbital anesthesia, perfusion fixation was performed using 1-4% paraformaldehyde and 0.1 M phosphate buffer solution (at pH 7.4) and the spinal cord (L4-6) was removed. The transverse sections were cut and processed for immunohistochemistry. Quantitative analysis was performed by NIH image software. Locomotor function recovery post SCI was monitored using the Basso, Beattie, and Bresnahan locomotor rating scale (BBB scale). Following SCI, the BBB scale was 2.4±0.6 at day 1, and increased to 12.4±0.6 four weeks after SCI, indicating the improvement of motor function. In the naïve rats we detected the expression of Cava2d-1 immunoreactivity mainly in lamina I and II in the dorsal horn. SCI significantly increased Cava2d-1 immunoreactivity in lamina I and II in the dorsal horn at four weeks after SCI significantly. These findings suggested that increase of Cava2d-1 in the L4/5 of dorsal horn after thoracic SCI was involved in the development of neuropathic pain in hindlimb. The increase of Cava2d-1 in superficial dorsal horn after SCI may be related to the pregabalin's effect on central neuropathic pain.

Disclosures: K. Kusuyama: None. **T. Tachibana:** None. **H. Yamanaka:** None. **K. Noguchi:** None.

Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

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Title: Role of NaVβ4 regulatory subunit and resurgent current in development of pathological pain

Authors: W. XIE[1], Z. TAN[2], C. M. BARBOSA-NUNEZ[2], *J. A. STRONG[1], T. R. CUMMINS[2], J.-M. ZHANG[1]
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Abstract: Abnormal spontaneous activity (SA) of sensory neurons plays a key role in many pain models. We previously showed that local inflammation of the L5 DRG (LID) causes mechanical and cold hypersensitivity and increases high frequency bursting SA in medium/large cells with high conduction velocities. Many neurons that express the Na channel isoform NaV1.6 pore-forming subunit have resurgent Na currents, which allow high frequency firing. *In vivo* knockdown of NaV1.6 with siRNA completely blocks pain behaviors and abnormal SA induced by LID. In expression systems, NaV1.6 does not exhibit resurgent currents, which in some neurons require the regulatory NaVβ4 subunit. We examined the role of NaVβ4 in the LID model. siRNA directed against NaVβ4, or nontargetting control siRNA, was injected into the L4 and L5 DRG in rats at same time of L5 DRG inflammation. Knockdown was verified with immunohistochemistry. NaVβ4 but not control siRNA reduced or abolished mechanical hypersensitivity (von Frey test) and allodynia (cotton wisp stroke test) on all days tested (POD1 - 28). Cold allodynia (acetone test) was not affected. Electrophysiological effects were first examined in isolated whole DRG with microelectrode current clamp. Cells were classified as C (unmyelinated), or A (myelinated) based on conduction velocity. On POD 3, SA incidence in A cells from NaVβ4 siRNA treated inflamed DRG was only 6.5%, similar to normal uninflamed DRG, reduced from 24% in inflamed DRG treated with control siRNA. DRG inflammation also reduced the rheobase and increased the fraction of cells showing multiple action potentials or subthreshold oscillations in response to supratheshold currents; these effects were largely blocked by NaVβ4 siRNA. Few effects of DRG inflammation or NaVβ4 siRNA were observed in C cells. Next, whole-cell patch clamp methods were used to record resurgent and transient Na currents in medium-sized DRG neurons (diameter: 35-45 μm) acutely cultured from normal DRG or DRGs isolated 3 days after local inflammation. In neurons with predominantly TTX-S

Na current, the transient current increased 27% after inflammation, resurgent current increased by 50%, and the ratio of resurgent to transient current increased 24%. In neurons with combined TTX-S and TTX-R currents (which generally lack TTX-S resurgent current), the TTX-S transient current density after LID was increased by 28% while the TTX-R transient current density was not altered. The results are consistent with a key role of spontaneous repetitive firing, and suggest that NaVβ4 mediated resurgent currents may play an important role in the hyperexcitability observed in this pain model.

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Poster

241. Pain: Ion Channels and Physiology

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Program#/Poster#: 241.08/CC26

Topic: D.08. Pain

Title: Roles of Acid-Sensing Ion Channels in neuropathic pain induced by experimental autoimmune encephalomyelitis

Authors: *I.-C. WANG[1], C.-H. LEE[2], S.-H. LIN[2], C.-Y. CHUNG[2], F. LIAO[2], C.-C. CHEN[2]
[1]Dept. of Life Science, Natl. Taiwan Univ., Taipei, Taiwan; [2]Inst. of Biomed. Sciences, Academia Sinica, Taipei, Taiwan

Abstract: Multiple Sclerosis (MS) is an inflammatory demyelinating disease in which neuropathic pain is now recognized as one of the major symptoms. To date, the underlying mechanism of neuropathic pain in MS is still a mystery. Previous studies have shown that tissue acidosis is induced by inflammation in a MS animal model. Furthermore, it's known that acidosis activates acid-sensing ion channels (ASICs) on the nociceptors. However, the relationships between ASICs and neuropathic pain in MS animal model remain unclear. Therefore, we hypothesize that ASICs may play a role in pain development in MS. We utilize experimental autoimmune encephalomyelitis (EAE), a well-established MS rodent model, to assess clinical deficits, mechanical response and pathological alterations in different ASIC subtypes knockout mice. All of the mice showed clinical deficits and mechanical hypersensitivity after EAE induction. Also, pathological studies demonstrated that dorsal root ganglion (DRG) neurons are injured after EAE induction by staining with neuron injury marker, ATF3. These results suggested that peripheral nerve may participate in the nociceptive

hypersensitivity in EAE. Moreover, analgesic drug application indicated that EAE-induced neuropathic pain is modulated by ASIC3 mediated anti-nociceptive pathway.

Disclosures: **I. Wang:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Dept. of life science, National Taiwan University. **C. Lee:** None. **S. Lin:** None. **C. Chung:** None. **F. Liao:** None. **C. Chen:** None.

Poster

241. Pain: Ion Channels and Physiology

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: D.08. Pain

Title: Discovery of a novel T-type calcium channel blocker for the treatment of neuropathic pain

Authors: *Y. HIRAI[1], T. KAWAZU[1], K. YASUTAKE[1], M. ADACHI[2], T. NAITOH[1], D. IKEGAMI[3], M. NARITA[3,4]
[1]Pharmaceut. Res. Dept., Biol. Res. Lab., Nissan Chemcal Industries, Ltd., Shiraoka-Shi, Saitama, Japan; [2]Pharmaceut. Res. Department, Chem. Res. Lab., Nissan Chem. Industries, Ltd., Funabashi-shi, Chiba, Japan; [3]Dept. of Pharmacology, Sch. of Pharm., Hoshi Univ., Tokyo, Japan; [4]Life Sci. Tokyo Advanced research center (L-StaR), Tokyo, Japan

Abstract: T (transient)-type calcium channels (TCCs) are low-voltage activated channels that open during a small depolarization of the cell membrane, which allows them to function at near-resting membrane potentials. TCCs are expressed in dorsal root ganglia (DRG), dorsal horn of the spinal cord and thalamus which related to pain signals. The excitability of the sensory neurons in DRG and dorsal horn caused by abnormal burst firing, has a critical function for hyperalgesia and allodynia. In diabetic neuropathy and the chronic nerve injury model of neuropathic pain, TCC current density is increased, and gene knockout or antisense knockdown of the Cav3.2 isoform, one of 3 subunits of TCCs, results in hyposensitivity to pain due to reduced excitability of neurons. Efonidipine is a dihydropyridine calcium channel blocker discovered by Nissan Chemical Industries, Ltd. We have investigated that efonidipine (racemate) blocks both TCC and L-type calcium channels (LCC), and R(-)efonidipine is reportedly a potent T-type calcium channel blocker (TCCB). We revealed that intrathecal administration of R(-)efonidipine reduced neuropathic pain behaviors, i.e., thermal hyperalgesia and mechanical

allodynia in the partial sciatic nerve ligation (Seltzer) model mouse. To further discover novel TCCBs, we conducted a high-throughput screening (HTS) campaign. Nissan Chemical's original focused library including about 20,000 small molecular compounds was assayed based on calcium influx assay using human Cav3.2 over-expressing HEK293 cells. Several active compounds (IC_{50} < 1 μM) were hit, and one of them was efficacious for the Seltzer model by intraperitoneal administration (30 mg/kg). Using these hit compounds, we started to perform optimization research for TCCB through SAR to improve their potency, selectivity, and effect in the formalin model. Finally, we discovered P11520031, a selective TCCB (IC_{50} for TCC: 0.20 μM, IC_{50} for LCC: 2.8 μM), which suppressed pain behaviors in the formalin test (55% inhibition @10 mg/kg p.o.) with no significant motor dyscoordination in the modified Irwin test (100 mg/kg p.o.). In conclusion, we eventually discovered a novel TCCB, which may be considered as a promising analgesic.

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Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Support: NIH Grant DE021847

Title: Thrombospondin-4 increase mediates disruption of intracellular calcium signaling in injured primary sensory neurons

Authors: *Y. GUO[1], H.-E. WU[1], Z. LUO[2], Q. HOGAN[1], B. PAN[1]
[1]Dept. of Anesthesiol., Med. Col. of Wisconsin, Milwaukee, WI; [2]Dept. of Pharmacol., Univ. of California Irvine, Irvine, CA

Abstract: Painful nerve injury disrupts calcium signaling in primary sensory neurons, including loss of sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) function, elevated plasma membrane Ca^{2+}-ATPase (PMCA) function and decreased endoplasmic reticulum (ER) Ca^{2+} storage. In the neuropathic pain model of spinal nerve ligation, expression of the extracellular matrix glycoprotein thrombospondin-4 (TSP4, non-glycosylated form) is increased, and TSP4 blockade can reverse or prevent behavioral hypersensitivity after SNL. TSP4 can decrease

calcium currents (I_{Ca}) through high-voltage-activated calcium channels and increase I_{Ca} through low-voltage-activated calcium channels in dorsal root ganglion (DRG) neurons, which mimic the change in axotomized peripheral sensory neurons. This study examines whether TSP4 can cause the disrupted levels of cytoplasmic and stored Ca^{2+} in primary sensory neurons that happened after injury. Concentration of intracellular calcium in dissociated DRG neurons was measured by microfluorometry (Fura-2). We find that TSP4 enhanced the store operated calcium entrance function, inhibited the SERCA function, accelerated the PMCA function and depleted calcium store in ER. Taken together, these findings indicate that TSP4 changes calcium signaling similar to that which happens in DRG neurons after injury, which suggests that TSP4 elevation may contribute to the pathogenesis of neuropathic pain.

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Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Support: Sunovion Pharmaceuticals Inc.

Title: Discovery and characterization of novel, potent and selective P2X4 receptor antagonists for the treatment of pain

Authors: *C. A. BOWEN[1], J. NEWCOM[1], W. YANG[1], M. ORSINI[1], L. HARDY[1], L. SCARABOTTOLO[2], A. DI SILVIO[2], J.-F. ROLLAND[2], F.-Y. ZHAO[3], H. WEI[3], D. C. SPANSWICK[3,4], T. LARGE[1]
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Abstract: Purinergic P2X4 receptors (P2X4R) are trimeric, ATP-activated cation channels that are widely distributed anatomically. Preclinical data indicate roles for P2X4R on CNS microglia and peripheral macrophages, respectively, in neuropathic and inflammatory pain. Consequently, P2X4R antagonists may be beneficial for the treatment of these pain conditions. Few potent and selective P2X4R antagonists have been described previously, and nonselective purinergic antagonists are reported to show species differences at P2X4R. Therefore, a campaign was

initiated to identify novel, potent and selective small-molecule antagonists that inhibit P2X4R across species and that have effects in multiple experimental pain models. Compounds were discovered which displayed a wide range of *in vitro* antagonist potencies (IC_{50}: <1 nM to >1 uM) with similar activities on human, mouse and rat P2X4R using FLIPR and electrophysiology assays, suggesting pharmacological similarities across species. Potent compounds were advanced into mouse models of neuropathic pain or peripheral inflammation after demonstrating selectivity against a broad panel of targets, including other P2X receptor subtypes. To assess potentially discrete roles of P2X4R in the CNS and periphery, two compounds (Compound 1: 280 nM and Compound 2: 12 nM IC_{50}) with low CNS exposures were chosen for further evaluation *in vivo*. Single intrathecal administration of Compound 1 at 3 and 10 nmol/5 uL selectively and significantly increased withdrawal thresholds of ipsilateral paws in a mouse spinal nerve ligation model, indicating a spinal anti-allodynic action. Repeated intraperitoneal dosing of Compound 2 at 100 mg/kg significantly attenuated intraplantar carrageenan-induced prostaglandin E2 concentrations, suggestive of peripheral anti-inflammatory action. In summary, novel, potent and P2X4R-selective small-molecule antagonists were discovered. Importantly, these molecules displayed similar *in vitro* potencies across species. Finally, two of these highly selective P2X4R antagonists demonstrated activity in experimental models of neuropathic pain or peripheral inflammation. Our data strongly support the hypothesis that P2X4R inhibition holds therapeutic promise for both centrally and peripherally mediated pain.

Disclosures: **C.A. Bowen:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **J. Newcom:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **W. Yang:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **M. Orsini:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **L. Hardy:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc. **L. Scarabottolo:** A. Employment/Salary (full or part-time):; Axxam SpA. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Sunovion Pharmaceuticals Inc. **A. Di Silvio:** A. Employment/Salary (full or part-time):; Axxam SpA. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Sunovion Pharmaceuticals Inc. **J. Rolland:** A. Employment/Salary (full or part-time):; Axxam SpA. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Sunovion Pharmaceuticals Inc. **F. Zhao:** A. Employment/Salary (full or part-time):; NeuroSolutions Ltd.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Sunovion Pharmaceuticals Inc. **H. Wei:** A. Employment/Salary (full or part-time):; NeuroSolutions Ltd..

B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Sunovion Pharmaceuticals Inc. **D.C. Spanswick:** A. Employment/Salary (full or part-time):; NeuroSolutions Ltd.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Sunovion Pharmaceuticals Inc. **T. Large:** A. Employment/Salary (full or part-time):; Sunovion Pharmaceuticals Inc..

Poster

241. Pain: Ion Channels and Physiology

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 241.12/CC30

Topic: D.08. Pain

Support: NIH Grant DA-K01-024751

NIH Grant NS42150

Title: Sigma-1 receptor regulates sarco-endoplasmic reticulum Ca2+-ATPase and store-operated Ca2+ entry activity in axotomized sensory neurons

Authors: *H.-E. WU[1], Y. GUO[1], B. PAN[1], Q. HOGAN[2]
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Abstract: Activation of the sigma-1 receptor (s1R) diminishes I_{Ca} and intracellular Ca^{2+} stores, which are also features of axotomy of primary sensory neurons, suggesting that sR1 actively regulates intracellular Ca^{2+} in painful nerve injury. The sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), plasma membrane Ca^{2+}-ATPase (PMCA), and store-operated Ca^{2+} entry (SOCE) are three critical pathways by which sensory neurons maintain cytoplasmic Ca^{2+} homeostasis. We therefore examined whether s1R activation modulates activity-induced cytoplasmic Ca^{2+} ($[Ca^{2+}]_c$) transients via regulating SERCA, PMCA or SOCE in injured sensory neurons. Sensory neurons were dissociated from dorsal root ganglia (DRGs) and recorded with Fura-2 microfluorometry. (**A**) SERCA function was isolated by blocking PMCA with pH8.8 bath solution and limiting mitochondrial Ca^{2+} buffering by evaluating small K^+-induced transients (35mM, 0.5s, without transient shoulder) in low bath Ca^{2+} (0.25mM). In uninjured neurons, s1R blocker BD1063 (10microM) and agonist (PTZ, 100microM) had no effect compared to vehicle.

Injured neurons (ligation of 5th lumbar spinal nerve, SNL) had slowed transient recovery (increased tau), which was further prolonged by BD1063 (10microM). (**B**) PMCA function was isolated by blocking SERCA with thapsigargin (TG, 1microM). Although injury slowed SERCA function (prolonged tau), there was no effect of BD1063 or PTZ on uninjured or injured neurons. (**C**) SOCE function was examined by Ca^{2+} readdition with bath change (0 to 2mM). A greater increase in $[Ca^{2+}]_c$ upon Ca^{2+} readdition was seen in injured neurons after BD1063 treatment compared with vehicle-treated neurons, indicating amplified SOCE function. These findings indicate that sR1 activity supports SERCA and SOCE function after injury, but has no affect on PMCA of injured neurons.

Disclosures: H. Wu: None. **Y. Guo:** None. **B. Pan:** None. **Q. Hogan:** None.

Poster

241. Pain: Ion Channels and Physiology

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 241.13/CC31

Topic: D.08. Pain

Title: A novel T-type calcium channel blocker P11520031 is effective for neuropathic pain without CNS-linked side effects

Authors: *Y. HAMADA[1], D. IKEGAMI[1], A. HAMADA[1], Y. HIRAI[2], T. KAWAZU[2], M. NARITA[1,3]
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Abstract: Recently, we newly synthesized a triazinone-derivatived T-type channel blocker (P11520031, IC50 = 0.20 ± 0.02 μM, calcium influx assay in hCav3.2/HEK293). We investigated the analgesic effect and the CNS-related side effects of P11520031 compared to pregabalin using the neuropathic pain model. Systemic administration of P11520031 (3, 10, 30, 100 mg/kg, p.o.) dose-dependently reduced neuropathic pain behaviors, i.e., mechanical allodynia and thermal hyperalgesia in PSNL (Seltzer's) model. Administration of P11520031 (3, 10 mg/kg, p.o.) also significantly reduced oxaliplatin-induced peripheral neuropathic pain. Next, we investigated the effect of P11520031 on somnolence and dizziness, known as adverse effects of pregabalin. In order to evaluate sleep liability, we analyzed electroencephalogram (EEG) and electromyogram in mice administered with pregabalin or P11520031. Analysis of EEG showed

that pregabalin at 10 mg/kg (p.o.) had a sleep-promoting profile with increased non-rapid eye movement (non-REM) and decreased wakefulness, whereas, P11520031 at 10 mg/kg (p.o.) had no effect on non-REM and wakefulness. In order to evaluate the effect on motor coordination, we performed the beam-walking test in rats. Administration of pregabalin (30 mg /kg, p.o.) prolonged the walking time and increased slipping counts, whereas P11520031 even at 100 mg/kg (p.o.) did not affect them. In conclusion, oral administration of P11520031 significantly reduced the neuropathic pain-like state without CNS-related side effects in rodents. These results suggest that this novel TCCB may be an attractive anti-neuropathic pain agent with better pharmacological profiles than pregabalin.

Disclosures: Y. Hamada: None. **D. Ikegami:** None. **A. Hamada:** None. **Y. Hirai:** None. **T. Kawazu:** None. **M. Narita:** None.

Poster

241. Pain: Ion Channels and Physiology

Location: Halls A-C

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Program#/Poster#: 241.14/CC32

Topic: D.08. Pain

Support: NIH Grant DE021847

Title: Thrombospondin-4 elevates sensory neuron excitability by decreasing N-type and increasing T-type calcium currents

Authors: *B. PAN[1], Z. LUO[2], Q. H. HOGAN[1]
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Abstract: Changes in both high-voltage–activated (HVA) and low-voltage–activated (LVA) calcium channels in primary sensory neuronsmay contribute to the generation of chronic neuropathic pain, and could be valuable targets for the development of novel analgesic drugs. Loss of HVA current and gain of LVA current cause elevated excitability in primary sensory neurons. Expression of the extracellular matrix glycoprotein thrombospondin-4 (TSP4, non-glycosylated form) in dorsal root ganglion (DRG) is increased after injury, and TSP4 blockade can reverse or prevent behavioral hypersensitivity after injury.TSP4 can decrease calcium currents (I_{Ca}) through HVA calcium channels and increase I_{Ca} through LVA calcium channels in DRG neurons, which mimic the change in axotomized peripheral sensory neurons.We therefore

investigated whether TSP4 regulates excitability of dorsal root ganglion neurons. We find that TSP4 decreased action potential generation following high frequency depolarization stimuli and both N-type and T-type calcium channel blockers attenuated TSP4's effect. In the neuropathic pain model of spinal nerve ligation, TSP4 application did not further increase the elevated excitability of injured DRG neurons. Taken together, these findings indicate that TSP4 elevation following peripheral nerve injury contributes to hypersensitivity of peripheral sensory systems by decreasing HVA and increasing LVA I_{Ca} in DRG neurons, making TSP4 in peripheral sensory neurons a potential target for analgesic drug development for neuropathic pain.

Disclosures: B. Pan: None. **Z. Luo:** None. **Q.H. Hogan:** None.

Poster

241. Pain: Ion Channels and Physiology

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NIH Grant NS050758 (BMD)

NIH Grant NS033730 (KMA)

NIH Grant NS023725 (HRK)

Title: Differential activation of cutaneous sensory neurons by optical activation of keratinocytes

Authors: *P. C. ADELMAN, K. M. BAUMBAUER, J. J. DEBERRY, R. H. MILLER, B. M. DAVIS, K. M. ALBERS, H. R. KOERBER
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Abstract: It has long been hypothesized that keratinocytes could play a significant role in the transduction of cutaneous stimuli. In order to test this hypothesis we developed a transgenic mouse model expressing channelrhodopsin-2 (ChR2) in keratinocytes by crossing Ai32 mice expressing ChR2 driven by the Rosa-26 promoter (ChR2-EYFP) with K14- keratin Cre mice. These mice exhibited robust expression of the EYFP tagged ChR2 in basal keratinocytes and hair

follicles of hairy skin and basal and suprabasal keratinocytes of glabrous skin. In addition intense staining was also found in Merkel Cells. Behavioral testing revealed that 10 applications of blue light (473nm; 39.7mW; 30s) per mouse elicited nocifensive responses in approximately 30% of the applications. This stimulation never evoked these responses in control groups (i.e. K14 Cre, Ai32 or WT). We next used an *ex vivo* skin/nerve/DRG/spinal cord preparation to record from individual cutaneous sensory neurons and characterize their sensitivity to mechanical and thermal stimuli. Fiber conduction velocities and the responses to natural stimuli were used to identify different types of nociceptors (e.g. CMH - C-fiber responding to mech., heat and cold stimuli). Next blue light (39.7mW; 5-10s) was applied to the cell's receptive field to determine whether keratinocyte activation elicited action potential (AP) firing in the nociceptive fiber. If no response was elicited, we paired blue light stimulation and natural stimuli (mechanical or thermal) to determine if keratinocyte activation potentiated responses in the nociceptive fibers. We found that keratinocyte activation elicited either suprathreshold or subthreshold responses in most types of cutaneous nociceptors. These included both C-fibers (CMH (5/5), CMHC (6/6), CH (4/7), CM (1/1), CMC (1/1), but not in CC (0/2) or CLTMR (0/1) and A-fiber nociceptors (A-HTMR (7/14)). Interestingly keratinocytes evoked responses in both polymodal nociceptors (mechanical and thermal) as well as those responding to a single modality. For A-LTMR fibers only the SA1 population responded to blue light stimulation. These results show that light activation of epidermal keratinocytes alone produces APs in multiple types of cutaneous sensory neurons, demonstrating that sensory transduction mechanisms may also reside in skin keratinocytes.

Disclosures: P.C. Adelman: None. **K.M. Baumbauer:** None. **J.J. DeBerry:** None. **R.H. Miller:** None. **B.M. Davis:** None. **K.M. Albers:** None. **H.R. Koerber:** None.

Poster

241. Pain: Ion Channels and Physiology

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Support: NIH grant NS075760

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NIH grant NS033730

NIH T32 NS073548

Title: Optogenetic control of neuromodulator release from keratinocytes

Authors: *R. H. MILLER, K. M. BAUMBAUER, P. C. ADELMAN, H. R. KOERBER, B. M. DAVIS, K. M. ALBERS
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Abstract: Recent studies have shown that the skin releases neuroactive substances that influence cutaneous afferent activity. Many of these substances, such as adenosine triphosphate (ATP), calcitonin gene related peptide (CGRP), acetylcholine, glutamate and neurotrophic growth factors are produced by epidermal keratinocytes. Evidence suggests these compounds directly and indirectly modulate the activity of sensory fibers that innervate the skin. Keratinocytes also express ligand gated and voltage-gated ion channels and growth factor/cytokine receptors, suggesting complex autocrine and paracrine signaling between epithelial and neural tissues. To advance understanding of the mechanisms regulating keratinocyte-neuronal communication we developed an optogenetic mouse model in which the light activated cation channel channelrhodopsin (ChR2) is targeted to K14 keratin expressing keratinocytes. Interestingly, K14-ChR2 mice exhibited nocifensive behaviors in response to blue light stimulation of the skin. Using an *ex vivo* skin-nerve preparation we found light activation of the skin also elicited action potentials (APs) in cutaneous sensory neurons. To investigate how activation of keratinocytes stimulates AP firing in sensory afferents, we established cultures of primary keratinocytes from K14-ChR2 expressing mice in order to assay neuroactivator release in response to light stimulation. We first measured the level of ATP, a neuroactivator released from keratinocytes that modulates afferent firing via activation of purinergic receptors. Results show that ChR2 activation results in a significant increase in ATP release following a brief (5s) blue light exposure. These findings suggest ATP contributes to the keratinocyte driven AP firing in afferents of K14-ChR2 mice.

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Poster

241. Pain: Ion Channels and Physiology

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 241.17/CC35

Topic: D.08. Pain

Support: NIH COBRE 2P20RR020145-06

Title: Stopping orofacial pain: Re-purposing old drugs for new use

Authors: *D. N. LYONS[1], T. KNIFFIN[2], L. ZHANG[1], F. MA[1], R. DANAHER[3], C. MILLER[4], C. CARLSON[2], K. WESTLUND HIGH[1]
[1]Physiol., [2]Psychology, [3]Microbiology, [4]Dent., Univ. of Kentucky, Lexington, KY

Abstract: Approximately 1/3 of the U.S. population suffers from a chronic orofacial/headache pain condition. Trigeminal neuropathic pain is one such unilateral orofacial pain syndrome characterized by constant aching and burning thought to be caused by unintentional injury or vascular mechanical irritation of the trigeminal nerve. Patients with this type of pain are currently treated with analgesics combined with anticonvulsants and/or antidepressants due to their anxiety related to pain. However, these drugs are proving to be unsatisfactory in relieving pain. The proposed studies demonstrate alleviation of chronic neuropathic orofacial pain- and anxiety-like behaviors by administering peroxisome proliferator-activated receptor gamma (PPARγ) agonist, pioglitazone (PIO), and NMDA receptor (NMDAR) partial agonist, D-Cycloserine (DCS) in the Trigeminal Inflammatory Compression (TIC) injury mouse model we devised to mimic chronic neuropathic orofacial pain. PIO is FDA approved as Actos® for treatment of Type II diabetes. Recent studies show that this drug is effective in reducing inflammation and other types of neuropathic pain. Likewise, DCS is FDA approved under the name Seromycin® as a broad spectrum antibiotic for tuberculosis which coincidentally also has unique binding ability for the glycine binding site of NMDAR. Studies show that DCS reduces hypersensitivity as well as anxiety through an NMDAR blocking mechanism in pre-limbic forebrain in certain neuropathy models. However, PIO and DCS have never been used to treat orofacial pain. Our findings determined that Pioglitazone and D-cycloserine each reduce trigeminal neuropathic pain in mice when administered solely and have a super additive effect when combined.

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Poster

241. Pain: Ion Channels and Physiology

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 241.18/CC36

Topic: D.08. Pain

Title: The effects of repeated forced exercise and/or caffeine on analgesia and analgesic tolerance

Authors: *J. W. PICKEL, V. SVYSTUN, G. PHANEUF, M. SHIELDS, E. GAUMER, J. A. SCHROEDER
Connecticut Col., New London, CT

Abstract: Caffeine containing supplements are widely and repeatedly used by athletes to enhance performance and resist fatigue. Caffeine has also been shown to be an effective analgesic adjuvant. In the current study, for ten consecutive days, rats received caffeine (5, 7.5 or 10 mg/kg, ip) or saline 5 minutes prior to being subjected to a 10 minute forced swim or no exercise. Tail flick nociception was measured at 15 (immediately post-swim), 30 and 45 minutes post injection. Results suggest that exercise alone significantly enhances analgesia immediately after swimming, however the effect dissipated by 15 minutes following exercise. Tolerance to exercise's analgesic effect did not develop over the course of ten days. Caffeine administered at 5.0 mg/kg did not enhance exercise induce analgesia. Preliminarty data suggests that higher doses of caffeine acts synergistically with exercise to enhance analgesia to tail flick nociception.

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Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Support: NIH R01 NS080954

Stanford Bio-X Neuroventures Program

Stanford Bio-X Interdisciplinary Initiatives Program

Title: Optogenetic tools for control of primary afferent neurons

Authors: *S. M. IYER[1], S. R. YOUNG[1], C. GORINI[1], K. L. MONTGOMERY[1], H. SCUTT[2], A. J. CHRISTENSEN[3], C. RAMAKRISHNAN[1], K. DEISSEROTH[1], S. L. DELP[1]
[1]Bioengineering, [2]Mechanical Engin., [3]Electrical Engin., Stanford Univ., Stanford, CA

Abstract: Primary afferent neurons are critical to how we perceive the world, mediating sensations ranging from touch to pain. Optogenetic tools have been used to great effect in controlling primary afferent sensory neurons, using both transgenic and virally mediated approaches. We have previously reported how intra-sciatic injections of AAV6-hSyn-ChR2-eYFP and AAV6-hSyn-NpHR3.0-eYFP may be used to optogenetically stimulate or inhibit pain perception respectively (Iyer, Montgomery, et al. 2014). The wide diversity of viral tropisms exhibited by adeno-associated viral vectors indicates that other AAVs may potentially be used to enable virally mediated optogenetic control over different classes of primary afferent neurons. We discuss preliminary results following intra-sciatic injection of scAAV8-CMV-GFP, which demonstrate that AAV8 transduces a largely non-overlapping set of primary afferent neurons when compared with AAV6. Neurons transduced by AAV8 project to the deep laminae in the dorsal horn of the spinal cord, send central projections rostrally through the dorsal columns in the spinal cord, and have large-diameter cell bodies and myelinated peripheral projections. We describe the immunohistological profile of these transduced neurons and effects of optogenetic activation of these neurons. We then extend our attempts to optogenetically control primary afferent nociceptors. We describe how the bicistronic vector AAV6-hSyn-NpHR-p2A-ChR2-eYFP may be used to achieve bidirectional control over pain perception through co-expression of ChR2 and NpHR in nociceptors in the same animal. Future studies using virally delivered ChR2 to optogenetically stimulate primary afferent nociceptors will require improved characterization of ChR2 expression. We describe dose-response curves for AAV6-hSyn-ChR2-eYFP injections, and discuss how sufficiently high titer viral injections (~1e11 vg) achieve persistent expression as late as 12 weeks following injection, while also characterizing the immune response that results from AAV6 injection.

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Poster

241. Pain: Ion Channels and Physiology

Location: Halls A-C

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Program#/Poster#: 241.20/DD2

Topic: D.08. Pain

Support: Grants-in-Aid and Special Coordination Funds from Kobe Gakuin University Joint Research (B)

Title: Exposure of early-life stress changes smechanical allodynia and depression-like behavior following nerve injury in mice

Authors: *T. NISHINAKA, K. NAKAMOTO, S. TOKUYAMA
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Abstract: Early life stress has recently been reported to be involved in the pathogenesis of psychiatric disorders and chronic pain in adult life. Maternal separation or deprivation is a widely used model of early life stress and induces abnormal behavior, such as an anxiety- or depression-like behavior in the adulthood of the offspring. In rats, maternal separation or deprivation has been shown to induce the change in the pain sensitivity and enhance the mechanical allodynia after nerve injury. However, there is a lack of information about the effect of early life stress on the pain modulation system in mice. In the present study, we evaluated the effect of early life stress on the pain sensitivity and mechanical allodynia induced by nerve injury in male and female mice. Furthermore, since chronic pain has been reported to induce emotional dysfunction, such as anxiety and depression, we evaluated the impact of early life stress on the emotional dysfunction after nerve injury. Maternal separation and social isolation (MSSI) used as early life stress. Mice were separated from dam and littermate for 6 h per day during postnatal days 15-21 and then were housed individually until the end of study. In 7 weeks of age, an anxiety-like behavior was evaluated by elevated plus maze test. In 9 weeks of age, the sciatic nerve was partially ligated to elicit neuropathic pain. Mechanical sensitivity and nerve injury-induced mechanical allodynia was evaluated by using von Frey test. Depression-like behavior was evaluated by using forced swim test at 3 weeks after nerve injury. Results revealed that in the elevated plus-maze test, anxiety-like behavior was enhanced in only female mice with MSSI. The von Frey test showed that the MSSI had no effect on mechanical sensitivity before nerve injury. However, nerve injury-induced mechanical allodynia in ipsilateral paw was enhanced by MSSI in male and female mice. Furthermore, the increasing response to mechanical stimulation in contralateral paw is observed in MSSI male and female mice. The forced swim test showed that depression-like behavior is observed in stressed female mice after nerve injury. Our findings suggested that neuropathic pain was exacerbated by MSSI in adult male and female mice. Overall, this mouse model may be useful for the understanding of the molecular mechanisms underlying the reciprocal relationship between early life stress and chronic pain.

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Poster

241. Pain: Ion Channels and Physiology

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Program#/Poster#: 241.21/DD3

Topic: D.08. Pain

Support: NMRC/1255/2010

NMRC/CBRG/0050/2013

Title: Cholinergic mechanism in posterior hypothalamus: Affects on neural activity in the forebrain and animal behavioral responses to noxious stimuli

Authors: *Z. WANG, M. ARIFFIN, S. KHANNA
Natl. Univ. of Singapore, Singapore, Singapore

Abstract: It has been proposed that the posterior hypothalamus is part of an arousal system. Indeed, acutely inactivating different areas of the region, including the supramammillary nucleus (SuM), attenuates electrophysiological indices of hippocampal and cortical information processing, prevents behavioral arousal during general anesthesia and prolongs the duration of anesthetic effect. Interestingly, we have earlier reported that cholinergic manipulation of the lateral SuM region (lSuM), affects neural activity in the dorsal hippocampus field CA1. In the present study, we have investigated whether (a) cholinergic activation of lSuM affect index of neural activity in cortex and hippocampus, and (b) alters animal behavioral responses to salient/affective stimuli. Using the induction of c-Fos, a transcription protein, as marker of synaptic excitation, we observed that unilateral microinjection of the cholinergic agonist, carbachol (0.156μg/μl, 0.1μl), into the lSuM region in anesthetized animals evoked a robust neuronal activation in ipsilateral cortex, and an increase in the hippocampus and the dentate gyrus. In experiments in awake animals, microinjection of cholinergic nicotinic-receptor antagonists, mecamylamine (2μg/μl, 0.1μl), into the lSuM on left partly attenuated nociceptive behaviors to hind paw injection of the algogen, formalin, into the contralateral hind paw. Thus, a decrease in nociceptive flinching, but not licking was observed in the 2nd phase of the formalin test. The cholinergic muscarinic-receptor antagonist, atropine (0.0068μg/μl, 0.1μl), however had no effect on nociceptive behaviors. Crucially, trends suggest that mecamylamine microinjected into the lSuM region also attenuates peripheral hypersensitivity in the contralateral hind paw in the complete Freund adjuvant (CFA) model of persistent inflammatory pain. However, mecamylamine did not affect reflexive withdrawal per se. Collectively the foregoing data (a)

points to a potential for cholinergic transmission in posterior hypothalamus in the mediation of neural activity in forebrain areas and (b) suggests that nicotinic-cholinergic mechanisms in the region modulate affective-motivational behaviors and tissue damage-induced peripheral hypersensitivity. The peripheral hypersensitivity is suggested to reflect an interaction between the external stimuli and the intrinsic state of brain activity (i.e. responsivity salience) that is altered due to the pain state.

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Poster

241. Pain: Ion Channels and Physiology

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Support: NIH Grant DA011471

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NIH Grant CA091007

Title: Inhibition of anandamide hydrolysis reduces nociceptor sensitization through CB1 receptors in a murine model of chemotherapy-induced peripheral neuropathy

Authors: *M. L. UHELSKI[1], C. HARDING-ROSE[2], D. SIMONE[2]
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Abstract: Painful chemotherapy-induced peripheral neuropathy (CIPN) is the major dose-limiting side effect of chemotherapy, which impacts the efficacy of therapy for patients who are unable to tolerate increasing doses. In addition, many patients continue to experience neuropathic symptoms for months or years after treatment has ended. The mechanisms underlying neuropathic pain due to chemotherapy has been explored in animal models. The platinum compound cisplatin produces mechanical, heat, and cold hyperalgesia in rodents and is associated with damage to primary sensory neurons. Cisplatin has been shown to sensitize nociceptors, evidenced by an increase in spontaneous discharge among both Aδ- and C-fiber nociceptors and an increase in evoked responses. Cannabinoids and drugs that inhibit hydrolysis of endocannabinoids reduce neuropathic pain, including pain from CIPN. Here we examined the effects of URB597, which inhibits the breakdown of anandamide by FAAH, on sensitized C-

fiber nociceptors following cisplatin treatment. Male C3H/HeJ mice (n = 53) received daily IP injections of cisplatin (1mg/kg) for 7d prior to electrophysiology experiments. A 10µl injection of URB597 (9µg, n = 19) or vehicle (n = 14) was administered into the receptive field of well-isolated, sensitized C-fibers. The CB1 and CB2 antagonists AM281 (10µg, n =10) and AM630 (10µg, n = 6), respectively, were administered 5 min prior to URB597 in order to determine the contribution of each receptor subtype to the drug effect. Fibers treated with URB597 demonstrated significant attenuation of spontaneous discharge (from 0.20±0.04 Hz to 0.03±0.01 Hz at 2 hr post-injection), an increase in response thresholds (from 19.6±6.9 mN to 147.1±54.0 mN at 2 hr post-injection), and a decrease in responses evoked by a standard suprathreshold mechanical stimulus (147 mN force; from 44.5±6.4 impulses to 8.2±2.7 impulses at 2 hr post-injection). These changes were mediated by CB1 receptors, as shown by inhibition of these effects in fibers pre-treated with AM281 but not AM630. These results provide evidence that the antihyperalgesic effect of URB597 demonstrated in behavior studies is mediated by effects of CB1 receptor activation. Continued research into the mechanisms underlying the involvement of the peripheral endocannabinoid system in the attenuation of cisplatin-induced hyperalgesia could contribute to the identification of novel targets for the treatment of chronic pain in CIPN patients.

Disclosures: M.L. Uhelski: None. **D. Simone:** None. **C. Harding-Rose:** None.

Poster

241. Pain: Ion Channels and Physiology

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 241.23/DD5

Topic: D.08. Pain

Support: DA021644

DA035068

Title: Optimization of a cisplatin model of chemotherapy-induced peripheral neuropathy in mice: Use of vitamin C and sodium bicarbonate pretreatments to reduce nephrotoxicity and improve animal health status

Authors: *J. GUINDON[1], L. DENG[2], B. FAN[2], J. WAGER-MILLER[2], A. G. HOHMANN[2]
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Abstract: Background: Cisplatin, a platinum-derived chemotherapeutic agent, produces antineoplastic effects coupled with toxic neuropathic pain and impaired general health status. These side-effects complicate long term studies of neuropathy or analgesic interventions in animals. We recently demonstrated that pretreatment with sodium bicarbonate (4% $NaHCO_3$) prior to cisplatin (3 mg/kg i.p. weekly up to 5 weeks) was associated with improved health status (i.e. normal weight gain, body temperature, creatinine and ketone levels, and kidney weight ratio) in rats (Guindon et al., 2013). To reduce the nephrotoxic effects of cisplatin treatment in mice, we compared pretreatments with sodium bicarbonate (4% $NaHCO_3$ s.c.), vitamin C (25 mg/kg s.c.), resveratrol (25 mg/kg s.c.) and saline (0.9 % NaCl). **Results:** Cisplatin-treated mice receiving saline pretreatment exhibited elevated ketone, creatinine and kidney weight ratios, representative of nephrotoxicity. Vitamin C and sodium bicarbonate lowered creatinine/ketone levels and kidney weight ratio whereas resveratrol normalized creatinine levels and kidney weight ratios similar to saline pretreatment. All pretreatments were associated with decreased ketone levels compared to saline pretreatment. Cisplatin-induced neuropathy (i.e. mechanical and cold allodynia) developed equivalently in all pretreatment groups and was similarly reversed by either morphine (6 mg/kg i.p.) or ibuprofen (6 mg/kg i.p.) treatment. RT-PCR showed that mRNA levels for IL-1β were increased in lumbar spinal cord of cisplatin-treated groups pretreated with either saline, $NaHCO_3$ or resveratrol/cisplatin-treated groups. However, IL-6 and TNF-alpha were elevated in the kidneys in all cisplatin-treated groups. Our studies also demonstrate that 60 days after the last cisplatin treatment, body weight, body temperature, kidney functions and mRNA levels have returned to baseline although the neuropathic pain (mechanical and cold) is maintained. **Conclusions:** Studies employing cisplatin should include $NaHCO_3$ or vitamin C pretreatment to improve animal health status and reduce nephrotoxicity (lower creatinine and kidney weight ratio) without affecting the development of chemotherapy-induced neuropathy or analgesic efficacy.

Disclosures: J. Guindon: None. **L. Deng:** None. **B. Fan:** None. **J. Wager-Miller:** None. **A. G. Hohmann:** None.

Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Support: NIH Grant R44NS062477

Title: *In vitro* electrophysiological screening with dorsal root ganglion neurons for pain related assays

Authors: A. M. NICOLINI, M. W. BROCK, C. M. ARROWOOD, *D. C. MILLARD
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Abstract: The sensation of pain is transmitted from nociceptive nerve endings to the central nervous system along axons of neurons in the dorsal root ganglion (DRG). Damage to these primary afferents, or inherited defects in the proteins underlying their electrical or sensory function, can cause neuropathic pain, a persistent sensation of pain caused by increased spontaneous activity of DRG neurons. Pain research has thus far been predominantly based on animal models, in part due to a lack of predictive *in vitro* screening methods. The development of a high throughput *in vitro* assay for pain thus stands to significantly impact the discovery of therapies for chronic pain. Here, we present a non-invasive technique for directly monitoring electrical responses in commercially-available rat primary DRG neurons cultured in 12- and 48-well microelectrode array (MEA) plates. PEI-Laminin coating of the MEA wells yielded excellent cell adhesion, clear neurite outgrowth, and a low rate of spontaneous action potential spikes between days 3 and 10 post-plating. Increased spike activity in DRG neurons could be reproducibly evoked with temperature changes, electrical stimulation, and application of the TRPV1 agonist capsaicin, demonstrating that cultured neurons recapitulate characteristic *in vivo* nociceptor electrophysiological phenotypes. Consistent with *in vivo* function, individual neurons displayed sensitivity to different temperature ranges, including cold, heat, and noxious heat. The response to capsaicin contained transient and persistent components, was dose-dependent, and could be blocked by the TRPV1 antagonists DHEA or capsazepin, illustrating the utility of this assay for screening potential pain inhibitors. In summary, we present an *in vitro* MEA-based model of nociceptor function that exhibits characteristic electrophysiological phenotypes and has applications in pain research and drug screening.

Disclosures: **A.M. Nicolini:** A. Employment/Salary (full or part-time):; Axion BioSystems, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axion BioSystems, Inc. **D.C. Millard:** A. Employment/Salary (full or part-time):; Axion BioSystems, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axion BioSystems, Inc. **M.W. Brock:** A. Employment/Salary (full or part-time):; Axion BioSystems, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axion BioSystems, Inc. **C.M. Arrowood:** A. Employment/Salary (full or part-time):; Axion BioSystems, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axion BioSystems, Inc.

Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Support: LEAD project, National Institute of Advanced Industrial Science and Technology (AIST)

Title: Development and characterization of a primate model of central post stroke pain

Authors: K. NAGASAKA[1,2], I. TAKASHIMA[2], K. MATSUDA[2], *N. HIGO[2]
[1]Grad Sch. Comp Human Sci., Univ. of Tsukuba, Tsukuba, Japan; [2]Systems Neurosci Group, Human Tech. Res. Inst, AIST, Tsukuba, Japan

Abstract: Central post stroke pain (CPSP) occurs after damage in the somatosensory system of the brain including thalamus. CPSP-induced allodynia, in which innocuous stimuli perceived as painful, decreases the quality of life of the affected patients. The development of animal models of CPSP is essential to investigate the underlying mechanisms. Animal models of thalamic CPSP are recently developed in rodents (Wasserman and Koeberle, 2009, Neuroscience 161, 173-183; Hanada et al., 2014, Neuroscience Research, 78, 72-80), while the model using nonhuman primates is also a favorable model to understand neural structures and circuits involved in the production of CPSP because of its close similarity with humans. Therefore, we attempted to establish a nonhuman primate model of thalamic CPSP using macaque monkeys. The location of ventral posterolateral nucleus (VPL) of the thalamus was determined by magnetic resonance imaging (MRI) and extracellular recording of neuronal activity during a tactile stimulation. Collagenase type IV, a proteolytic enzyme, was then injected to induce a hemorrhagic lesion in the identified VPL. T2- MRI imaging and histological analysis using Nissl staining confirmed that the lesion is localized to a small region within VPL. A mechanical withdrawal test using von Frey filaments showed a significant attenuation of the withdrawal threshold in the hand contralateral to the lesion at several weeks after the lesion. A thermal stimulation test also showed a significant decrease in the withdrawal latency to the thermal stimulation of 50℃. These changes lasted for at least 3 months after the lesion induction. The behavioral analyses indicated that both tactile and thermal allodynia were induced in the VPL-lesioned monkey. In addition, we investigated the brain areas with significantly increased activities for thermal stimulation at 50℃ compared with control thermal stimulation at 37℃ using functional MRI (fMRI). The fMRI analysis indicated that the thermal stimulation at 50℃ showed significantly increased activity in the cortical areas that are identical to those observed in the fMRI investigations of CPSP patients,

such as the primary (S1) and secondary somatosensory cortices (S2) and the insular cortex of the lesional hemisphere. The present CPSP model using macaque monkey is useful for studying plastic changes of neural structures and activities that underlie CPSP, especially those occurred in the cortical neurons after thalamic hemorrhage.

Disclosures: **K. Nagasaka:** None. **I. Takashima:** None. **K. Matsuda:** None. **N. Higo:** None.

Poster

241. Pain: Ion Channels and Physiology

Location: Halls A-C

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Program#/Poster#: 241.26/DD8

Topic: D.08. Pain

Title: Mentha Piperita (peppermint) anti-nociception: Measurement in rats in the hotplate assay of the nociception

Authors: ***W. MICHNO**, E. P. WIERTELAK
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Abstract: Mentha piperita (peppermint), a cross-hybrid of Mentha Aquatica (water mint) and Mentha Spiciata (spearmint), is an aromatic herbaceous plant from the Labiatae family that for centuries has been used in folk medicine for the treatment of various disorders. Peppermint has been shown to possess anti-microbial, anti-fungal, and anti-inflammatory properties; possible anti-nociceptive properties of peppermint have also been suggested. The main constituent of the plant, menthol, is often used to counteract pain, typically due to its cooling effect, however peppermint oil has also reduced symptoms of post-herpetic neuralgia. Previous work in mice suggests dosages 200mg/kg and 400mg/kg of peppermint solutions (either ethanol extraction or decoction) produce an anti-nociceptic effect to thermal stimulation. The present study aimed to investigate whether peppermint exhibits the same anti-nociceptive properties in rat models of nociception. The fresh plant was first air-dried away from sunlight, then freeze-dried; the handpicked and selected for quality leaves were then extracted with 99.5% ethanol. Animals received either of the two dosages that have been shown to be effective in mice, or vehicle, using a blinded method. Nociceptive sensitivity was tested via the hotplate assay of nociception. Animals receiving either of the dosages exhibited significantly longer latencies in the hotplate test compared to controls. Further studies employing the five main bioactive constituents of the plant are ongoing; elucidation of the nociceptive effect of peppermint and the underlying nociceptive circuitry involved might lead to novel treatments for the control of pain in humans.

Disclosures: **W. Michno:** None. **E.P. Wiertelak:** None.

Poster

241. Pain: Ion Channels and Physiology

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Program#/Poster#: 241.27/DD9

Topic: D.08. Pain

Title: Kappa-Opioid receptor agonist analgesia and antianxiety: Effects of Salvinorin A in rat

Authors: ***E. P. WIERTELAK**, S. A. ANDERSON, Jr., H. C. GEMRICH, T. M. NICHOLS-MEADE, S. SANGGAARD
Neurosci. Studies, Macalester Col., SAINT PAUL, MN

Abstract: Salvinorin A is the main active component of Salvia Divinorum, a plant indigenous to Oaxaca, Mexico. The subjective effects of S. Divinorum are described as being akin to lysergic acid diethylamide (LSD) and other classical hallucinogens that interact with the serotonin 5-HT2A receptor subtype. However, Salvinorin A does not interact with this receptor (Listos et al., 2011). Therefore, Salvinorin A can be defined as a structurally unique, non-nitrogenous, highly selective kappa opioid receptor (KOR) agonist (Roth et al, 2002). Results from various studies exemplify a range of effects similar to known KOR agonists, although reports of analgesia from Salvinorin A have been somewhat varied and collectively limited (McCurdy et al., 2006). Braida et al. (2009) found no dose-dependent effects of Salvinorin A in the Elevated Plus Maze, which tests for anxiety. Their results suggest that Salvinorin A could possess a slight anxiolytic effect. This present study expands upon previous work by testing the analgesic and anxiolytic effects of Salvinorin A on rats using the hot plate, tail-flick, formalin and Elevated Plus Maze assays at three different dosages of Salvinorin A: 0.25 mg/kg, 0.50 mg/kg, and 0.75 mg/kg. Significance was found in open-arm time between the control and 0.50 mg/kg and between 0.50 mg/kg and 0.75 mg/kg. Behaviorally, rats given the 0.50 mg/kg dosage exhibited exploratory activity, as opposed to the 0.75 mg/kg, which induced hypolocomotion and avoidance of the open-arms. These results suggest that 0.50 mg/kg of Salvinorin A has a significant anxiolytic effect in rats. Studies of the potential analgesic effects are currently ongoing. In the future, Salvinorin A could serve as a template for non-addictive opioids, provided dose-dependent dysphoria and hallucinations are eliminated. More research needs to be done to understand the mechanisms behind Salvinorin A, in order to utilize its possible anxiolytic and analgesic therapeutic effects.

Disclosures: **E.P. Wiertelak:** None. **S.A. Anderson:** None. **H.C. Gemrich:** None. **T.M. Nichols-Meade:** None. **S. Sanggaard:** None.

Poster

241. Pain: Ion Channels and Physiology

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Topic: D.08. Pain

Support: Migraine Research Foundation

Title: Use of a light dark choice paradigm for defining migraine like pain in mice after mast ell degranulation

Authors: ***T. L. YAKSH**, R. RAMACHANDRAN
Univ. California, LA JOLLA, CA

Abstract: Background: Photophobia leading to light-averse behavior is commonly reported by migraineurs during acute attacks and, to a lesser degree, between attacks. Therefore as an alternative to the measurement of tactile thresholds, we sought to assess the development of light aversive behavior in mice as a surrogate of photophobia. **Methods:** Mice (male and female, C57Bl6) were placed in a test system composed of two equally sized chambers. Animal could move freely between the two chambers through a small portal. One chamber was exposed to light from the top (100 lux) while the other chamber was completely dark. The animals were acclimatized for 20 min in the chamber one day before testing. On the day of testing, mice were tested for 10 min prior to the injection with saline or the drug i.p. Following saline/drug administration, animals were tested for 10 mins at different time points (15 min, 1 hr, 2 hrs and 4 hrs). The following parameters were tested using the software: 1) Time spent in the light and dark box 2) Transition of animals from dark to light. Presence and time spent in each chamber was assessed by the obscuration by the animal of red light LEDs. On test day, mice received i.p injections of vehicle (control), the NO donor sodium nitroprusside (1 mg/kg) or the mast cell degranulating agent compound 48/80 (2mg/kg) which has been shown to induce a prolonged state of excitation in primary afferent nociceptors followed by activation of second order neurons. **Results:** After adaptation, the animal's relative time in the light / dark chambers was approximately 40/60 at each time point. After the injection of sodium nitroprrusside, the relative time spent in the light dark chamber over the 4 hrs period did not differ from saline control. In contrast, after compound 48/80, there was a significant decrease in the time spent in the light

chamber and a reduction in the number of crossing between the light dark chamber through 2 hrs with a complete reversal to the 40/60 ratio by 4 hrs but not the longest time points. **Significance:** These findings suggest the utility of the mouse in the light averse behavioral end point. (RR fellowship funded by Migraine research foundation).

Disclosures: T.L. Yaksh: None. **R. Ramachandran:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

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Topic: D.08. Pain

Support: NIH Grant RO1 NS031680

Title: Different types of ROS are involved in opposing forms of synaptic plasticity in the spinal cord

Authors: *J. JUN, J. WANG, A. BITTAR, K. CHUNG, J. M. CHUNG
Neurosci. and Cell Biol., Univ. of Texas Med. Br., Galveston, TX

Abstract: The synaptic plasticity in the spinal dorsal horn neurons is an important factor producing central sensitization and thus pain. Our previous studies demonstrated that the direction of synaptic plasticity in dorsal horn neurons in response to nociceptive inputs are cell-type specific by showing long term potentiation (LTP) in spinothalamic track neurons (STTn) and long term depression (LTD) in GABAergic neurons (GABAn). We also demonstrated reactive oxygen species (ROS) are critically involved in both forms of synaptic plasticity. We hypothesize that the opposing forms of neuronal plastic changes are due to the involvement of different types of ROS - superoxides in LTP in STTn and hydroxyl radicals in LTD in GABAn. To test this hypothesis, the effects of specific ROS scavengers are examined on the LTP in STTn and the LTD in GABAn by using whole-cell patch-clamp recordings. From young naïve mice (GAD67-GFP mice, FVB-Tg(GadGFP)45704Swn/J; male, 2-3 week old), spinal cord slices are prepared and excitatory postsynaptic currents (eEPSCs) are recorded from identified STT and GABA neurons. The test stimuli (0.5 ms, 30-70μA) and the afferent conditioning stimuli (ACS; 2Hz for 40 sec, 80 pulses, 30-70μA) with a holding potential of +30mV) are applied to the dorsal root entry zone. GABAn are identified by green fluorescent in GAD67-GFP mice. STTn are identified by labeling with FAST-DiI, a retrograde tracer which was injected into the thalamus.

Three different types of ROS scavengers were used: a superoxide scavenger, TEMPOL (1mM); two hydroxyl radical scavengers, dimethyl sulfoxide (DMSO, 100mM) and dimethylthiourea (DMTU, 20mM). The scavengers were applied through the perfusion solution. LTD is consistently induced in GABAn following ACS application. Pretreatment with DMSO or DMTU prevented development of LTD after ACS. Furthermore, an application of TEMPOL also blocks LTD development in GABAn. In STTn, LTP is consistently induced following ACS. While an application of TEMPOL blocks LTP development in STTn, either DMSO or DMTU had no effect on LTP development in STTn. The results indicate that development of LTP in STT neurons requires the presence of superoxides but not hydroxyl radicals. On the other hand, the development of LTD in GABA neurons depends on both superoxides and hydroxyl radicals. Hydroxyl radicals in GABA neurons could have originated from superoxides. In summary, the results indicate that the different types of ROS may determine the direction of synaptic plasticity in spinal dorsal horn neurons: superoxides on LTP in STTn and both superoxides and hydroxyl radicals on LTD in GABAn.

Disclosures: J. Jun: None. **J. Wang:** None. **A. Bittar:** None. **K. Chung:** None. **J.M. Chung:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Topic: D.08. Pain

Support: NIH Grant DP2 OD008380

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Title: Treatment of neuropathic pain with a highly selective monoclonal antibody targeting sodium channel NaV1.7

Authors: *R.-R. JI[1,2], J.-H. LEE[3], C.-K. PARK[1], G. CHEN[1], R.-G. XIE[1], S.-Y. LEE[3]
[1]Pain Res. Division, Anesthesiol., Duke Univ. Med. Ctr., Durham, NC; [2]Neurobio., [3]Biochem., Duke Univ., Durham, NC

Abstract: Human genetic evidence from both gain-of-function and loss-of function studies suggests a critical role of voltage-gated sodium (NaV) channel subtype 1.7 (NaV1.7) in human pain sensation. Furthermore, NaV1.7 is expressed by nociceptor neurons in dorsal root ganglion (DRG) and their central terminals in the spinal cord dorsal horn. Thus, NaV1.7 represents one of the most attractive targets for pain medicine. However, it has been a real challenge to develop specific therapeutics against NaV1.7, due to high sequence homology among the nine NaV subtypes. Instead of using a traditional small molecule screening method, we have come up with a unique strategy of developing monoclonal antibodies (mAbs) to target a previously unexplored voltage sensor paddle of NaV1.7 channels. We have successfully raised a monoclonal antibody (SVmab1) that not only inhibits NaV1.7 with high selectivity in isolated heterologous cells but also effectively (70-300 nM) suppresses transient and persistent sodium currents and action potentials in isolated small-sized DRG neurons. Furthermore, application of SVmab1 to spinal cord slices inhibits excitatory synaptic transmission (spontaneous EPSCs) in lamina II neurons. In contrast, the control antibody targeting different domains of NaV1.7 (CTmab) had no effects on sodium currents and EPSCs. Interestingly, SVmab1 is more effective in suppressing persistent sodium currents and EPSCs in a mouse model of neuropathic pain following chronic constriction injury (CCI). Systemic injection of SVmab1 (50 mg/kg, i.v.) attenuated CCI-induced mechanical allodynia for 24 hours. Intrathecal injection of SVmab1 (50 μg) also reduced CCI-induced neuropathic pain. Taken together, our data suggest that NaV1.7-targeting monoclonal antibody (SVmab1) can attenuate neuropathic pain via both peripheral and central mechanisms. Given the specificity and long-lasting effects of monoclonal antibody therapies, humanized NaV1.7-targeting monoclonal antibody could be developed for treating neuropathic pain as well as other chronic pain conditions.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Topic: D.08. Pain

Support: DA037673

DA021644

Title: Preserved antinociceptive efficacy following chronic administration of a brain impermeant inhibitor of the anandamide hydrolyzing enzyme fatty-acid amide hydrolase (FAAH) in a mouse model of chemotherapy-induced peripheral neuropathy

Authors: *R. SLIVICKI[1], L. DENG[2], A. HOHMANN[2]
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Abstract: We recently reported that Δ9-tetrahydrocannabinol (THC), the major psychoactive ingredient in cannabis, produces anti-allodynic efficacy in a model of chemotherapy-induced peripheral neuropathy induced by paclitaxel treatment in mice (Deng et al. (2014) Biological Psychiatry, in press). However, with repeated dosing, tolerance developed to anti-allodynic efficacy of the cannabinoid. By contrast, inhibitors of the endocannabinoid hydrolyzing enzyme fatty-acid amide hydrolase (FAAH) exhibit antinociceptive efficacy in animal models of chronic pain without producing cardinal signs of CB1 receptor activation. Previously, our lab showed that inhibition of FAAH can reverse behavioral sensitivities to mechanical and cold stimulation in a neuropathic pain state induced by administration of the chemotherapeutic agent cisplatin (Guindon et. al (2013) Pharmacol Res. 67: 94-109). In the present study, we asked whether efficacy of a peripherally restricted FAAH inhibitor would be preserved following chronic administration in a mouse model of chemotherapy-induced neuropathy induced by paclitaxel. Paclitaxel induces a neuropathic pain state when administered systemically and produces behavioral hypersensitivities to both mechanical and cold stimulation. Acute treatment with URB937 produced a long duration of anti-allodynic efficacy that was maintained for at least 4 h following administration of the drug. URB937 suppressed paclitaxel-induced hypersensitivity to both mechanical and cold stimulation and normalized cold responsiveness to pre-paclitaxel levels. Moreover, anti-allodynic efficacy of URB937 was preserved throughout the chronic dosing regimen, suggesting that tolerance did not develop to the therapeutic effects of peripheral FAAH inhibition. Finally, the CB1 antagonist rimonabant (10 mg/kg i.p.) did not precipitate CB1-dependent withdrawal signs in animals subjected to chronic dosing with URB937. Our results suggest that inhibition of FAAH outside the central nervous system produces preserved anti-allodynic efficacy without unwanted CNS side-effects in a mouse model of paclitaxel-induced neuropathic pain.

Disclosures: **R. Slivicki:** None. **L. Deng:** None. **A. Hohmann:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Support: National Institutes of Health NS046606

National Cancer Institute CA124787

Title: Paclitaxel sensitizes trpv1 via activation of tlr4 contributing to chemotherapy induced peripheral neuropathy

Authors: *Y. LI, H. ZHANG, A. K. KOSTURAKIS, P. M. DOUGHERTY
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Abstract: Peripheral neuropathy is dose limiting in cancer chemotherapy with paclitaxel and can often induce persistent pain and discomfort in cancer survivors. This project tested the hypothesis that chemotherapy agents produce this side effect by sensitization of TRPV1 through Toll-like receptor 4 signaling. Immunohistochemistry showed that TLR4 is expressed in TRPV1 positive DRG sensory neurons and western blot studies showed that membrane TRPV1 in DRG and spinal cord is increased in rats treated with paclitaxel. TRPV1 sensitization in DRG neurons was evaluated using calcium imaging and whole cell patch clamp recordings in dissociated DRG neurons. Perfusion of paclitaxel directly activated DRG neurons in both types of experiments and showed an acute sensitization of TRPV1 via a TLR4-mediated mechanism in naïve rats. Moreover, paclitaxel significantly sensitized TRPV1 to capsaicin measured by calcium image and dissociated DRG patch clamp. In paclitaxel treated rats, the acute sensitization of capsaicin evoked increases in intracellular calcium accumulation and inward currents by paclitaxel co-administration was not apparent, but capsaicin responses alone were sensitized and did not show desensitization with repeated application as observed in vehicle treated rats. Paclitaxel-induced behavioral hypersensitivity to mechanical stimuli was prevented and reversed by systemic administration of a TRPV1 antagonist (AMG9810). In summary, Toll-like receptor (TLR) 4 is activated by paclitaxel and leads to sensitization of TRPV1. This mechanism could contribute paclitaxel-induced painful neuropathy.

Disclosures: Y. Li: None. **H. Zhang:** None. **A.K. Kosturakis:** None. **P.M. Dougherty:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Topic: D.08. Pain

Support: Sao Paulo Research Foundation Grant 2011-23764-0

Title: Dorsal root ganglia transcriptome profile of early painful diabetic neuropathy in a rat model of type-i diabetes

Authors: *M. P. ATHIÉ[1], A. S. VIEIRA[2], F. C. PRADO[1], E. V. DIAS[1], C. A. PARADA[1]
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Abstract: Peripheral Diabetic Neuropathy (PDN) manifests in 50-60% of types I and II diabetic patients and is the major cause of limb amputation. Although electrophysiological and morphological aspects are well described, little is known about its development and progression, undermining effective therapies. Hyperglycemia and insulin signaling impairment are considered the triggering events of oxidative stress production observed in the dying nerves. Several hypotheses try to explain the phenomenon, but until now there are many gaps in the pathogenesis and the plastic changes it generates. Transcript changes can help understand the plethora of symptoms and molecular events observed in DPN. We studied a rat model for type I diabetes, treated with low-dose of Streptozotocin to explore, by Next-Generation Sequencing of transcriptome, the main pathways and molecules differentially expressed in dorsal root ganglia (DRG) cells in early painful diabetic neuropathy. Glucose concentration and an electronic analgesimeter were used detect diabetes onset and the first modifications in mechanical sensitivity during 28 days after drug injection in STZ-treated rats and its respective controls. RNA from L4 and L5 DRG was submitted to transcriptome sequencing at Illumina HiSeq2500 System. STZ treatment induced hyperglycemia already in day 3, which persisted throughout the duration of experiment, while mechanical hypersensitivity started to appear two weeks after first STZ injection. Although transcriptome mapped ~ 20.000 genes for control and diabetic rats, comparative analysis found a great similarity between transcriptomes, with only 66 differentially expressed genes between the groups (43 down and 23 up-regulated in diabetic group). Molecular function enrichment showed a significant alteration in pathways related to cholinergic nicotinic transmissions, which are enrolled in pain modulation. Some genes encountered may relate to new altered pathways in the context of early painful diabetic neuropathy, and need to be further investigated. Nonetheless, the small number of differentially expressed genes between diabetic and control groups suggests that other mechanisms, such as post-transcriptional changes, may play an important role in this phase of DPN and complement the transcriptome information in explaining sensorial alteration.

Disclosures: **M.P. Athié:** None. **A.S. Vieira:** None. **F.C. Prado:** None. **E.V. Dias:** None. **C.A. Parada:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 242.06/DD16

Topic: D.08. Pain

Title: Involvement of the opioid system in the analgesic effect of celecoxib in rats with diabetic neuropathy

Authors: *I. E. JUAREZ-ROJOP, JR[1], C. A. TOVILLA-ZARATE[2], H. AGUILAR-MARISCAL[1], D. Y. BERMUDEZ-OCAÑA[2], T. RAMON-FRIAS[1], J. L. BLE-CASTILLO[1], J. C. DIAZ-ZAGOYA[3]
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Abstract: Diabetic neuropathy is one of the most frequent complications of diabetes mellitus. In patients with diabetic neuropathy pain relief is incomplete even taking high doses of analgesics and modulators. Furthermore, it is known that celecoxib, a selective inhibitor cyclooxygenase-2 (COX-2) attenuated pain in rats with diabetic neuropathy. Previous studies suggest that celecoxib can be attached to other cellular targets that are different from COX. In this regard, ion channels and the opioid system are identified as new markers for the molecular basis of some of the pharmacological actions of celecoxib. The purpose of this study was to assess the participation of naltrexone (a non-selective opioid receptor antagonist) in the anti-nociceptive and anti-allodynic effect of Celecoxib in rats with diabetic neuropathy. Streptozotocin (60 mg/kg; intraperitoneal) injection caused hyperglycemia within 1 week a Wistar rats. After 4 weeks the pain behavior was assessed using tactile allodynia and formalin test (Formalin 0.5%). Four to six weeks after diabetes induction, hyperalgesia and tactile allodynia was observed in the streptozotocin-injected rats. Results indicate that Intraperitoneal (i.p.) administration of celecoxib (0.3-100 mg/kg) is able to produce a dose-dependent decrease formalin-induced nociception and tactile allodynia in streptozotocin-injected rats. Moreover, on this condition, i.p administration of naltrexone (3 mg/kg) significantly reverses anti-nociceptive and anti-allodynic effects of Celecoxib in diabetic rats. These results suggest that the analgesic effect of Celecoxib in rats with diabetic neuropathy

is mediated through opioid receptors. In conclusion, Celecoxib (i.p) has beneficial effects for the management of diabetic neuropathic pain.

Disclosures: I.E. Juarez-Rojop: None. **C.A. Tovilla-Zarate:** None. **H. Aguilar-Mariscal:** None. **D.Y. Bermudez-Ocaña:** None. **T. Ramon-Frias:** None. **J.L. Ble-Castillo:** None. **J.C. Diaz-Zagoya:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 242.07/DD17

Topic: D.08. Pain

Support: Korea Health technology R & D Project, Ministry of Health & Welfare, Republic of Korea (A120254)

Title: Modulation of spinal AMPA receptor phosphorylation by PI3-Kinase activation in a rat model of neuropathic pain

Authors: J. JUN[1], S. JUNG[1], H. KIM[1], *J. W. LEEM[2]
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Abstract: Reactive oxygen species (ROS) in the spinal cord, which plays a crucial role in sensitization of dorsal horn neurons, has been implicated in neuropathic pain. ROS is an important mediator for regulating spinal AMPA receptor phosphorylation to induce persistent pain. ROS is involved in inducing neuropathic pain via PI3-kinase activation in spinal dorsal horn. In this work, we investigate whether PI3-kinase activation modulates the phosphorylation of spinal AMPA receptors in the neuropathic state. Mechanical hyperalgesia of hind paw, evaluated by measuring paw withdrawal threshold upon the application of von Frey hairs, was induced by L5 spinal nerve ligation (SNL). PI3-kinase activity was analyzed by western blotting, and PIP_3 protein level by ELISA. L5 SNL-induced mechanical hyperalgesia was attenuated by pretreatment with either ROS scavenger alpha-phenyl-N-tert-butyl nitron (PBN) or PI3-kinase inhibitor wortmannin. Both PIP3 level and PI3-kinase activity in the lumbar spinal dorsal horn were increased in rats with L5 SNL, and such increases were attenuated by pretreatment with either PBN or wortmannin. In rats with L5 SNL, the phosphorylation of spinal AMPA receptors at GluA1 (S831) and GluA2 (S880) subunits was increased. The increased AMPA receptor phosphorylation was reversed by inhibition of PI3-kinase. The results suggest that PI3-kinase-

dependent changes in the phosphorylation of spinal AMPA receptors, through the action of ROS, are crucial for the development of neuropathic pain.

Disclosures: **J. Jun:** None. **J.W. Leem:** None. **S. Jung:** None. **H. Kim:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

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Topic: D.08. Pain

Support: NIH Grant R01 NS031680

Title: Anti-hyperalgesic effects of ROS scavengers in neuropathic mice

Authors: ***A. BITTAR**, J. JUN, J. WANG, K. CHUNG, J. M. CHUNG
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Abstract: Our previous study has shown that the direction of neural plasticity in the spinal cord is cell-type specific in that afferent conditioning stimulation produces long term potentiation (LTP) in spinothalamic tract neurons (STTn) and long term depression (LTD) in GABAergic neurons (GABAn). We hypothesize that intense afferent stimulation induces LTP in excitatory STTn, which contributes to persistent pain. Furthermore, simultaneous production of LTD in inhibitory GABAn further intensifies persistent pain. We further hypothesize that two different types of reactive oxygen species (ROS) are involved as intracellular signaling molecules and produce these two opposing synaptic plasticity. To test this hypothesis, the effects of scavengers for specific ROS on pain behaviors are examined in the spinal nerve ligation (SNL) model of neuropathic pain in mice. SNL are produced by a tight ligation of the left L5 spinal nerve in C57/B6 mice. Mechanical hypersensitivity of the hind paw is assessed by measuring the frequencies of foot lifting and shaking in response to mechanical stimulation with Von Frey filaments (VF #2.44 and #3.00) to the affected paw. Sham operated mice are used as the control group. The effects of 4 different ROS scavengers injected intrathecally (i.t., 5μl) on mechanical hypersensitivity are examined at various times after neuropathic surgery. The tested drugs include PBN (nonspecific ROS scavenger, 100μg), TEMPOL (superoxide scavenger, 100μg), DMSO (hydroxyl radical scavenger, 0.55mg) and DMTU (hydroxyl radical scavengers, 1.04mg). To test whether the effect of ROS scavengers is related to GABA dysfunction, the effects of GABA receptor inhibitors (Bicuculline, 0.5μg; CGP46381, 0.25μg) in combination with ROS

scavengers are also examined. The results show that SNL mice produce mechanical hypersensitivity evidenced by increasing the frequencies of foot lifting and shaking of the affected hind limb. An intrathecal injection of ROS scavenger reduces all those measured behaviors representing the mechanical hypersensitivity. All tested ROS scavengers reduce pain behaviors at different degrees of magnitude. The GABA receptor inhibitors reverse the DMSO- and DMTU-induced anti-hyperalgesic effect. The results show that ROS scavengers reduce neuropathic pain behaviors. The data also suggest that an important component of the ROS scavenger-induced anti-hyperalgesic effect is by restoring impaired GABA inhibitory function.

Disclosures: **A. Bittar:** None. **J. Jun:** None. **J. Wang:** None. **K. Chung:** None. **J.M. Chung:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Title: Phosphorylation of transcriptional factors CREB and C/EBPβ was mediated by mitochondrial ROS in the HIV gp120 neuropathic pain model in rats

Authors: *S. HAO, H. YI, W. ZHENG, W. HUANG, S. LIU, R. LEVITT, K. CANDIOTTI, D. A. LUBARSKY
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Abstract: Despite the wide research of HIV/AIDS, the mechanisms underlying the manifestation of HIV neuropathic pain remain poorly understood. HIV neuropathic pain may be initiated by gp120 and sustained by spinal neuropathology. Mitochondria are the primary source for the generation of reactive oxygen species (ROS), a byproduct of oxidative phosphorylation. The production of ROS is known to be upregulated under inflammatory conditions and can induce

nociceptor sensitization. Transcriptional factor CREB is involved in the neuropathic pain state. However, the role of C/EBPβ (a downstream factor of CREB) is not clear in the HIV pain state. Here, we investigated the neurochemical mechanisms of HIV neuropathic pain in rats. HIV neuropathic pain was induced by gp120 peripheral application into sciatic nerve. Mechanical allodynia was evaluated using Von Frey fibers. HIV gp120 peripheral application into sciatic nerve increased spinal mitochondrial superoxide, upregulated phosphorylation of CREB and C/EBPβ, and lowered the MnSOD (mitochondrial SOD) activity. Either intraperitoneal PBN (a pan-ROS scavenger) or intrathecal PBN suppressed mechanical allodynia induced by HIV gp120. Intrathecal injection of a new mitochondria-targeted superoxide scavenger (Mito-Tempol) suppressed mechanical allodynia and reversed the lowered MnSOD activity. Intrathecal Mito-Tempol also suppressed the upregulated pCREB and pC/EBPβ. Double Immunostaining show the co-localization of MnSOD or pCREB and pC/EBPβ with NeuN, suggesting that MnSOD or pCREB and pC/EBPβ were located in the spinal neurons. The results suggest that mitochondrial ROS, and transcriptional factors pCREB and pC/EBPβ are involved in the HIV pain model, and that mitochondrial ROS mediate the phosphorylation of transcriptional factors CREB and C/EBPβ in the rat model.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Support: NIH grants R21NS82895 to Z.Z.X. and RO1 DE17794, DE22743, and NS67686 to R.R.J.

Title: Resolution of chemotherapy-induced neuropathic pain by protectin D1 via peripheral and central mechanisms

Authors: *Z. XU, R. XIE, S. BANG, G. CHEN, R.-R. JI
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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is the dose-limiting toxicity for many commonly used classes of anti-cancer agents. CIPN can lead to dose reductions or

discontinuation of cancer therapy. Taxanes, such as paclitaxel, are among the most effective and extensively used drugs in human chemotherapy; unfortunately, they cause painful neuropathy in most cancer patients receiving chemotherapy. Currently, there are no FDA-approved interventions or prevention strategies for CIPN. CIPN results in peripheral and central sensitization, neuroinflammation in the PNS and CNS, and loss of epidermal innervations. Protectin D1 (PD1) is a novel pro-resolution and anti-inflammatory lipid mediator biosynthesized from omega-3 fatty acid DHA. Our previous studies showed that PD1 potently inhibited inflammatory pain and nerve trauma-induced neuropathic pain. In this study, we investigated whether PD1 could prevent and reverse chemotherapy-induced neuropathic pain and further address the underlying mechanisms. We found that pretreatment with PD1 largely prevented chemotherapy-induced neuropathic pain. Mechanistically, PD1 protected chemotherapy-induced axonal degeneration *in vivo* and promoted axonal outgrowth *in vitro*. PD1 also prevented chemotherapy-induced neurochemical changes in DRGs. Interestingly, chemotherapy-induced hyperexcitability in large-sized DRG neurons, as revealed by whole-cell patch clamp recordings in whole-mount DRGs, was normalized by PD1. Furthermore, post-treatment of PD1, via intrathecal route, dose-dependently attenuated chemotherapy-induced neuropathic pain. Patch clamp recordings in spinal cord slices showed that PD1 also reversed chemotherapy-induced spinal cord synaptic plasticity (sEPSC increase). Collectively, our data suggest that pre- and post-treatment of PD1 can effectively attenuate chemotherapy-induced neuropathic pain, by resolving chemotherapy-induced axonal degeneration, neuroinflammation, DRG neuronal hyperexcitability, and spinal cord synaptic plasticity. Thus, PD1 and its mimetics may offer novel therapeutics for preventing and treating neuropathic pain following chemotherapy.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

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Topic: D.08. Pain

Support: Intradepartmental fund, The University of Texas MD Anderson Cancer Center

Title: Pentoxifylline decreases Paclitaxel-increased free radicals level in the primary dorsal root ganglia cells in culture

Authors: *H. KIM, S. ABDI
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Abstract: Chemotherapy agents including taxanes, vinca alkaloids, and platinum complexes are used to treat cancer patients, however, their neurotoxic side effect has a detrimental impact on the quality of life of patients. We previously reported that pentoxifylline (PTX), phosphodiesterases 1-5 inhibitor, ameliorates paclitaxel (PAC)-induced neuropathic pain in rats. The purpose of this study is to investigate the effects of PTX on the PAC-induced free radicals production using primary cultured rat dorsal root ganglion (DRG) cells. The lumbar DRGs were dissected from adult male Sprague-Dawley rat and then dissociated with collagenase and mechanical trituration. Isolated DRG cells were placed in poly-D-lysine and laminin coated 24-well plate and cultured with complete culture medium (DMEM, 10% FBS, 1% antibiotics, 4 mM glutamine) in a CO2 incubator for 1 weeks. To measure the free radicals level, DRG cells was treated with PAC solution for 1 day and the free radicals level was measured by flow cytometry using CellROX Deep Red Reagent. PAC (10 uM, 1 day) significantly increased the fluorescent intensity by 46% compared to vehicle (0.5% DMSO in culture medium) treated group. Further, PAC-induced increase fluorescent intensity was decreased by PTX (1, 10 mM). This result indicates that PAC increases the production of free radicals in the DRG cells and inhibition of PAC-induced free radicals level in DRG cells may be the mechanism underlying the analgesic effect of PTX on chemotherapy-induced neuropathic pain in rats.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

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Topic: D.08. Pain

Support: NRS|F 2007-2013 Aristeia 1361 Hellenic Ministry of Education

Title: Regulator of G protein signaling – 9 modulates the actions of analgesic drugs in neuropathic pain models

Authors: *V. MITSI[1], S. GASPARI[1], D. TERZI[1], L. MANOURAS[1], G. DESCALZI[2], J. FENG[2], I. PURUSHOTHAMAN[2], L. SHEN[2], V. ZACHARIOU[3]

[1]Basic Sci., Univ. of Crete, Heraklion, Greece; [2]Neurosci., [3]Neurosci. and Pharmacol. and Systems Therapeut., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Regulator of G protein signaling 9-2 (RGS9-2), is a striatal enriched modulator of GPCR signal transduction, which has been shown to be a potent modulator of monoamine and mu opioid receptors. Rgs9-2 modulates the actions of several clinically applied medications, including opiate analgesic, antipsychotic and antiparkinsonian drugs. Our previous work revealed a potent role of signal transduction complexes containing RGS9-2 in the nucleus accumbens in the rewarding and analgesic actions of opiates. Here, we use the Spared Nerve Injury (SNI) paradigm of neuropathic pain, to determine the role of RGS9-2 in neuropathic pain related symptoms and in responses to antidepressant medications used for the treatment of this disorder. Our findings suggest that while Rgs9-2 plays a minor role in the expression and intensity of neuropathic pain symptoms, it is a potent modulator of anxiety and depression behaviours induced by neuropathic pain. Specifically, Rgs9-2 plays a protective role in neuropathy - related depression, as indicated by the social interaction and the forced swim phenotypes of Rgs9 knockout mice. Notably, knockout of the Rgs9 gene accelerates the onset of the antiallodynic actions of monoamine targeting antidepressants used for the treatment of neuropathic pain symptoms. Specifically, Rgs9 knockout mice show an earlier and enhanced response to the tryciclic antidepressant desipramine (DMI) compared to their wildtype controls. Respectively, overexpression of RGS9-2 in the nucleus accumbens (NAc) via viral mediated gene transfer, blocks the antiallodynic effects of DMI. Western blot and immunoprecipitation assays indicated that the mechanism of DMI action in this brain region involves changes in RGS9-2 complexes as well as in downstream signal transduction events, including nuclear shuttling of G beta subunits and changes in protein kinase A (PKA) mediated phosphorylation of protein phosphatase 2A (PP2A). We finally applied RNA sequencing methodology in order to understand the impact of RGS9-2 on gene regulation induced by chronic antidepressant treatment and/or neuropathic pain. The RNA-sequencing studies revealed that modulation of GPCR activity by RGS9-2, controls the expression of several molecules with a key role in the long term actions of antidepressant medications, including several GPCRS, transcription factors and ion channels. Together our findings illustrate a dynamic role of RGS9-2 in the actions of monoamine targeting antidepressants and point to new intracellular targets for the treatment of neuropathic pain.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: D.08. Pain

Support: BI contribution to Innovative Medicines Initiative

Title: Behavioral characterization of ZDF rat as model for neuropathic pain

Authors: *N. GORODETSKAYA, A. PEKCEC, L. CORRADINI, H. N. DOODS
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Abstract: Neuropathic pain is a severe late complication of diabetic polyneuropathy which is diagnosed within 15-20 years after onset of diabetes. Preclinical evaluation of novel analgesics is mostly based on the rat Streptozotocin-induced model that mimics features of diabetes type 1 and is characterized by an acute severe development. However, there are currently no rodent models that reflect a more chronic phase of diabetic polyneuropathy. Objective of the current study is the evaluation of evoked pain-like and natural behaviors in Zucker Diabetic Fatty (ZDF) rat, a genetic model of spontaneously developed diabetes type2, from onset until the late phase of the diabetes using age-matched genetically linked Zucker Lean (ZL) and genetically independent Wistar rats as controls. Methods Six weeks old male ZDF, ZL and Wistar rats (n=32 per group) were used. Hind paw sensitivity (mechanical allodynia and hyperalgesia) and non-evoked behavior (burrowing and locomotor activity) were measured at 4 time points: at the age of 6-8, 15-17, 31-33 and 37-38 weeks. Blood glucose concentration were measured weekly during diabetes development and every second week after diabetes had been established. Results All but two ZDF rats developed diabetes at the age of 8 weeks. Withdrawal thresholds to tactile stimuli did not differ between ZDF and ZL rats (3.3±0.7g vs. 3.2±0.6g, respectively) but were significantly lower as in Wister rats (10±1.1g) starting already at the first time-point measurement and continued throughout the study. Sensitivity to mechanical pressure stimuli at onset of the study was similar in all 3 groups (withdrawal thresholds for ZDF, ZL and Wistar rat: 140±10.5g, 141±7.9g and 145±8.9g). At the follow up measurements at the age of 17 and 33 weeks the withdrawal threshold to pressure stimuli in ZDF rat was significantly lower than in ZL and Wistar rats (115±3.5g and 107± 4.8g vs 164±7.6g and 168± 9.5g, respectively) and was again increased to 157±11g at 38 week of age. Burrowing did not differ between groups at the age of 8 weeks (ZDF, ZL, Wistar: 800±78g, 1026±58g, 1266±85g), but was significantly lower in diabetic ZDF rats compared to ZL and Wistar rats at all later evaluation time-points (e.g. at age of 16 weeks for ZDF, ZL and Wistar: 783±88g, 1463±55g, 1409±52g). Locomotor activity as assessed by distance moved and rearing frequency was at all evaluation time points high in ZL rats compared to ZDF and Wistar rats. ZDF rats differ from Wistar rats only in rearing frequency at the age of 31 weeks. Conclusions Both kind of read-outs, based on reflex-withdrawal reactions

and on innate behavior have revealed a significant difference between diabetic and age-matched control rats.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Topic: D.08. Pain

Support: NIH Grant NS042150

NIH Grant DA-K01-024751

Title: Painful nerve injury reduces mitochondrial calcium buffering in axotomized sensory neurons but elevates calcium buffering in adjacent sensory neurons

Authors: ***Q. H. HOGAN**, C. SPRICK, Y. GUO, S. MUELLER, M. BIENENGRAEBER, B. PAN, H.-E. WU
Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Mitochondria play a critical role in coordinating neuronal energy metabolism and cytoplasmic Ca2+ concentration ([Ca2+]c). Initial observations have suggested that mitochondrial dysfunction may be a causative factor contributing to neuropathic pain. Mitochondrial uptake, storage, and release of Ca2+ is engaged by activity-induced [Ca2+]c transient elevation, but the effects of injury have not been examined. Using the application of FCCP (1μM) to eliminate mitochondrial Ca2+ uptake combined with oligomycin (10μM) to prevent ATP depletion, we first identified features of the depolarization-induced neuronal [Ca2+]c transient that are sensitive to blockade of mitochondrial Ca2+ buffering in order to assess mitochondrial contribution to [Ca2+]c regulation. This established the loss of a shoulder (plateau phase) during the recovery of the depolarization (K+)-induced transient, increased transient peak and area, and elevated level of the shoulder as evidence of diminished mitochondrial Ca2+ buffering. We then examined transients in Control neurons and neurons from the 4th lumbar (L4) and L5 dorsal root ganglia after spinal nerve ligation (SNL) at the L5 level. The SNL L4 neurons showed decreased transient peak and area compared to control

neurons, while the SNL L5 neurons showed increased shoulder level. Additionally, SNL L4 neurons developed shoulders following transients with lower peaks than Control neurons. Application of FCCP/Oligo elevated resting [Ca2+]c in SNL L4 neurons more than in Control neurons. Whereas application of FCCP/Oligo 2s after neuronal depolarization initiated mitochondrial Ca2+ release in most Control and SNL L4 neurons, this usually failed to release mitochondrial Ca2+ from SNL L5 neurons. For comparable cytoplasmic Ca2+ loads, the releasable mitochondrial Ca2+ in SNL L5 neurons was less than Control while it was increased in SNL L4 neurons. These findings show diminished mitochondrial Ca2+ buffering in axotomized SNL L5 neurons but enhanced Ca2+ buffering by neurons in adjacent SNL L4 neurons.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

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Topic: D.08. Pain

Support: JSPS Grant 25221309

Title: Netrin acts as a pain-inducing factor in the adult spinal cord

Authors: *Y. HAYANO[1], K. KITADA[2], T. YAMASHITA[1]
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Abstract: Neuropathic pain, a debilitating syndrome that occurs post-nerve damage, can lead to hypersensitivity in the peripheral and central nervous systems. The underlying mechanism is poorly understood, and currently available treatments remain inefficient. Here, we demonstrated that Netrin-4, a member of axon guidance molecule family, enhances the sensitivity to sensory input and contribute to neuropathic pain. A lack of the Netrin-4, which was expressed in dorsal horn lamina IIi neurons of the adult spinal cord, attenuated allodynia in neuropathic pain. In contrast, intrathecal administration of Netrin-4 to naïve rats induced allodynia, being mediated by Unc5B and intracellular tyrosine phosphatase SHP2. These findings suggest that suppression of Netrin-4-Unc5B-SHP2 signal may prove as an efficient strategy for the treatment of

neuropathic pain. Our findings provide evidence for the previously unidentified function of Netrin in adult nervous system.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Topic: D.08. Pain

Support: NIH P20GM103643

Title: Deletion of Sox11 in nociceptive neurons inhibits nerve regeneration and prolongs neuropathic pain after nerve injury

Authors: ***L. LEI**[1], M. ANDERSON[1], B. ROY[1], L. CAO[2], E. BILSKY[3]
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Abstract: The transcription factor Sox11 is highly expressed in sensory neurons in dorsal root ganglia (DRG) and trigeminal ganglia during development, and controls the survival and axonal growth of embryonic sensory neurons *in vivo* and *in vitro*. In adult mice, Sox11 is highly up-regulated in DRG after peripheral nerve injury. We hypothesize that the upregulation of Sox11 after peripheral nerve injury is an adaptive change on the part of the nervous system to promote axonal regrowth and reinnervation of distal territories, thus protecting against neuropathic pain. To test this hypothesis, we generated nociceptor-specific Sox11 conditional knockout (CKO) mice using the Cre-loxP-mediated gene targeting system with the Nav1.8-Cre line that expresses the Cre recombinase specifically in nociceptors. These Sox11 CKO mice were viable, fertile and grossly normal. We tested these Sox11 CKO mice in two neuropathic pain models - sciatic nerve crush (SNC) which allows for axonal growth and nerve regeneration, and L5 spinal nerve transection (L5Tx) which does not allow for nerve regeneration. The ablation of Sox11 in nociceptors led to a prolonged period of hypersensitivity in the SNC model, while had no effect in the L5Tx model. Furthermore, retrograde tracing experiment indicated that axonal growth and regeneration was inhibited in the Sox11 CKO mice after SNC.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Topic: D.08. Pain

Support: Innovative Medicines Initiative Joint Undertaking, under grant agreement No. 115007

Title: Electrophysiological characterization of the ZDF rat model of type 2 diabetes using conventional nerve conduction studies and microneurography

Authors: E. GARCIA[1], N. GORODETSKAYA[2], A. PEKCEC[2], R. SOLA[1], M. SUMALLA[1], *J. SERRA[1]
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Abstract: Painful neuropathy is a prevalent condition in type 2 diabetic patients. However, existing animal models of the disease are poorly characterized. We have used electrophysiological techniques to examine peripheral nerve function in the Zucker diabetic fatty (ZDF) rat model of type 2 diabetes Conventional nerve conduction techniques and microneurography were used to evaluate the electrophysiological features of both large and small caliber nerve fibers in ZDF, ZDF lean, and normal Wistar rats; animals were obtained from Boehringer Ingelheim Pharma GmbH & Co (see companion poster). All animals were maintained on high fat diet until recordings were made at age 38-41 weeks. Under urethane anesthesia, amplitude and conduction velocity of sensory nerve action potentials (SNAP) and compound motor action potentials (CMAP) were recorded from the tail. Alternatively, microneurographic recordings of unmyelinated C-fibers were obtained from the sciatic nerve. Eleven ZDF rats (336±6g), 14 ZDF lean rats (454±8g), and 15 Wistar (492±12g) age-matched rats were used in the study. ZDF rats had significantly reduced weights and higher blood glucose levels (499.5±17.9mg/dl; $P<0.001$). No differences in blood glucose levels were detected in ZDF lean (135.93±4.03mg/dl) and Wistar (129.47±10.44 mg/dl) rats. ZDF rats exhibited significantly reduced conduction velocities but normal amplitude of the SNAP and CMAP. The difference in conduction velocity was more prominent for sensory than motor fibers. A total of 605 C-fibers were recorded using microneurography: 104 (17.2%) mechano-sensitive nociceptors, 367 (60.7%) mechano-insensitive nociceptors, and 134 (22.1%) other types (including sympathetic and thermoreceptor fibers). Overall, abnormal spontaneous activity in nociceptors was observed

in 56 (15.26%) mechano-insensitive C-nociceptors from which 25% were recorded in the ZDF group, 11.9% in ZDF lean and 12.9% in Wistar rats. Although the ZDF group had a higher percentage of spontaneously active fibers, this difference did not reach statistical significance. In summary, we have found altered functional properties in peripheral nerves in the ZDF rat model of type 2 diabetes. The reduction in conduction velocities parallel changes found in other diabetic models and patients. Furthermore, C-nociceptors become hyperexcitable and spontaneously active in old hyperglycemic ZDF rats, but also in age-matched and apparently normoglycemic ZDF lean and Wistar rats fed with a high fat diet.

Disclosures: **E. Garcia:** A. Employment/Salary (full or part-time):; Neuroscience Technologies. **N. Gorodetskaya:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma GmbH & Co. KG. **A. Pekcec:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma GmbH & Co. KG. **R. Sola:** A. Employment/Salary (full or part-time):; Neuroscience Technologies. **M. Sumalla:** A. Employment/Salary (full or part-time):; Neuroscience Technologies. **J. Serra:** A. Employment/Salary (full or part-time):; Neuroscience Technologies, Boehringer Ingelheim Pharma GmbH & Co. KG. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Innovative Medicines Initiative Joint Undertaking, under grant agreement No. 115007.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 242.18/DD28

Topic: D.08. Pain

Support: Post-Doc fellowship NRF-2011-35B-C00034

National Honor Scientist program

Title: Protein synthesis of synaptic proteins in the anterior cingulate cortex maintains chronic pain

Authors: ***H.-G. KO**[1], J.-H. CHOI[1], X. LI[2], G.-C. BAEK[1], J. PARK[1], J.-I. KIM[1], S.-E. SIM[1], J. DO[1], J. SHIM[1], T. CHEN[2], M. ZHUO[2], B.-K. KAANG[1]
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Abstract: Synthesis and degradation of synaptic proteins mediated reorganization of synaptic structure, which in turn partly regulate synaptic strength. Change in synaptic strength is believed to be the fundamental mechanism of pain. In the present study, we examined whether change of synaptic proteins in the ACC is involved in chronic pain. To do this, we investigated the effect of protein synthesis/degradation blockade in pain response, immunoblot analysis, spine structure analysis and electrophysiological measurements in the ACC suffering from chronic pain.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Title: Sensory neuron subpopulation specific dysregulation of intracellular calcium in a rat model of chemotherapy-induced peripheral neuropathy

Authors: *E. YILMAZ[1,2], M. S. GOLD[1,2,3]
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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a common side effect of cancer treatment responsible for dose limitation if not cessation of therapy. CIPN is initially manifest with numbness and tingling followed by persistent pain typically localized in distal appendages. Current explanations for CIPN include the primary mechanisms of chemotherapeutic action, immune cell activation, and mitotoxicity. Unfortunately none of these mechanisms alone can explain stocking-glove manifestation of CIPN and the sensory neuron selectively. We and others have previously documented the heterogeneity among subpopulations of sensory neurons including those defined by target of innervation. Ca^{2+} regulatory mechanisms are one of the many differentially distributed properties that contribute to this heterogeneity.

Given recent evidence implicating dysregulation of intracellular Ca^{2+} ($[Ca^{2+}]_i$) as a mechanism of peripheral neuropathy and the importance of mitochondria in the regulation of $[Ca^{2+}]_i$ we hypothesized that the clinical signs of CIPN are due, at least in part, to a dysregulation of $[Ca^{2+}]_i$ in subpopulations of sensory neurons. We therefore assessed the impact of paclitaxel administration *in vivo*, on the regulation of $[Ca^{2+}]_i$ in subpopulations of sensory neurons *in vitro* defined by target of innervation and the presence of criteria used to identify putative nociceptors. There were differences among subpopulations of sensory neurons defined by the presence of nociceptive properties as well as by target of innervation with respect to resting, magnitude, and decay of high K^+-evoked Ca^{2+} transients. Two weeks after paclitaxel administration, when hypersensitivity was fully manifest, there was no detectable influence of target of innervation or paclitaxel treatment on putative non-nociceptive neurons. However, there was a significant influence of paclitaxel on the decay of the high K^+-evoked Ca^{2+} transient in putative nociceptive neurons. Transients decayed faster with a trend toward a larger decrease in transient duration in neurons innervating the glabrous skin on the hindpaw relative to the hairy skin on the thigh and the dorsal surface of the hindpaw. Preliminary results of experiments to identify the basis for the paclitaxel effects suggest an increased activity of sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) may contribute to these differences in putative nociceptors from paclitaxel treated rats. Our results suggest a model whereby neurotoxicity may reflect a decrease in the transport of mitochondria that results in neurotoxicity in distal endings of putative nociceptors as a result of a decrease in SERCA-mediated Ca^{2+} reuptake.

Disclosures: **E. Yilmaz:** None. **M.S. Gold:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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KJS09003

Title: Activation of cAMP-PKA signaling pathway in rat dorsal root ganglion and spinal cord contributes to development of bone cancer pain

Authors: X. TAN[1], *G. ZHU[2], X.-M. HE[1], P. CHEN[1], X.-J. SONG[1]
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Abstract: Treating cancer pain possesses a major clinical challenge and mechanisms underlying cancer pain remain elusive. The cAMP-PKA signaling pathway, which impinges on many essential cellular processes and is crucial for synaptic plasticity, is widely involved in inflammatory and neuropathic pain. Here we report that activation of cAMP-PKA signaling in rat dorsal root ganglion (DRG) and the spinal cord contributes to the development of bone cancer pain. Studies were performed on adult, female, Sprague−Dawley rats. Bone cancer pain in rats was produced by tumor cell (1×10^5 cells/μl, 5μl) implantation (TCI) into the intramedullary space of the right tibia. TCI treatment induced significant painful behaviors manifested as thermal hyperalgesia and mechanical allodynia. TCI treatment also increased the expression of PKA mRNAs in DRG and increased levels of cAMP concentration and PKA activity in both DRG and the spinal cord. *In vivo* delivery of PKA inhibitor Rp-cAMPS (1 mM/ 20 μl) intrathecally to interfere cAMP-PKA signaling pathway in the early (3-5 day) and late phase (9-10 days) after TCI treatment, respectively, significantly delayed and suppressed TCI-induced thermal hyperalgesia and mechanical allodynia. The increased level of cAMP concentration and PKA activity was also greatly suppressed by the PKA inhibitor. These findings indicate that TCI treatment activates the cAMP-PKA signaling pathway and continuing activation of this pathway is required for TCI-induced hyperalgesia and allodynia. This study reveals a critical role of the cAMP-PKA signaling pathway in the production and maintenance of bone cancer pain and suggests that targeting the activated cAMP-PKA pathway may be an effective therapeutic approach for suppressing cAMP-dependent neural hyperexcitability and bone cancer-induced behaviorally expressed hyperalgesia and allodynia.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Topic: D.08. Pain

Support: VA Merit Review Fund

Title: Ventrobasal thalamic burst firing after selective spinal cord tractotomies

Authors: J. OVELMEN-LEVITT[1], *P. G. SHINKMAN[2]
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Abstract: In human subjects, spontaneous dysaesthetic sensations, often described as painful, frequently follow spinal cord injury. Peripherally-evoked dysaesthesias are also reported, referred to the same or different body locations. Monkeys and rats also show signs of chronic spontaneous and evoked dysaesthesias following midthoracic spinal tractotomies that involve the anterolateral column (ALC). Our ALC lesions included primarily the spinothalamic tract. These signs are shown by sensory testing, neurological testing, and behavioral observation. Sprague-Dawley rats were observed chronically for over a year for signs of dysaesthesia. After these observations were complete, various spinal cord lesions led to different changes in firing patterns in the hindlimb area of the middle ventral posterolateral thalamic nucleus (mVPL), including burst firing as indicated by the burst index (BI) and the distribution of interspike intervals (ISI). Five groups were studied with extracellular unit recordings in anesthetized animals: intact control (C), dorsal column section (DC), anterolateral column (ALC), DC+ALC, and hemisections (Hemi). Large increases (10-50%) in long-duration (25-50 msec) multispike (7-10 APs) burst firing in hind-limb mVPL was found in ALC subjects when compared to C subjects (Mann-Whitney $U = 14$, $p<0.01$) in the hindlimb area. There was a high correlation between burst firing and the presence of objective signs of dysaesthesias, measured many months post-op (Pearson's correlation, $r = 1.00$). In DC subjects there was a marked increase in firing frequency compared to C ($U = 2.5$, $p<0.001$), with no accompanying spontaneous dysaesthesias. Overall, spinal cord lesions resulted in significant long-term changes among groups in the BI and ISI (chi-square = 17.6 and 18.9, respectively, with 4 df; $p<0.005$ and 0.001). We conclude that spinothalamic tract deafferentation (ALC) produces increased bursting in mVPL neurons. Based on ISI criteria and known mechanisms of burst discharges in thalamic nuclei, we interpret these bursts as consistent with the involvement of T-type calcium channel activation. This disruption in firing pattern occurs coincident with spontaneous behavioral dysaesthesia.

Disclosures: J. Ovelmen-Levitt: None. **P.G. Shinkman:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Topic: D.08. Pain

Support: NIH Gant NS072206

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Title: Contribution of dorsal root ganglion myeloid zinc finger protein 1 to neuropathic pain in rats after nerve trauma

Authors: *Y. TAO, Z. LI, X. GU, L. SUN, S. WU, L. LIANG, B. M. LUTZ
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Abstract: Peripheral nerve injury-induced changes in gene transcription and translation in primary sensory neurons of the dorsal root ganglion (DRG) are considered to contribute to neuropathic pain genesis. Transcription factors control gene expression. Peripheral nerve injury increases expression of myeloid zinc finger protein 1 (MZF1), a transcription factor, in the injured DRG. However, whether DRG MZF1 participates in neuropathic pain is unknown. Here, we report that pre-knockdown of DRG MZF1 prevents nerve injury-induced mechanical, cold, and thermal hypersensitivities in rats. Post-knockdown of DRG MZF1 also markedly reduces established neuropathic pain. Mimicking nerve injury-induced increase in DRG MZF1 produces neuropathic pain symptoms in naïve rats. Mechanistically, MZF1 blocks Kcna2 mRNA and protein expression, reduces total potassium current, and increases neuronal excitability through activation of Kcna2 antisense RNA gene in DRG neurons. Thus, MZF1 might serve as a potential target for preventing and treating neuropathic pain.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Support: NIH R01-NS079166-01A1

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NS081121

NS038261

NS011255

Title: Wnt5a signaling pathway in the pathogenesis of HIV-associated pain

Authors: *S.-B. YUAN, B. LI, G. JI, V. NEUGEBAUER, S.-J. TANG
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Abstract: Pain is a high incidence neurological complication in HIV-1/AIDS patients, but the underlying pathologic mechanism is unclear. We recently found that Wnt5a was aberrantly up-regulated in the spinal cord dorsal horn (SDH) in HIV patients who developed neuropathic pain. Further, intrathecal injection (i.t.) of HIV gp120, a protein implicated in HIV-1-associated pain, rapidly up-regulated Wnt5a expression in the SDH of C57BL6 mice. The objective of this study was to investigate the role of Wnt5a in the pathogenesis of HIV-associated pain. Using a HIV-related pain mouse model generated by i.t. gp120, we found that a Wnt5a-specific antagonist (Box5) attenuated gp120-induced mechanical allodynia. Conversely, either a Wnt5a agonist (Foxy5) or purified Wnt5a facilitated gp120-induced allodynia. Next, we tested the role of the JNK/TNF-α pathway, which can be activated by gp120 in the SDH in a Wnt5a-dependent manner. We found that a specific JNK inhibitor (SP600125) prevented the expression of allodynia induced by gp120 and Foxy5. Similarly, a specific TNF-α antagonist (Enbrel) was able to reverse gp120 and foxy5 induced allodynia. These data suggest that JNK and TNF-α are the downstream proteins that mediate the biological effects of Wnt5a in regulating gp120-induced pain. To determine neuronal effects, we performed extracellular single-unit recording on the SDH neurons in anesthetized mice. Gp120 increased background activity (action potentials) and responses to brief innocuous and noxious test mechanical stimuli applied to the hindpaw. Box5 or SP600125 blocked the facilitatory effects of gp120. Foxy5 also increased background and evoked activity, and this effect was blocked by SP600125 or Enbrel. The electrophysiological data suggest that Wnt5a, JNK and TNF-α mediate the facilitatory effects of gp120 on SDH neurons. Collectively, our findings implicate the Wnt5a-JNK-TNF-α pathway as a critical signaling mechanism in the development of gp120-induced pain.

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Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Title: A spinal analogue of memory reconsolidation enables the reversal of hyperalgesia

Authors: *R. P. BONIN, Y. DE KONINCK
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Abstract: The development of persistent pain through the sensitization of pain relays in the spinal cord dorsal horn shares many mechanistic and phenotypic parallels with memory formation. Memory reconsolidation, in which the reactivation of memories renders them labile and susceptible to erasure by inhibition of protein synthesis, may thus be of particular relevance to the treatment of persistent pain. Here we test the hypothesis that the reactivation of sensitized pain pathways initiates a process similar to memory recall and reconsolidation to render hyperalgesia labile and erasable. We find that both acute and long-lasting mechanical hyperalgesia could be reversed after reactivation of the sensitized pain pathway and the concomitant inhibition of spinal protein synthesis. This process was dependent on the activation of spinal AMPA, NMDA, and NK1 receptors during reactivation of the sensitized pain pathways, and on the activation of CaMKII and ERK. Additionally, the activation of spinal AMPA or NMDA receptors without peripheral stimulation was sufficient to render hyperalgesia labile, suggesting this process was mediated by post-synaptic activation of dorsal horn neurons. In electrophysiological experiments, synaptic LTP in the superficial dorsal horn, a cellular model of hyperalgesia, was similarly rendered labile and reversible by reapplying the LTP induction stimulus in the presence of the protein synthesis inhibitor anisomycin. These findings provide the first demonstration of a reconsolidation-like phenomenon in spinal pain processing pathways and the sensory system in general, suggesting that reconsolidation may exist more broadly throughout the CNS than previously known. These findings may further provide a novel therapeutic strategy for the treatment and erasure of persistent pain.

Disclosures: R.P. Bonin: None. **Y. De Koninck:** None.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Program#/Poster#: 242.25/EE3

Topic: D.08. Pain

Title: Painful hypersensitivity is attenuated by treatment with a novel CSE inhibitor in a rat model of chemotherapy induced peripheral neuropathy

Authors: *S. SYDSERFF, B. FRANCHINI, T. SACH, S. DURON, J. CHAPMAN
Sova Pharmaceuticals, La Jolla, CA

Abstract: Chemotherapy induced peripheral neuropathy (CIPN) is a significant treatment limiting side effect of taxane or platinum based chemotherapeutics. CIPN develops in around 40% of patients during a course of chemotherapy and is the dose-limiting toxicity. Initially presenting as a tingling or burning sensation in the extremities it can quickly develop into a painful debilitating condition. Reducing the onset of CIPN or alleviating the symptoms would both enable the continuation of aggressive cancer therapy for longer periods and reduce the painful sequelae. We have developed novel inhibitors of Cystathionine-γ-lyase (CSE), an enzyme involved in the production of hydrogen sulfide gas (H2S). H2S has been implicated in the regulation of ion channel pharmacology in pathophysiological states and has been shown to sensitize or activate both TRP and voltage gated ion channels involved in the nociceptive pathway. We hypothesize that inhibiting H2S production will remove this molecular sensitizer and effectively reset the pain pathway to a normal/baseline state. In order to test this hypothesis we have developed a dual chemotherapeutic model of CIPN in the rodent, which we describe here. Male SD rats were administered 2 mg/kg Paclitaxel on alternate days for 1 week and 10 mg/kg Carboplatin daily for 4 days. 23 days after beginning chemotherapy, hypersensitivity to von Frey hairs was assessed. The novel CSE inhibitor SV250 was administered either daily for 1 week prior to testing or acutely 5 hours prior to testing. Paclitaxel + carboplatin treated animals were extremely sensitive to the application of von Frey filaments, much more so than in animals treated with Paclitaxel alone. The 50% withdrawal threshold (g) being approximately a sixth of that seen in naïve rats. SV250 normalized the withdrawal response in both hind paws when administered for 7 days prior to testing at 3 mg/kg daily or when administered at a dose of 30 mg/kg 5 hours prior to testing. In the latter instance reduction in hypersensitivity to filaments was observed up to 48 hours post dosing. We have demonstrated that inhibiting CSE is highly effective at reducing painful hypersensitivity associated with chemotherapy induced peripheral neuropathy in the rat.

Disclosures: **S. Sydserff:** A. Employment/Salary (full or part-time):; Sova Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sova Pharmaceuticals. **B. Franchini:** A. Employment/Salary (full or part-time):; Sova Pharmaceuticals. **T. Sach:** A. Employment/Salary (full or part-time):; Sova Pharmaceuticals. **S. Duron:** A. Employment/Salary (full or part-time):; Sova Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sova Pharmaceuticals. **J. Chapman:** A. Employment/Salary (full or part-time):; Sova Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sova Pharmaceuticals.

Poster

242. Mechanisms of Neuropathic Pain: Signaling Pathways and Models

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Title: AKAP-dependent cAMP-PKA signaling maintains pain-related spontaneous activity in nociceptor somata after spinal cord injury

Authors: A. BAVENCOFFE[1], Z. WU[2], Q. YANG[1], J. DU[3], Y. LI[1], E. J. KENNEDY[4], S. M. CARLTON[3], C. W. DESSAUER[1], *E. T. WALTERS[1]
[1]Dept Integrative Biol & Pharmacol, Univ. Texas Med. Sch. At Houston, HOUSTON, TX; [2]Scripps Res. Inst., La Jolla, CA; [3]Univ. Texas Med. Br, Galveston, TX; [4]Univ. of Georgia, Athens, GA

Abstract: Chronic, intractable pain is a major problem for many patients with spinal cord injury (SCI), but the underlying mechanisms are not understood. In a rat model of contusive SCI at T10 we found that pain-related behavioral changes were associated with persistent hyperexcitability and spontaneous activity (SA) in the cell bodies of primary nociceptors recorded *in vitro* and *in*

vivo (Bedi et al. J Neurosci, 30:14870, 2010; Wu et al. Pain, 154:2130, 2013). Because prolonged activation of cAMP-PKA signaling has been linked to persistent nociceptor hyperexcitability and pain (e.g., Song et al., J Neurophysiol 95:479, 2006) and nociceptor responsiveness involves the complexing PKA and associated proteins to AKAP scaffolds (e.g., Efendiev et al., J Biol Chem 288:3929, 2013), we are testing the hypothesis that AKAP-dependent cAMP-PKA signaling continuously contributes to SA in nociceptor cell bodies acutely and chronically after SCI. Neurons from L4 and L5 DRG dissociated between 3 days and 3 months after SCI showed increased incidence of SA (21 of 39 cells showed SA, 72% incidence) compared to neurons from sham-operated (3 of 15, 20%) or naive animals (5 of 30, 17%), without apparent changes in incidence across this period (replicating Bedi et al., 2010). Blocking PKA activation with Rp-cAMPS blocked the increase in SA (4 of 21 with SA, 19%). Disrupting the binding of PKA to AKAP with two different blocking peptides, Ht31 and a novel stapled peptide STAD-2, blocked the increase in SA (5 of 24, 21% for Ht31; 3 of 16, 19% for STAD-2) compared to the effect of a corresponding inactive peptide, Ht31P (6 of 10, 60%) or STAD-2scr (8 of 11, 73%). A peptide that specifically blocks binding of adenylyl cyclase to AKAP also blocked the increase in SA (2 of 13, 15%) compared to the effect of a corresponding inactive peptide (8 of 12, 67%). Moreover, application of forksolin to capsaicin-sensitive DRG neurons from naive animals depolarized resting potential (9 of 9, 100%; 1,9-dideoxyforskolin, 0 of 9). To test whether PKA-AKAP association is also necessary for chronic SA generated in DRG neuron cell bodies *in vivo*, we have begun to test the effects of blocking peptides delivered locally to lumbar DRG in anesthetized rats. Preliminary results suggest that exposure to stearated Ht31 reduces SCI-induced SA generated in neurally isolated but otherwise intact DRG *in vivo*. These observations support the hypothesis that continuing activation of an AKAP-dependent cAMP-PKA signaling pathway maintains nociceptor SA that promotes pain in acute and chronic phases after SCI.

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Poster

243. Pain Imaging and Perception

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 243.01/EE5

Topic: D.08. Pain

Support: NSERC Scholarship CGSM

Title: Functional MRI reveals emotional modulation of pain processing in the human brainstem and cervical spinal cord

Authors: *T. A. MCIVER[1], J. KORNELSEN[2], S. D. SMITH[3], R. L. BOSMA[1], H. S. KHAN[1], A. I. COTOI[1], R. H. Y. LEUNG[1], P. W. STROMAN[1]
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Abstract: Emotions can modulate pain perception. In line with Motivational Priming Theory, painful stimuli are generally experienced as more painful when accompanied by, or associated with a negative emotional influence, while pain ratings decrease with positive emotional influence. Functional magnetic resonance imaging (fMRI) has been used to investigate pain and its underlying neural mechanisms throughout the central nervous system. Although fMRI studies have examined emotional modulation of pain processing in the brain, this provides an incomplete representation of the process. Pain processing areas are widely distributed across the brain, brainstem, and spinal cord. To gain a complete understanding of how emotion influences pain, we need to characterize the neural response in the brainstem and spinal cord. The present study used fMRI of the spinal cord and brainstem to examine the effect of emotional modulation on thermal pain processing and neural function at this level. Behavioral testing identified healthy, heterosexual, right-handed adult females who exhibited emotional modulation of pain perception. Painful heat stimulation (via Medoc® TSA-II Thermal Sensory Analyzer) was calibrated for each participant to produce a moderately painful sensation and applied on the thenar eminence of the right hand, corresponding to the C6 dermatome. Imaging sessions entailed 12 functional runs, with the painful stimulus applied in a block paradigm. For the duration of each run, participants viewed images selected from the International Affective Picture System of negative, neutral, or positive valence, with image valence held constant throughout each run. Participants reported the sensation's intensity and unpleasantness using pain rating scales ranging from 0 -100. Data analysis for fMRI included use of a general linear model, with subsequent contrasts to compare BOLD responses between conditions. We observed significant differences in pain perception, reflecting the influence of emotional valence of the visual stimuli on pain intensity and unpleasantness. Similarly, contrast analysis revealed significantly greater BOLD percent signal change, in the ipsilateral dorsal horn (C6) corresponding to the painful stimulus during negative picture viewing, relative to both positive and neutral picture viewing conditions. Image valence and arousal appear to have varying effects on BOLD responses to pain in different regions of the brainstem, including the periaqueductal gray, rostral medulla, and the nucleus tractus solitarius. These results provide evidence for descending emotional modulation of brainstem and spinal mechanisms involved in pain processing.

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Poster

243. Pain Imaging and Perception

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Pittsburgh Tissue Engineering Initiative Interface Seed Grant

Title: Nanotheranostics as a tool for search, rescue and discovery: Understanding chronic pain processing in the peripheral nervous system

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Abstract: Chronic pain has been an inadequately managed condition in the clinic, for a number of reasons: the cause is not always apparent, it is complex in terms of its pathogenesis, the cognitive processing of pain is highly variable in individuals, and current treatments are not yet targeting underlying pain mechanisms. Hence, new ways of tracking and manipulating the biology of chronic pain – in a personalized context - is critical for developing therapies that treat, rather than mask chronic pain. Combining diagnostics with therapeutics, the field of nanotheranostics provides both potent imaging capabilities and a directed therapeutic delivery modality, following systemic administration. In certain chronic pain conditions, there is a dynamic interplay between the nervous and immune systems. By way of mechanisms currently under intense research, the immune system and glia play a role in initiating and maintaining aspects of neuropathic pain - which is characterized by allodynia (normally innocuous stimuli causing pain), among other symptoms. Learning more about the way that chronic pain develops in the context of this multi-system cross-talk will offer the best strategy for its treatment. To explore the relationship between the immune system and neuropathic pain, – a type of chronic

pain that affects the nervous system - we have adapted a nanotheranostic 'search' tool, with the aim of imaging the inflammation associated with chronic pain. We intravenously injected a novel nanotheranostic emulsion – using a chronic constriction injury (CCI) rat model of neuropathic pain – which enables neuroinflammation to be imaged by a near infrared (NIR) lipophilic fluorescence reporter (DiR) and separately by a 19F fluorine MRI tracer. The nanotheranostic emulsion is internalized by circulating inflammatory cells, such as monocytes and macrophages. We have demonstrated NIR fluorescence, a 19F MRI signal, as well as the infiltration of CD68 positive macrophages at the site of CCI tissue damage. Our work paves the way for utilizing nanotheranostics to target the inflammatory events involved in chronic pain initiation, progression and maintenance - both in the context of treatment, as well as furthering our understanding of pain biology in the neuron-glia network.

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Poster

243. Pain Imaging and Perception

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P01 AT006663 (NIH/NCCAM)

Title: Neuromodulation of conditioned placebo/nocebo cue effect in heat pain: Anodal vs. cathodal tDCS to the right DLPFC

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Abstract: Placebo and nocebo play an important role in clinical practice and medical research. Modulating placebo/nocebo responses using noninvasive brain stimulation methods, such as transcranial direct current stimulation (tDCS), offers a great potential for harnessing these effects

in a clinical setting. Right dorsolateral prefrontal cortex (DLPFC) has been previously implicated in both placebo and nocebo effects. Its excitability can be successfully modulated by tDCS, as reported in several studies, in which anodal tDCS enhanced learning and consolidation. In the present study we assessed the effect of anodal and cathodal tDCS over the right DLPFC on conditioned placebo/nocebo cue response to heat pain. Two matched groups of healthy volunteers (N=15 per group) were subjected to an identical 30-minute session of conditioning, during which low and high cues (abstract images) were associated with low and high pain levels, respectively. Following the conditioning phase, 20-minute 2mA tDCS (either anodal or cathodal) over the right DLPFC (F4 in 10-20 coordinate system) was applied. The influence of tDCS current polarity (anodal vs. cathodal) on placebo/nocebo cue effect was assessed, using the change in subjects' pain ratings in response to identical pain stimuli preceded by the conditioned high or low cues. A significantly greater difference ($p=0.045$) between the high and low cue ratings was observed in the anodal tDCS group compared with the cathodal tDCS group, where the effect was reduced. This study provides evidence that it is possible to modulate conditioned placebo/nocebo cue effect by changing the brain excitability of the right DLPFC using tDCS.

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Poster

243. Pain Imaging and Perception

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Topic: D.08. Pain

Title: Changes in the functional connectivity of pain related brain areas during mental stress

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Abstract: Introduction: The cold pressure test (CP) induces a long lasting pain which changes during its application from an acute sensation to a deep and dull pain. It has been shown that mental stress as it is induced by a false color stroop task (ST) changes the pain perception. This is expressed by a change in the activation pattern of the affected brain areas. In this work we further studied these changes regarding the functional connectivity between these brain areas and

hypothesized that the connectivity of pain related brain areas will be diminished when the subjects were distracted from the pain by mental stress. Materials and methods: 14 healthy subjects were included in the study. They participated on psychophysical pre-examination where pain rating, blood pressure and heart rate were recorded. In the second run fMRI was assessed using a block design with alternating stimulus and baseline. Each stimulus lasted 2 minutes and was either CP alone or CP with ST (CP+ST). Each combination was given twice. For CP the right foot was immersed into cold water (4°C = 40°F), during the baseline periods it was changed to warm temperature (14°C = 57°F). Whole brain fMRI EPI sequences (TR=3000ms) were acquired on a 1.5 T Siemens MAGNETOM Espree. Predefined anatomical regions (ROI) were used and the mean MRI time courses in these regions were extracted for each subject. These signals were z-transformed and Pearson's correlation coefficients (r) were calculated between the ROI for the periods CP and CP+ST, respectively. Results: The psychophysical studies and the fMRI showed significant lower pain ratings during CP+ST as compared with CP. Blood pressure was increased during the painful stimuli of CP. Nearly all connectivity values were higher during CP than during CP+ST. The highest r values were found between S1 and S2 (BA 40) on both hemispheres ($r>0.8$, $p<0.00$) with only little decrease during CP+ST as compared with CP. There were also high connectivity values ($r>0.6$, $p<0.00$) between the areas of the medial system (insular cortex, anterior cingular cortex, amygdala). The most prominent decreases of connectivity (CP+ST compared to CP) were found in the network consisting of amygdala, caudate and BA 40. These areas are known to be involved in processing of the affective components of pain. Conclusion: CP activates the previously described network of pain related brain areas which is reflected by an increased functional connectivity between these areas. Distraction from the pain by a stroop task reduces the synchronism. This effect is pronounced most in areas which are known to be involved in processing of the affective components of pain.

Disclosures: **R. Ringler:** None. **P.V. Keylen:** None. **K. Detmar:** None. **R. Loose:** None. **C. Forster:** None.

Poster

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Topic: D.08. Pain

Support: IMI Europain Grant

Title: An objective human brain biomarker of target engagement by gabapentin in central sensitization

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Abstract: Aim Development of new analgesics for neuropathic pain is hindered by translational failure of their efficacy from animals to patients. Detecting such failures in large phase III studies is expensive and unsustainable. Demonstrating target engagement objectively in mechanism-based models in early phase II studies might avoid such failures. Functional magnetic resonance imaging (FMRI) is a powerful tool for providing such objective evidence. Resting state (RS) functional connectivity (Fc) between different brain regions can be gathered over a few minutes with little effort from individuals, making it an ideal tool for patient studies. We used Fc in a human model of central sensitization (CS) to study target engagement of a clinically effective drug, gabapentin (Gb) in neuropathic pain; CS being a key mechanism that contributes to neuropathic pain. We hypothesised that CS induced Fc between nociceptive processing areas would be modulated by Gb but not by ibuprofen (Ib), a drug that is ineffective in neuropathic pain or by placebo (Pl), irrespective of changes in subjective pain ratings which are influenced by context and expectation. Method We used a double blind placebo controlled crossover study design with 3 visits. In 24 healthy volunteers we induced CS using 1% topical capsaicin on the right leg, 90 min after 600mg of Ib, 1200mg Gb or Pl (balanced for order). Subjects rated spontaneous ongoing pain (OGP) 60 and 100 min after capsaicin using a visual analogue scale (anchors “no pain” and “severe pain”). RS data were acquired 90 min after capsaicin over 6 min using a 3T MRI scanner and analysed using FMRIB Software Library. We used a whole brain Fc analysis with the contralateral (left) thalamus (Th) as the seed region as it is a structure that is central to nociceptive impulse transmission. Plasma drug levels were measured 3.15 hrs after dosing. Results There were no significant differences between the visits in OGP scores but Gb significantly reduced CS induced Fc between the left Th and the left somatosensory cortex (SII) when compared to Ib and to Pl (mixed effects analysis; cluster corrected; $z > 2.3$; $p<0.05$). This Fc during Gb showed a significant -ve correlation (rho 0.59; $p = 0.002$) with Gb plasma levels. There were no significant differences in Fc between Pl and Ib. Conclusion Only Gb suppressed CS induced Fc between left Th and SII; key nociceptive processing areas implicated in chronic neuropathic pain patients, in a plasma drug level dependent manner. RS Fc in a relevant human model differentiated Gb, an effective analgesic from Ib, an ineffective analgesic in neuropathic pain despite low subject numbers, while the subjective pain scores did not.

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Poster

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Basic Research Program of Shenzhen (JC201005270293A)

The peacock plan of Shenzhen (KQC201109050100A)

Title: Temporal summation of sensory and affective dimensions of pain in humans: A fMRI study

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Abstract: Aim of investigation: To explore temporal summation (acute, tonic) of sensory and affective dimensions of deep muscle pain by using functional magnetic resonance imaging (fMRI). Methods: 16 healthy subjects participated to this study. We used fMRI to measure BOLD signal changes between acute and tonic pain condtions. Acute pain anticipation followed by pain are induced by masseter injection of a single bolus of isotonic saline (0.9% 0.2ml) and hypertonic saline (5% 0.2ml), respectively. A 20 min tonic pain state is induced by masseter injection of hypertonic saline by the application of a computer-controlled closed-loop system which could adjust the hyper saline infusion rate based on the VAS pain intensity rating score. And tonic pain of anticipation followed by the tonic pain, which induced by masseter injection of isotonic saline with a constant rate of 75ul/min. The visual analog scale (VAS) and SF-McGill pain questionnaire (MPQ) are used to assess the sensory and affective dimensions of pain. Results The significant difference was only found in the MPQ affective subscale in comparisons the subjective rating scores of the MPQ, PANAS and POMS scales between acute pain and tonic pain. The fMRI BOLD signal differences were found in regions of ipsilateral mid-prefrontal cortex, contralateral hippocampus and ipsilateral cerebellum in the tonic pain compared to acute pain conditions, and the signal differences were found in regions of bilateral insula, bilateral primary somatosensory (SI) and contralateral primary motor cortex (MI) in the acute pain compared to tonic pain conditions. Conclusions Temporal summation of muscle pain can affects sensory and affective dimensions of pain. Comparing with acute pain, the fMRI signal of tonic pain has more correlation with sensory and affective dimensions of pain in human brain areas such as mid-prefrontal cortex, hippocampus and cerebellum.

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Poster

243. Pain Imaging and Perception

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Support: Ministry of Science, ICT and Future planning Grant 10033812, South Korea

Ministry of Science, ICT and Future planning Grant 10033657, South Korea

Title: Motor cortex stimulation on neuropathic pain with positron emission tomography

Authors: ***J. KIM**[1], S. LEE[2], J. SHIN[1], H. JUNG[1], S. KIM[2], J. CHANG[1]
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Abstract: Neuropathic pain is one of severe diseases. Neuropathic pain is induced by direct or indirect nerve damage. Neuropathic pain is different from the other types of pain. It cannot be easily modulated by general pain treatments. The Motor cortex stimulation (MCS) is one of the treatment modalities for neuropathic pain. However, the mechanism of MCS is still unclear. We have studied to find the action mechanism of MCS using positron emission tomography (PET). We used Sprague-Dawley rats and induced neuropathic pain. To measuring tactile allodynia, behavior tests were carried on. We carried on immunohistochemical study, imaging study using PET. MCS modulates secretion of c-Fos and Serotonin (5-HT). In mPET study, the brain activity of the Striatum, Thalamic area and Cerebellum was changed. From this study, we can demonstrate that MCS suppresses neuropathic pain effectively, and we suggest that MCS modulates the ascending and descending aspects of the pain pathway in modulating pain.

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Poster

243. Pain Imaging and Perception

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Topic: D.08. Pain

Support: Office of Director, NIH. Grant number: 1DP2OD006469-01

Title: A magnetoencephalography study of pain anticipation in patients with thalamic pain syndrome

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Abstract: Thalamic pain syndrome (TPS) may occur after thalamic strokes or lesions, with numbness and pain in the contra-lateral side of the body. Not purely a somatosensory experience, chronic pain has significant affective components, including anticipatory phenomena (Melzack 1990). Evaluation of the neural mechanisms underlying pain anticipation is important to avoid maladaptive pain conditioning and injury, in addition to serving as biomarker to evaluate the results and progress of therapy. In this study we evaluated for the first time, with magnetoencephalography (MEG), the neurophysiological activity during pain anticipation in patients with TPS using visual countdown cues. Patients were evaluated before and after DBS therapy targeting affective sphere of pain (Plow et al 2013). Testing was performed with 7 adult patients seated upright in a 306 channel MEG array (Elekta AB). The paradigm (Machado et al 2014) consisted of 4 blocks of 60 randomized trials of distinct visual cues signaling the arrival of a painful stimulus (60% chance) or of no stimulus. Each trial was 9s long with 1s of baseline, 3s of pre-stimulus countdown and 5s post-stimulus period. All data analysis was performed at the sensor level using Fieldtrip (Oostenveld et al 2011) software in the time (power) domain. Data (from 204 orthogonal gradiometers) were tSSS filtered, parsed, down-sampled, anomalies rejected and filtered to 1-70 Hz. Additionally, head movement confounds were corrected. Here, we looked at the 300 ms response (P300) to the first visual cue as predictor of anticipation to upcoming painful stimulus. Data show high anticipatory phenomena around P300 both in control population (n=10) and patients with DBS OFF (n=7) stimulated on the painful limb. Data in 2 patients with DBS ON show this anticipatory phenomena was disrupted.

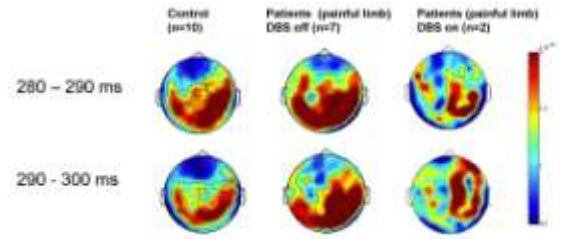

Fig.1. Topographies of MEG data at 300ms after the first visual cue preceding painful stimulus. Negativity in the anterior regions and positivity in the posterior regions is indicative of anticipatory behaviour. Dotted sensor locations showed significant activity ($p<0.05$) compared to a condition when visual cue indicated upcoming non-painful stimulus.

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Poster

243. Pain Imaging and Perception

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Support: DFG Grant PL 321/10-1

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Title: Neurophysiological correlates of bottom-up and top-down mediated modulations of pain

Authors: L. TIEMANN[1], E. MAY[1], M. POSTORINO[1], E. SCHULZ[1], M. NICKEL[1], U. BINGEL[2], *M. PLONER[1]
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Abstract: The perception of pain is highly variable. It depends on bottom-up mediated factors like stimulus intensity and top-down mediated factors like expectations. In the brain, pain is associated with a complex pattern of neuronal responses including evoked potentials and induced

responses at alpha and gamma frequencies. Although they all covary with stimulus intensity and pain perception, responses at gamma frequencies can, under certain conditions, be particularly closely related to the perception of pain. It is, however, unclear whether this generalizes to fundamentally different modulations of pain. Here, we used electroencephalography to directly compare bottom-up and top-down mediated modulations of pain, which were implemented by changes in stimulus intensity and placebo analgesia, respectively. The results show that stimulus intensity modulated pain-evoked potentials and pain-induced alpha and gamma responses. In contrast, placebo analgesia was associated with changes of evoked potentials, but not of alpha and gamma responses. These findings reveal that pain-related neuronal responses are differentially sensitive to bottom-up and top-down modulations of pain indicating that they provide complementary information about the perception of pain. The results further show that pain-induced gamma oscillations do not invariably encode pain perception but may rather represent a marker of local sensory processing whose influence on pain perception varies with behavioral context.

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Poster

243. Pain Imaging and Perception

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Topic: D.08. Pain

Support: Pfizer

Title: Investigation of the neural processes involved in cognitive modulation of pain in the human cervical spinal cord and brainstem using functional MRI

Authors: *R. H. LEUNG, A. I. COTOI, R. L. BOSMA, T. A. MCIVER, H. T. KHAN, P. W. STROMAN
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Abstract: Pain is a highly subjective sensory experience involving elaborate neural pathways, and the perception of pain can vary across and even within individuals. Studies have shown that descending pathways originating from cortical regions can modulate ascending nociceptive stimuli. For example, engaging executive functions, such as working memory can alter the

salience and intensity of pain, and behavioural studies have shown that increased attentional load reduces the perception of pain. This cognition-dependent suppression of pain perception illustrates a mechanism of modulation in which top-down attentional selection can mitigate the bottom-up salience of noxious stimuli via increased distraction or cognitive load. This study aims to assess the neural correlates of cognitive modulation of pain within the brainstem and cervical spinal through blood-oxygenation level dependent (BOLD) responses during functional magnetic resonance imaging (fMRI). Cognitive modulation of pain was behaviourally confirmed in a group of 13 healthy, female adults ages 18 to 45 using a robust heat pain paradigm and the N-back working memory cognitive task. All participants completed questionnaires regarding social desirability, depression, anxiety, and pain catastrophizing as a means of control. Calibrated thermal pain was applied to the right thenar eminence, which corresponds to the C6 dermatome, via a Medoc® TSA-II Thermal Sensory Analyzer as participants performed the task pseudo-randomly varied between three levels (no-, 1-, and 2-back). Participants rated their pain intensity according to a rating scale ranging from 0 (no sensation) to 100 (intolerable pain). The no-back yielded the highest pain ratings while the 2-back yielded the lowest pain ratings, and pair-wise comparisons between each condition was significant with Bonferroni adjustment. Task performance also differed significantly between the 1- and 2-back levels. To investigate this further, ongoing fMRI studies are examining the neural correlates involved in cognitive modulation of pain. BOLD responses in the brainstem and spinal cord are recorded while participants perform a cognitive task and undergo thermal stimulation. We hypothesize significant differences in subjective pain responses as well as signal intensity changes in key regions such as the periaqueductal gray, rostral ventral medulla, and the dorsal horn of the C6 spinal cord segment, when comparing between N-back levels. The results of this study will help us better understand how pain experiences are formed in healthy individuals by elucidating the role cognitive function plays in the neural processing of pain.

Disclosures: **R.H. Leung:** None. **A.I. Cotoi:** None. **R.L. Bosma:** None. **T.A. McIver:** None. **H.T. Khan:** None. **P.W. Stroman:** None.

Poster

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Title: Effects of visual body image on pain perception

Authors: *M. OSUMI, R. IMAI, K. UETA, S. MORIOKA
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Abstract: [Objective]In recent years, there has been a rise in changing the visual body image as a method of rehabilitation for chronic pain. However, there are inter-individual differences in the analgesic effect changing the visual body image. We made a hypothesis that negative emotion on self-body perception associated with changing the visual body image. Now we investigated, using a rubber hand illusion, whether these unpleasant caused by changing the visual body image of a healthy person are able to modulate pain. [Methods] Twenty-three healthy subjects participated in this study (8 male, 15 females, mean age 21.6; SD 1.5years). To evoke negative emotion on self-body perception associated with changing the visual body image, we applied the method of rubber hand illusion. In order to create these unpleasant, we created a "injured rubber hand", a "hairy rubber hand", and a "twisted rubber hand". We also created a “normal rubber hand” as a control. The both of participant’s real hand and rubber hand were touched synchronously with paint brush to elicit body ownership experience for rubber hands in each condition. After that, the pain threshold of the hidden real hand was measured while the participant observed the rubber hand using a device that measured pain caused by thermal stimuli. In the between conditions, values of illusory ownership, unpleasantness on self-body perception and pain threshold were analyzed using one-way repeated-measures ANOVA. The Bonferroni method was used for post hoc comparisons. An alpha level of 5% was considered as statistically significant. [Result]Body ownership experiences were elicited by observation of the injured rubber hand and hairy rubber hand as well as the normal rubber hand. Participants felt more unpleasantness by observing the injured rubber hand and hairy rubber hand than the normal rubber hand and twisted rubber hand ($p < 0.001$). The pain threshold was lower under the injured rubber hand condition than with the other conditions ($p < 0.001$). [Conclusions] This result supports the view that negative emotion on the visual body image associated with pain can increase pain sensitivity. We can conclude that rehabilitation for pain by changing the visual body image will succeed if there is no negative emotion associated with the pain.

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Poster

243. Pain Imaging and Perception

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Title: Cathodal transcranial direct current stimulation over primary somatosensory cortex increases joint flexibility

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Abstract: Is the primary somatosensory cortex (S1) involved in joint flexibility? Joint flexibility is dependent on both mechanical and neural factors. However, the contribution of neural factors is not fully understood. Transcranial direct current stimulation (tDCS) can modulate excitability of the cerebral cortex; anodal tDCS can upregulate excitability of the cerebral cortex under the electrode, whereas cathodal tDCS can downregulate excitability. Herein, we assessed whether tDCS over the S1 of the lower limb can modify the joint flexibility of the ankle joint in healthy subjects. In eight male subjects, range of motion (ROM) of the left ankle joint and left wrist joint were measured during the passive-dorsiflexion test. The ankle and wrist were passively dorsiflexed at a speed of 1°/s to the maximal dorsiflexion angle. We defined the ROM as the angle at which the subject felt discomfort in the calf or the wrist. To determine changes of due to mechanical factors, the passive torque, which represents involuntary resistance torque to dorsiflexion, was also measured at the ankle joint. Subjects performed the left ankle and the left hand passive-dorsiflexion tests before and after anodal, cathodal and sham-controlled tDCS over the S1 of the lower limb. The current was applied for 10 min with an intensity of 2.0 mA during anodal and cathodal tDCS. Cathodal tDCS was significantly increased by 10.5% (from 26.5 ± 7.7° to 29.4 ± 8.1°) of ROM for the ankle joint, but not for the wrist joint. Both anodal and sham tDCS had no significant effect. Cathodal tDCS over the S1 of the lower limb might have changed neural factors such as perception of joint angle or pain, because the mechanical factors indicated by the passive torque at 0°, 5°, 10° and 15° were not changed. These results suggest that the S1 is involved in joint flexibility.

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Poster

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Topic: D.08. Pain

Support: CIHR

Pfizer

Title: Pain facilitation in the spinal cord and brainstem of patients with fibromyalgia syndrome: An fMRI study

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Abstract: Fibromyalgia syndrome (FMS) is a highly prevalent wide-spread chronic pain condition, which afflicts primarily females. Although the etiology of this disease is not completely understood, FMS pain is believed to be maintained by both peripheral mechanisms, through tonic deep muscle inputs, and central mechanisms, by facilitation of the spinal cord and brain. Temporal summation of second pain (TSSP) paradigms evoke a C-fiber transmitted enhancement (or "wind-up") of the dorsal horn neurons that coincides with an increase in pain perception. Abnormal behavioral responses to temporal summation paradigms in patients with FMS indicate central pain processing abnormalities. Using this paradigm and spinal cord functional MRI, we can, for the first time, non-invasively probe the central mechanisms of this pain response in the spinal cord and brainstem of patients with FMS. The aim of this study is to characterize the fMRI BOLD responses in the spinal cord and brainstem that correspond to TSSP in patients with FMS and in healthy controls. Healthy female adults (N = 15) and females with FMS (N = 15) completed a quantitative sensory testing session and two imaging sessions. In the first session, heat pain thresholds were determined, and participants became familiar with the study procedures, as well as the MRI environment. As a measure of control, participants completed questionnaires to assess depression, anxiety, pain catastrophizing and social desirability. Functional MRI studies of the spinal cord and brainstem were conducted at 3T. A thermode was placed on the right thenar eminence (C6 dermatome) and TSSP (0.33 Hz) and non-TSSP (0.17 Hz) heat pain paradigms were employed. The stimulus intensity was calibrated for each individual in order to produce the wind-up effect and achieve comparable TSSP ratings of moderate pain at 0.33 Hz. Participants rated the pain on a scale from 0 (no sensation) to 100 (intolerable). Data were analyzed by means of the general linear model. In the TSSP condition, all participants rated the pain intensity of the last stimulus (FMS M_{rating} = 51, Control M_{rating} = 50) more painful than the first (FMS M_{rating} = 24, Control M_{rating} = 18). Temporal summation to a final rating of moderate pain (~50) was achieved at lower temperatures in the FMS group compared to the controls. Preliminary fMRI results in both groups indicate that, compared to the

non-TSSP condition, the BOLD response to the TSSP condition was more robust and was sustained beyond the duration of the stimulation block. Results from this study indicate the presence of wind-up in the human dorsal horn and will provide more information about changes in pain processing in patients with FMS.

Disclosures: R. Bosma: None. **E. Mojarad:** None. **L. Leung:** None. **C. Pukall:** None. **R. Staud:** None. **P. Stroman:** None.

Poster

243. Pain Imaging and Perception

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 243.14/EE18

Topic: D.08. Pain

Support: Eli Lilly Principal Investigator's Award

Title: Chronic non-resolving nerve injury in the rat causes behavioral changes and global and cellular abnormalities in brain morphology

Authors: *L. A. LOW[1], B. WANG[1], S. J. THOMPSON[2], M. BUSHNELL[1]
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Abstract: Aim: To monitor nerve injury-induced changes on behavior in the rat, and investigate global and cellular changes in the brain 7 months after nerve injury. **Methods:** 12 male Sprague-Dawley rats received a spared nerve injury, where 2 of the 3 branches of the sciatic nerve are ligated and cut. 12 males received sham surgery. From 3 to 7 months, animals were regularly tested for sensory hypersensitivity (mechanical and cold hypersensitivity), as well as exploratory behaviors in the open field and elevated plus maze. At 7 months, rats were euthanized and post-mortem MRI scans were performed on a 7T Bruker Pharmascan machine, then MR signal intensity across the whole brain compared using AFNI software. In addition, brains were retained for histological analysis. Initially, prefrontal cortex was sectioned and stained for GFAP (astroglia) immunofluorescence. **Results:** Nerve-injured rats remained significantly hypersensitive to both mechanical and cold (acetone) stimulation at all time points ($p<0.0001$), with no resolution of sensory hypersensitivity. Additionally, nerve-injured rats showed reductions in rearing in an elevated plus maze and open field ($p<0.05$), suggesting decreased exploratory behaviors. 7 months after nerve injury, post-mortem MR analysis showed reductions

in MR signal intensity in 4 clusters (>20 voxels) in primary somatosensory areas, dentate gyrus and ventral tegmental area (VTA) in nerve-injured animals compared to controls ($t=3.64$, $p=0.003$). Contralateral prefrontal cortex (infralimbic and prelimbic) also showed increases in GFAP immunofluorescence ($p<0.05$, $n=8$ per group), suggestive of the presence of activated astrocytes (gliosis). **Conclusions:** Chronic (7 months) nerve injury causes non-resolving sensory hypersensitivity and long-lasting changes in exploratory behaviors. Reductions in MR signal are seen in brain areas involved in pain processing and changes in cellular morphology are seen in prefrontal brain regions. Ongoing work is characterizing further cellular changes in brain integrity in ROIs suggested by MR results.

Disclosures: L.A. Low: None. **B. Wang:** None. **S.J. Thompson:** None. **M. Bushnell:** None.

Poster

243. Pain Imaging and Perception

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Program#/Poster#: 243.15/EE19

Topic: D.08. Pain

Support: CIHR

Title: Measuring the effect of pain on sensorimotor integration using short-latency afferent inhibition

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Abstract: INTRODUCTION: The effects of pain on the motor system are not yet well described. In the presence of pain, motor cortex excitability is reduced. In the context of rehabilitation, where pain is often present during motor re-training, such reduced excitability could potentially have a negative impact on sensorimotor integration and thereby reduce treatment effectiveness. The aim of the present study was therefore to measure if a pain-induced decrease in motor cortex excitability interferes with sensorimotor integration. METHODS: Ten healthy subjects (aged 25.8 +/- 4.1) came to the laboratory for a single session. They were exposed to the same sensorimotor integration protocol either in the presence or absence of pain. Sensorimotor integration was assessed with a short latency afferent inhibition (SAI) paradigm. SAI consisted in measuring the amount of inhibition of a cortically evoked motor response in the

first dorsal interosseus muscle (FDI) with transcranial magnetic stimulation (TMS) when preceded by a brief transcutaneous electrical stimulation of the ulnar nerve (200μs pulse). The intervals between electrical and magnetic stimulations were based on the latency of the afferent sensory volley measured with electroencephalography (N20) in each subject and were set to N20-5ms, N20+2ms, N20+4ms and N20+10ms. TMS intensity was set to induce a 1mV FDI motor evoked potential (MEP) in the no Pain condition and ulnar nerve stimulation intensity was set at 5-10% Mmax in FDI. Pain was induced by applying heat pulses (50°C) with a thermode to the lateral border of the stimulated hand. Statistical analysis consisted of a two-way repeated measures ANOVA with factors Condition and Stimulation and a Sidak adjustment for multiple comparisons was used in post-hoc tests. RESULTS: show a main effect of Condition ($p=0.005$) as overall, MEPs in the NoPain condition had a larger amplitude (0.91 vs. 0.59 mV). There was also a main effect of Stimulation ($p=0.012$). Pairwise comparisons revealed that MEPs at N20+2 were inhibited compared to Test ($p=0.016$). These results support that: 1) heat pain reduces corticospinal excitability; and 2) as there were no interaction effects, pain does not interfere with sensorimotor integration as measured with SAI. CONCLUSION: While pain reduces corticospinal excitability, these results suggest that under controlled conditions, such reduction in excitability does not interfere with sensorimotor integration.

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Poster

243. Pain Imaging and Perception

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Topic: D.08. Pain

Support: Pfizer

Title: Investigating the neural processing of Conditioned Pain Modulation (CPM) in the human brainstem and spinal cord using functional MRI

Authors: *A. COTOI, R. H. Y. LEUNG, R. L. BOSMA, T. A. MCIVER, M. VALENCIA, H. S. KHAN, P. W. STROMAN
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Abstract: Pain is an unpleasant experience that varies in perception depending on the degree of activity in neural pain networks that span the entire central nervous system (CNS). Studies

suggest that when a noxious “conditioning” stimulus is applied to a remote area of the body prior to the onset of a “test” stimulus, the perception of pain is diminished. This phenomenon, termed Conditioned Pain Modulation (CPM), is suggested to activate descending inhibitory pathways which dampen the sensation of pain. Although functional MRI studies have shown cortical responses related to the CPM effect, pain inhibition by means of this paradigm is yet to be studied in the spinal cord and brainstem. This study investigates the neural processing at the level of the brainstem and spinal cord by means of blood-oxygenation level dependent (BOLD) responses to CPM with functional MRI. Studies of 11 healthy, adult women, confirmed the behavioral effect of CPM on pain perception. As a measure of control, all participants completed a series of questionnaires regarding depression, anxiety, pain catastrophizing, and social desirability. Two Medoc® TSA-II Thermal Sensory Analyzers were used to deliver cold and hot pain to the left and right thenar eminence, respectively, as the conditioning and test stimuli. Participants received either the test stimulus alone, or both test and conditioning stimuli, and were instructed to focus their attention either towards the hot (test) or cold (conditioning) pain. Participants then rated the heat pain intensity according to a visual scale ranging from 0 (no sensation) to 100 (intolerable) at the end of each pseudo randomized trial. The results indicate a significant reduction in heat pain ratings when the conditioning and test stimuli were applied simultaneously and focus was directed on the cold pain (CPM condition), compared to receiving the test stimulus only, with focus directed on the hot pain (control condition). To investigate BOLD responses in the brainstem and spinal cord, participants underwent functional MRI while exposed to the CPM paradigm described above. We hypothesize that a decrease in pain perception will correspond to the BOLD signal change in the dorsal horn of the spinal cord at the C6 segment, the periaqueductal gray matter of the midbrain, and in the rostral ventromedial medulla in the CPM condition compared to the control condition. We further hypothesize that the connectivity between these regions will be greater during CPM. This study elucidates the neural mechanisms of pain modulation and pain inhibition, furthering our understanding of pain processing.

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Poster

243. Pain Imaging and Perception

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Program#/Poster#: 243.17/EE21

Topic: D.08. Pain

Title: Demonstration and validation of a new MRI-safe pain tolerance device

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Abstract: One of the barriers to studying the behavioral and emotional effects of pain using functional Magnetic Resonance Imaging (fMRI) is the absence of a commercially available, MRI compatible pressure-based algometer to elicit pain. The present study sought to address this barrier through creation and validation of a novel MRI-safe apparatus capable of delivering incremental, measurable amounts of pressure inside a scanning bore. This device was created using a sphygmomanometer, sealed plastic tubing (to allow for metal portions of the device to be kept outside the scanner room), and a hard plastic disc with attached round nub. The disc was attached to participants' downward facing right hand such that the nub was positioned to touch the skin between middle and pointer fingers with the sphygmomanometer cuff wrapped around it. During each of 10 consecutive trials the experimenter systematically increased the pressure applied to the participant's hand by inflating the cuff at a rate of roughly 10 mmHg per second. A timer and the pressure gauge attached to the sphygmomanometer were used to standardize application of pressure across participants. Participants were instructed to signal when the "pain was too uncomfortable to continue," at which point, air pressure was released from the cuff. If participants did not signal to discontinue pressure was released at 2000 kPa, a cutoff selected following consultation with previous literature (e.g. Neziri et al., 2013). Using the noted procedure the device was first tested outside the scanner to ensure that its functioning was comparable to the AlgoMed, a portable, electronic algometer widely used in literature exploring pain-tolerance (N = 308). Results of this initial study will be presented to support the validity and reliability of the novel device. The device was then piloted inside a MAGNETOM 7T Siemens MRI scanner (Siemens Healthcare, Malvern, PA, USA), where participants' (N = 10) blood-oxygen level dependent (BOLD) signal was recorded while pressure was administered using the noted procedure with the addition of an infrared button-box used to signal when participants reached their pain threshold. The use of this device yielded BOLD activation within the insula (BA 13) and the anterior cingulate gyrus (BA 24); with each increase in pressure, activation in these areas parametrically increased. These findings correspond to other studies using thermal, electrical, mechanical, or pin-prick pain applications. This behavioral and functional data demonstrates that this new device is a valid method of administering pressure-related pain in MRI environments.

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Poster

243. Pain Imaging and Perception

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Topic: D.08. Pain

Support: UMSOD ORC Grant

Title: An fMRI study of estrogen-dependent visceral hypersensitivity following stress in rats

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Abstract: Irritable bowel syndrome (IBS) is a functional, multifactorial disorder characterized by abdominal pain, altered bowel habits, and visceral hypersensitivity, which can be influenced by psychological factors including stress and/or anxiety. Even after accounting for health-care seeking differences, this condition is more prevalent among females, suggesting hormonal influences on visceral pain sensitivity may, in part, underlie the etiology of IBS. Moreover, recent imaging studies have demonstrated that patients with IBS have altered brain activity in emotional-arousal and stress-related circuitry and subchronic stress (forced swim, FS) has been shown to induce visceral hypersensitivity that is estrogen (E2) dependent in a rat behavioral model. The major aim of the current study was to use a novel fMRI paradigm to examine brain activation associated with colorectal distension (CRD) and the role of gonadal hormones in a model of visceral hypersensitivity. Ten female, Sprague Dawley rats (10-12 weeks) were ovariectomized 15 days prior to the baseline scan and singly housed following surgery for the duration of the study. E2 or vehicle was injected s.c. every 4 days. Stress was produced by a 3 day FS paradigm (26°C water, 10 min first day, 20 min on following 2 days). Three scans were performed for each rat: baseline (no visceral hypersensitivity), 2 days post-stress (E2-dependent visceral hypersensitivity), and 18 days post-stress (no visceral hypersensitivity). Rats were fasted for 24 hrs prior to undergoing MRI in a Bruker BioSpec 70/30USR Avance III 7-Tesla scanner (Bruker Biospin MRI GmbH, Germany). Rats were sedated with ≤ 1.5% isoflurane for the entire scanning session. fMRI scans were performed during CRD (80 mmHg for 15 s with a 60 s

interstimulus interval, 15 trials, ~15 min, TR = 1.5 s, resolution = 0.35 x 0.35 x 1 mm). All image preprocessing and statistical analyses were conducted using SPM8. Across all rats, CRD resulted in activation of bilateral insula, bilateral hippocampus, retrosplenial cortex, anterior cingulate cortex (ACC), periaqueductal gray (PAG), and hypothalamus. E2-dependent hypersensitivity was associated with an enhanced response of the right amygdala and bilateral insula, indicating an upregulation in stress-related emotional-arousal circuitry may be estrogen-dependent. Understanding the brain circuitry involved in the IBS model could allow us to define targets for pharmacological and nonpharmacological therapies in humans.

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Poster

243. Pain Imaging and Perception

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Topic: D.08. Pain

Support: Division of Intramural Research, National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, Maryland

Title: *In vivo* opioid receptor availability in rat brain

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Abstract: Introduction: Human brain imaging studies using positron emission tomography (PET) have demonstrated that the cerebral opioid system is altered in patients with chronic pain. With the view of investigating rodent pain models in the future, we investigated the *in vivo* distribution of opioid receptors in the rat brain using PET imaging and compared it to the distribution in humans to elucidate potential species differences. Methods: Each rat (n=10, male Sprague-Dawley) was briefly anesthetised with sevoflurane and received a tail vein injection of [18F]-fluoroethyl-diprenorphine ([18F]-FDPN), a PET tracer with comparable affinity for mu,-

kappa-, and delta opioid receptors, and a subcutaneous hind paw injection of saline (acting as a control for a different experiment). Anesthesia was removed and each rat was placed in an observation chamber for 35 minutes. After behavior observation, each rat was anesthetised with sevoflurane and placed in a Siemens Inveon preclinical PET scanner. At minute 45, the scan began and the rat remained anesthetized for 30 minutes of dynamic image acquisition. Using SPM8 brain imaging tools, PET data were aligned to a common space and coregistered to an anatomical MRI of a weight-matched rat. Data were normalized to the visual cortex, a region relatively devoid of opioid receptors, using the reference tissue method. A set of regions known to be involved in the processing of nociceptive stimuli were defined on the anatomical MRI in Paxinos space and used to calculate the mean tracer binding within each region. Results: The highest levels of [18F]-FDPN binding were seen in the thalamus, caudate putamen, anterior cingulate cortex and amygdala. The level of tracer binding within these regions was in alignment with the human literature. Conclusion: The level of [18F]-FDPN binding in the rat brain is similar to levels seen in the human brain for multiple brain regions of interest for pain-related research.

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Poster

243. Pain Imaging and Perception

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Topic: D.08. Pain

Support: Swedish Research Council 2010-2120

Title: Human nerve growth factor mutation alters neural pathways for behavioral responses to acute pain

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Abstract: Acute pain coding goes beyond sensory processing to encompass the selection and modification of behavioral responses to a noxious event. We used fMRI and white matter tractography (tract-based spatial statistics, TBSS) to investigate such pain-action. We compared

healthy subjects to patients with a rare nerve growth factor beta (NGFB) mutation that results in a reduction in unmyelinated and thinly-myelinated afferent nerves (including nociceptive C and Aδ afferents). These patients show impaired pain sensitivity and indifference to pain, and have been diagnosed with hereditary sensory and autonomic neuropathy type V (HSAN-V; Minde et al 2004). However, they are able to report thermal pain thresholds, which do not differ from those of controls. During fMRI, participants pressed a button at the appearance of a visual cue during painful or nonpainful thermal stimulation, with task instructions changing by block. This allowed modeling of voluntary motor responses and painful stimulation separately (Perini et al, J Neurosci, 2013). For the main effect of pain regardless of motor response, the neurologically healthy control group showed a robust bilateral activation of anterior insula (AI). The main effect of motor action activated the midcingulate/anterior cingulate cortex (MCC/ACC). This activation pattern suggests that anterior insula signals motor-independent aspects of the noxious stimulus, whereas MCC/ACC is more closely tied to the production of a voluntary motor response. In contrast, the mutation carrier group's AI responses were markedly reduced for the main effect of pain, and their MCC/ACC responses were absent for the main effect of motor action. Carriers also rated the "urge to move" their hand away from the painful thermal stimulus as significantly lower than controls. When this "urge to move" was included as a covariate in the statistical model for the healthy group, pain-related signal changes revealed activation in the posterior insula contralateral to stimulation. "Urge" itself covaried with responses in the AI contralateral to stimulation. In carriers, fractional anisotropy (FA) in ACC positively correlated with the density of C afferents in the cornea, which more generally indexes nerve density in the skin. These results suggest a caudal-rostral coding of pain and "urge" information in insula which culminates in action-related processing in the MCC/ACC. The NGFB mutation's consequences in the brain may not affect pain perception per se but lead to less efficient translation of pain information into action, resulting in failure to make appropriate behavioral adjustments in potentially tissue-damaging situations.

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Poster

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Support: NINDS R01NS065051

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Radiology and Anesthesia Foundations, Boston Children's Hospital

Title: Intrinsic brain networks normalize with treatment in pediatric complex regional pain syndrome

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Abstract: Complex regional pain syndrome (CRPS) is a neuropathic pain condition affecting the peripheral and central nervous system characterized by the continuing presence of pain that is disproportionate to the inciting event and it is not curable in adults. Pediatric patients with CRPS typically recover with standard medical treatment. In this study, we evaluated intrinsic brain networks measures in the CRPS disease state and after an intensive multidisciplinary pain treatment program to delineate (a) network abnormalities in the disease state and (b) changes induced by treatment. Twelve patients with unilateral lower extremity CRPS were recruited (n=12; 14.1 ± 0.7 yrs.) and 12 age/sex matched controls (n=12; 14.2 ± 0.8 yrs.). Patients and Healthy Controls were scanned twice, before treatment (patients-Visit 1) and at discharge (3 weeks later: Visit 2). A 3T Siemens Tim Trio was used. Subjects underwent a structural scan (MPRAGE, 256x256 mm 192-1.33 mm slices) and a RSN scan (EPI, 41 slices isotropic 3.5 mm3 voxels, TR/TE=3s/30ms 200 volumes). fsl tools were used for preprocessing and an ICA analysis was done. Group ICA were first calculated and then dual regression was applied to determine differences. The main contrasts of interest were: (i) Patients vs. Controls visit 1 (Disease Effect); (ii) Patients visit 1 vs. Patients visit 2 (Treatment Effect) (iii) Patients vs. Controls Visit 2 (Residual Effects). Patients indicated a reduction of pain symptoms and functional disability. Disease Effect: Intrinsic networks associated with chronic pain in adults displayed abnormal connectivity: salience network: increased connectivity with prefrontal (PFC), cingulate and insula cortices. Default mode network: increased connectivity with frontal, postcentral and precuneus. Central executive network: increased connectivity with PFC, post central and temporal areas. Sensorimotor network: increased connectivity with frontal and parietal areas, decreased connectivity with cingulate and cerebellum. Following treatment, abnormalities in most of these networks tended to be non-significant, however, in some networks (sensorimotor network), they still persisted (Residual Effects). Pediatric pain imaging studies are very sparse and difficult to perform. This is one the first studies of a chronic pain condition in children. We detected changes in intrinsic brain networks in children suffering from CRPS. After a relatively short treatment of 3 weeks, most of the observed changes were diminished. The plasticity of a young brain could be a significant factor in the recovery, as it does not occur in adults.

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Poster

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Title: White matter neuroplasticity following cognitive behavioral therapy for chronic pain

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Abstract: Objectives: Chronic musculoskeletal pain is a leading cause of disability in the United States, however, efforts to provide patients with therapeutic relief have remained insufficient. Novel biobehavioral and psychotherapeutic interventions such as cognitive behavioral therapy (CBT) may provide a more successful long-term treatment option. In this study we hypothesize that changes in potentiation due to CBT intervention will act to neuroplastically alter white matter pathways in the pain matrix measured by diffusion tensor imaging (DTI). Furthermore, we speculate that there will be an increase in fractional anisotropy (FA) and a decrease radial diffusivity (RD) that will correlate with clinical self-efficacy behavioral scores. **Methods:** 49 adult patients with a primary diagnosis of chronic musculoskeletal pain were evaluated by a physician to confirm eligibility. Additionally, 33 age and gender matched controls were recruited. Chronic pain patients were randomized to one of two groups: group 1 received an 11 week CBT intervention, and group 2 received educational mailings pertaining to healthier living habits but not pain coping mechanisms. Magnetic resonance imaging (MRI), including DTI assessment, was acquired before and after 11 week CBT. Furthermore, behavioral self-efficacy questionnaires were completed before and after completion of CBT. DTI analysis was conducted using FSL tract based statistical software (TBSS) followed by subsequent region of interest (ROI) cross-group voxel-based statistical analysis using behavioral clinical measures as covariates. **Results**: There were significant group by time interactions between CBT and education. A decrease in FA was observed in the right

cingulum adjacent to the hippocampus (p<0.01), the right superior longitudinal fasciculus (SLF) (p=0.03) as well as an increase in RD in the left cerebral peduncle (p=0.04). Additionally, significant improvements in self-efficacy behavioral scores were correlated with a decrease in FA and increase in RD. **Conclusions**: CBT intervention showed decreased FA and increased RD in pain matrix white matter tracts, however, the same trend was also noted in the education group. Antithesis to our hypothesis, these changes indicate a further amplification of both FA and RD post-intervention opposed to attenuation. These changes may be attributed to learning to cope with chronic pain over time. Alternatively recent molecular mechanisms have been proposed implicating ATP release by tonically active neurons modulating astrocyte cytokine activity that could have downstream effects on myelinating oligodendrocytes.

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Poster

243. Pain Imaging and Perception

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KUMC clinical pilot

KUMC School of Health Professions Pilot Funding

Title: Brain responsiveness to mechanical pressure in patients with sub-acute low back pain

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Abstract: Low Back Pain (LBP) is one of the most common chronic conditions affecting millions of people worldwide with significant economic impact. Currently, there is no clear understanding of how nociceptive regions of the brain respond to different pain stimuli. Nociceptive brain region reactivity has been examined with functional magnetic resonance imaging (fMRI) to thermal or noxious stimuli. Critically, patients with back pain report pain with mechanical stress. Understanding how the brain responds to mechanical stress could improve our understanding of nociception in LBP. In this study we used fMRI to investigate brain activation patterns resulting from a mechanical stimulus applied to the lumbar spine via fMRI. We developed a MRI compatible device capable of delivering a range of pressures. Thirteen healthy and thirteen LBP subjects (average duration of pain: 8 months) were included in this preliminary study. Using a block design, subjects received 3 levels of mechanical pressure to the lower back with our custom-made pressure device. Whole-brain voxel-wise t-tests comparing each pressure condition to rest were used to examine brain responses to mechanical pressure in healthy and LBP patients separately. Results show significant differences in brain activation patterns between healthy and LBP subjects. Healthy subjects demonstrated increased brain activation in the cerebellum and parts of the frontal and parietal lobes, and decreased activation in the hippocampus, pre and post central gyrus compared to rest. In contrast, LBP subjects demonstrated decreased activation in cingulate, caudate and post central gyrus compared to rest. Our preliminary results suggest that subjects with LBP have altered sensory processing and respond differently to mechanical stimuli compared to healthy subjects.

Disclosures: Z. Mansour: None. **L.E. Martin:** None. **R.J. Lepping:** None. **S. Kanaan:** None. **B. Cowley:** None. **W. Brooks:** None. **N.K. Sharma:** None. **V. Papa:** None.

Poster

243. Pain Imaging and Perception

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: D.08. Pain

Support: NCCAM

NIH

R01-AT004714

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P01-AT002048

Title: Cortical thickness change following acupuncture therapy predicts long-term pain improvement in idiopathic hand pain patients

Authors: *Y. MAEDA[1,2], N. KETTNER[2], J. KIM[1], S. CINA[1], C. MALATESTA[3], J. GERBER[1], C. MCMANUS[3], A. LIBBY[1], R. ONG-SUTHERLAND[3], P. MEZZACAPPA[1], L. R. MORSE[4], J. AUDETTE[5], V. NAPADOW[1]
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Abstract: Introduction: Patients with carpal tunnel syndrome (CTS) and idiopathic hand pain (IHP) both suffer from pain and paresthesia over median-nerve innervated regions of the hand. While CTS presents with decreased median nerve conduction, IHP does not. We have previously shown maladaptive structural neuroplasticity in CTS, and acupuncture has been reported to relieve symptoms associated with hand pain and paresthesia, as well as producing brain plasticity (Napadow et al. 2006, 2007). However, the effects of acupuncture on symptomatology and structural brain plasticity in hand pain disorders devoid of peripheral nerve pathology have not been reported. Moreover, the link between structural brain plasticity and long-term outcomes in such patients is also unknown. Methods: We evaluated the brains of 19 IHP patients with structural T1-weighted MRI at 3T (Siemens Trio) before and after 8 weeks of acupuncture treatment. Pain and paresthesia were evaluated with the Boston Carpal Tunnel Syndrome Questionnaire (BCTSQ, 0-5 scale) before and after acupuncture, as well as 3 months following therapy. Acupuncture treatment included 2Hz electrical stimulation at acupoints PC7 and TW5 near the affected wrist, with an additional 3 acupoints, manually stimulated, chosen by the acupuncturist. Structural data were preprocessed using specific algorithms for longitudinal cortical thickness measurement (Freesurfer, ver. 5.3). Gray matter thickness data were contrasted before versus after acupuncture, and a paired difference map was calculated, cluster corrected for multiple comparisons at $p=0.05$. Cortical thickness data from the most significant vertex were correlated with BCTSQ change. Result: BCTSQ pain (baseline: 3.0±0.6, post-acup: 2.1±0.7, 3-months: 1.9±0.7, mean±SD) and paresthesia (baseline: 2.6±0.7, post-acup: 1.7±0.5, 3-months: 1.5±0.3) decreased following acupuncture treatment and at 3-month follow-up ($p<0.01$). Following acupuncture, cortical thickness was significantly reduced in primary somatosensory cortex (S1) contralateral to the most affected hand. Furthermore, S1 thickness change after acupuncture was negatively correlated with long-term change in BCTSQ pain (3month follow-up vs. post-acupuncture, $r=-0.78$, $p=0.01$). Therefore, greater increase in S1 cortical thickness rate,

the greater the continued reduction in BCTSQ pain at long-term follow-up. Conclusions: Acupuncture reduced pain and paresthesia, while S1 thickness change was correlated with long-term follow-up reductions in pain. Thus, longitudinal cortical thickness change following acupuncture therapy may serve as a predictor for long-term treatment outcome in IHP.

Disclosures: Y. Maeda: None. **N. Kettner:** None. **J. Kim:** None. **S. Cina:** None. **C. Malatesta:** None. **J. Gerber:** None. **C. McManus:** None. **A. Libby:** None. **P. Mezzacappa:** None. **L.R. Morse:** None. **J. Audette:** None. **V. Napadow:** None. **R. Ong-Sutherland:** None.

Poster

243. Pain Imaging and Perception

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 243.25/FF1

Topic: D.08. Pain

Title: 10 hz-modulation low level laser stimulation of k1 acupoint induced brain ipislateral activations: a rodent functional mri study

Authors: C.-W. HSIEH[1], Y.-A. HUANG[2,3], S.-H. YANG[4], C.-H. HSIEH[2,3], C. WU[4], *D.-Y. CHEN[5]
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Abstract: Laser acupuncture (low level Laser) stimulated on acupoint Yongquan (K1) with the different modulation induced different brain activations in the human study. However, according to the higher heart rate and respiratory rate of rodents, we hypothesis the effect of modulated laser acupuncture would conduct similar results in the low frequency modulation in a rodent study. In this study, we are testing the hypothesis by stimulating 10Hz modulated laser acupuncture on the rat K1 located at right backpaw. All the MR experiments were conducted on a 7T Biospec MRI system (Bruker, Germany). Six male Sprague Dawley rats were scanned under institutional approval. During fMRI, rats were anesthetized with α-chloralose. The functional data were analysis by using SPM8 (UCL, U.K.). Our results demonstrated the greater brain activation at right somatosensory cortex S1 in the hind limb region, left motor cortex M1, thalamus and paraventricular thalamic nucleus. Firstly, the activation founded in R-S1 suggested the undefined ipislateral pathway that conducted by laser acupuncture. Secondly, the activation

in thalamus suggested the similar activation to the human 2Hz modulation laser acupuncture study. In conclusion, this study firstly demonstrated the laser acupuncture effect on the rodent K1. The ipislateral activation area and the similar activation of human study suggested under discover pathway conducted by laser acupuncture.

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Poster

243. Pain Imaging and Perception

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Program#/Poster#: 243.26/FF2

Topic: D.08. Pain

Support: NIH NINDS NS035115

NIDCR DE022746

CIHR 301236

Title: Structural and functional network topologies of the limbic system shape chronic pain

Authors: *E. VACHON-PRESSEAU, M. N. BALIKI, P. TETREAULT, T. J. SCHNITZER, A. V. APKARIAN
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Abstract: Neuroimaging studies in humans show that limbic brain properties are involved in motivation, aversive learning, and memory, and might predispose individuals to develop chronic pain. Here we test the role of functional and anatomical properties of the limbic brain in the transition to chronic back pain. We collected fMRI and DTI in 69 subacute back pain (SBP, pain duration < 12 weeks), repeatedly over one year, and examined the functional and structural topological properties of the limbic brain. We constructed structural brain graphs of 1068 nodes (4 mm3 voxels) belonging to the frontal cortex, nucleus accumbens, amygdala, and the hippocampus with edges drawn between nodes representing white matter tracks. Edges were thresholded at a density of 0.1 and binarized. Functional brain graph was also constructed from Pearson correlation coefficients, thresholded at a density of 0.1 and binarized. Both structural and functional networks were sparse and displayed small world properties. A modularity analyses on the structural limbic network revealed the presence of three modules, with dense

within module interconnections and sparse across module interconnections. These modules corresponded to the corticostriatal circuit, the orbitofrontal cortex and the hippocampo-amygdala complex. Over one year, 39 of SBP persisted with back pain (SBPp) and 30 recovered (SBPr). SBPp showed stronger structural connectivity in the corticostriatal module than SBPr at all visits ($F = 9.7$; $p = .003$) and no group differences or interaction were observed in the other modules. SBPp showed stronger functional connectivity in the corticostriatal module compared to SBPr ($F = 4.6$; $p = .04$) and an interaction of time by group was observed in the orbitofrontal module ($F = 3.3$ $p = .02$) and the hippocampo-amygdala module ($F = 4.3$ $p = .007$). These findings suggest that the density of structural and functional connectivity in the corticostriatal circuit predispose individuals to chronic pain; while the density of functional connections in the other two limbic modules change in time in relation to the clinical profile of the patients. Therefore, these results indicate the critical role of the limbic brain in transition to chronic pain.

Disclosures: E. Vachon-Presseau: None. **M.N. Baliki:** None. **P. Tetreault:** None. **T.J. Schnitzer:** None. **A.V. Apkarian:** None.

Poster

243. Pain Imaging and Perception

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Program#/Poster#: 243.27/FF3

Topic: D.08. Pain

Support: NIH NIDCR DE022746

Title: Resting state and stimulus evoked brain activity in awake or isoflurane-anesthetized rat

Authors: *P.-C. CHANG, D. PROCISSI, A. APKARAIN
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Abstract: Functional imaging in rodents generally uses anesthetics to control stress levels and excessive motion. However, the use of anesthetics or paralyzers introduce confounds that can hamper our ability to assess brain network properties and/or stimulus representation. In this work, we describe an innovative design for assessing resting state and task related fMRI in awake, conscious rats. To demonstrate the value of the awake rat fMRI, we compare within animal fMRI results in the awake and anaesthetized conditions. To reduce the stress of the animals in the MRI environment, the rats were trained seven continuous days prior to MRI scans. Each day half hour training with fMRI scanning sound and head-post constraint conditioning

was administered. Custom made snuggle were adjustable around the rats for effective yet comfortable restraint, with an opening that provided access to the rat's hindpaw. A resting state fMRI (rs-fMRI) and stimulus-evoked fMRI were first acquired when the rats were in awake state, followed by repeat scans under isoflurane anesthesia. Rs-fMRI was acquired when rats were resting in the scanner for 8 minutes with no external stimulation. Air-puff stimuli, applied to the hindpaw, were used as mechanical stimulation to examine somatosensory activity. Stimulus-evoked fMRI had 10 repetitive stimulus blocks. Each block consisted of stimulation alternated with 15s-off/15s-on/10s-off. While the rats were awake, Nucleus of Accumbens (NAc) core and shell showed significant connection to various cortical regions, including the cingulate (ACC), somatosensory (S1, S2) and motor cortices (M1, M2). After the rats went under anesthesia, the core and shell mainly display local connections. In the awake state, we observed a significant activation in contralateral S1, S2, thalamus (Tha), and deactivation in ipsilateral S1, and bilateral amygdala (Amy). While anesthetized, the same stimulus only resulted in deactivations in ipsilateral S1, and bilateral Amy. In summary, NAc core and shell in the awake rats show more widespread functional connectivity, compared to the state when the rats were anesthetized; in addition, rats had more fMRI activation in response to air-puff stimulation. These results demonstrate that awake rat fMRI will permit better evaluation of brain networks and brain responses to external stimuli.

Disclosures: P. Chang: None. **D. Procissi:** None. **A. Apkarain:** None.

Poster

243. Pain Imaging and Perception

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 243.28/FF4

Topic: D.08. Pain

Title: Cell to cell coupling in dorsal root ganglion (DRG) neurons amplifies pain sensation

Authors: *Y. KIM[1], K. PARK[2], S. JILAFU[2], L. HAN[2], Z. LI[2], L. YOUNG[2], P. LAVINKA[2], S. HE[2], F. ZHOU[2], Y. GUAN[2], M. CATERINA[2], X. DONG[2]
[1]Neurosci., [2]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Hyperalgesia/allodynia, heightened pain sensitivity in uninjured and/or injured tissues, is widely accepted to be mediated primarily by sensitization of spinal cord and brain neurons, a process called central sensitization. The contribution of peripheral sensitization of primary sensory neurons to hyperalgesia/allodynia remains controversial and largely unknown. Here we

have generated Pirt-GCaMP3 mice in which GCaMP3, a genetic-encoding Ca2+ sensitive indicator, is expressed robustly and specifically in almost all primary sensory neurons. The advantages of GCaMP3 imaging using these mice include facile, parallel direct visualization of activity in many afferents, excellent spatial resolution, and preservation of somatotopic organization. Application of this technique to DRG hyperalgesia/allodynia and inflammatory model permitted us to visualize robust increase of neuronal hypersensitivity in cell bodies of DRG neurons *in vivo* in an anesthetized Pirt-GCaMP mouse. Peripheral sensitization was observed not only as of increasing activated number of neuronal cell bodies in DRG but also as of apparently increasing cell to cell coupling events. Gap junctions are specialized transmembrance channels that allow rapid electrical signaling and direct intercellular communication for maintenance and coordination of normal cellular activities and homeostasis. Strikingly, augmentation in cell to cell coupling in DRG was mediated by the dynamic plasticity of gap junctions in response to nervous system injury and modulated neuronal hypersensitivity and pain in animal models of inflammatory and/or sciatic nerve chronic constriction model (SN-CCI). Blockade of gap junction modulated DRG cell to cell coupling and also chronic and inflammatory pain and is the potential target as a novel modality for the treatment of intractable pain syndromes. Pirt-GCaMP3 mice thus represent a unique tool with which to visualize primary sensory neuron activity under physiological and pathological conditions of pain, itch, and any somatosensations *in vivo* and to reveal peripheral sensitization mechanisms associated with hyperalgesia, allodynia, and possibly referred pain. Therefore, this study creates a new way of characterizing the physiological properties and functions for developing new pain- or other modality-specific drug target for a treatment with few side effects.

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Poster

244. Opioids and Other Analgesics

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 244.01/FF5

Topic: D.08. Pain

Support: NIDA T32 DA00797-32

Title: A bivalent ligand that targets μ opioid and mGluR5 receptor heteromers reduces neuropathic pain responses when given intrathecally to mice

Authors: *C. PETERSON[1], K. F. KITTO[2], E. AKGUN[3], M. LUNZER[3], P. S. PORTOGHESE[4], C. FAIRBANKS[2]
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Abstract: Background: Both the mu opioid receptor (MOR) and mGluR5 receptor are expressed in the spinal cord and are involved in analgesia and pain. The association of the two receptors as MOR-mGluR5 heteromers has been noted in HEK293 cells coexpressed with MOR and mGluR5. Therefore, it is possible that this putative heteromer may be present in the central nervous system (CNS). Such a heteromer could be involved in the hyperalgesia associated with chronic pain given the known functional interaction of mGluR5 with the NMDA receptor. Thus, a bivalent ligand (MMG22) that contains both mGluR5 antagonist and mu agonist pharmacophores should antagonize the mGluR5 protomer and activate the MOR protomer of the putative MOR-mGluR5 heteromer. In fact, intrathecal MMG22 has recently been reported to produce profound antinociception without tolerance in mouse models of inflammatory pain (Proc. Nat Acad Sci. USA, 2013, 110, 11595). In the present studies, we show that MMG22 also reduces nerve-injury-induced tactile hypersensitivity. **Methods:** Baseline responses to electronic von Frey apparatus were collected in ICR-CD1 male mice, after which they were subjected to the spared nerve injury model of neuropathic pain. Several weeks post injury, multiple doses of MMG22 were intrathecally injected at a wide dose range (0.01-10 nmol). Time points assessed included 5, 12, 20, 90, 120 minutes post-injection. Additionally, morphine, mGluR5 antagonist (MPEP), and the combination of morphine + MPEP were assessed for interactions. **Results:** This study demonstrated that intrathecal delivery of the bivalent ligand MMG22 attenuates nerve-injury induced hypersensitivity with high potency. This effect was clear at 5 minutes post injection. Higher doses (1, 3, 10 nmol) demonstrated a longer duration of action. The monovalent ligands, morphine and MPEP (upon which the bivalent structure is based), both reduced hypersensitivity in nerve-injured mice when given individually at 1, 3, and 10 nmol. The potency of coadministered monovalents was substantially less than that of MMG22. **Conclusion**: These results suggest that the MMG22 compound targets a heteromer that likely is comprised of both mu opioid and mGluR5 protomers expressed in the spinal cord.

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Poster

244. Opioids and Other Analgesics

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Program#/Poster#: 244.02/FF6

Topic: D.08. Pain

Support: NIH DA020110

Japan Society of Promotion Science: Grant-in-Aid for Scientific Research (B)26861247

Title: Antinociceptive mechanisms of spinal beta-endorphine with opioid recepter antagonists on acute pain

Authors: *T. TERASHIMA[1], S. YAMAGUCHI[1], T. TAKASUSUKI[1], Y. HORI[2], T. L. YAKSH[3]
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[3]Anesthesiol., UCSD, San Diego, CA

Abstract: Background: An endogenous antinociceptive peptide, which highly binds to mu- and delta-opioid receptors, is β-endorphin. These opioid receptors regulate the neurotransmitter release from primary afferents and hence control analgesia in spinal dorsal horn. The aim of this study is to disclose an ability of intrathecal β-endorphin on acute pain induced behavior and neurotransmitter release from primary afferents. Materials and methods: Rats (male Holtzman Sprague-Dawley, 250g) with intrathecal (IT) catheter administrated IT saline, β-endorphin (1, 3 and 10μg), β-endorphin (10μg) + intraperitoneal (IP) naltrindole (3mg/kg) or β-endorphin (10μg) + IP naloxone (3mg/kg). Animals received intraplantar formalin (50μl) to the left hindpaw. Formalin-evoked flinches were counted for an hour by an automated machine. Rats were transcardially perfused at 2 hours after formalin and c-Fos expression in the lumbar spinal dorsal horn were measured by immunohistochemistry. In separated groups, rats were perfused at 10 min after formalin and the incidence of neurokinin 1 receptor (NK1r) internalization in the superficial dorsal horn were quantified by fluorescent immunohistochemistry. Results and discussion: Formalin injection to the paw resulted in an intense, bi-phasic flinching behavior in the injected side paw, which was reduced by IT β-endorphin pretreatment dose-dependently. Robust NK1r internalization in the ipsilateral L3 to L5 dorsal horn was showed following formalin injection. IT β-endorphin significantly decreased NK1r internalization. Formalin also caused c-Fos expression in the superficial and deep dorsal horn neurons, which reversed by IT β-endorphin. The regulations of IT β-endorphin on formalin-induced flinching behavior, NK1r internalization and c-Fos were demised by naltrindole. Conclusion: IT β-endorphin displayed potent antinociceptive effects in acute pain model. At the dose of analgesia, IT β-endorphin suppressed the release of substance P from primary afferents likewise c-Fos expression in dorsal horn neurons. The effects were at least in part regulated through the delta-opioid receptors.

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Poster

244. Opioids and Other Analgesics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 244.03/FF7

Topic: D.08. Pain

Title: SEO-16: An orally active opioid analgesic with rapid onset of activity and reduced CNS-side effects

Authors: ***S. D. HARRISON**[1], N. ANAND[2], I. CHOI[2], J. EVANS[2], K. GOGAS[2], M. HENNESSY[2], H. GURSAHANI[2], G. KIM[2], M. LEE[2], P. QUACH[2], W. RUBAS[2], J. RIGGS[2]
[1]Nektar Therapeut. Inc., San Francisco, CA; [2]Nektar Therapeut., San Francisco, CA

Abstract: Opioids are widely prescribed for the treatment of moderate to severe acute pain but are limited by their CNS-mediated side effects. Stable polymer conjugation technology has been successfully applied in the development of NKTR-181. NKTR-181 is an opioid receptor agonist intended for the chronic treatment of moderate to severe pain. The molecule is designed to have a slow rate of entry into the CNS, to markedly reduce abuse liability and CNS side effects. Nektar is also developing a novel opioid molecule intended to have low CNS side-effects (SEO-16). This program targets treatment of acute pain, hallmarked by rapid onset of analgesic activity. SEO-16, binds to the mu opioid receptor (Ki = 191 nM) and displays full agonist efficacy *in vitro*. Oral SEO-16 produces full efficacy in the mouse acetic acid writhing model of pain (ED50 = 7 mg/kg po). Activity in the formalin paw test was seen within 15 min, consistent with rapid attainment of plasma exposure (concentrations peak 20 mins after oral dosing in rats), suggesting a rapid onset of analgesic effect. Doses of SEO-16 that produced a greater than 50% reduction in the time spent on the rotarod were 60-80 times higher than ED50 doses that were associated with efficacy in the acetic acid writhing models, suggesting a significant separation of the analgesic and CNS side effects. These data were consistent with *in vivo* pharmacokinetic and *in situ* brain perfusion data in rats showing a 40 fold reduction in brain:plasma ratio and a 4-fold reduction in brain entry rate compared with marketed opioids. These preclinical data suggest that SEO-16 is a mu-opioid agonist with low CNS side effects that produces a rapid onset of analgesic activity. The reduced brain entry rate may also lead to reduced abuse potential compared to currently marketed products for this indication. Taken together, these data indicate

that Nektar's polymer conjugation technology can be used to alter opioid pharmacology to enable treatment of both chronic and acute pain while minimizing undesirable CNS side effects.

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Poster

244. Opioids and Other Analgesics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 244.04/FF8

Topic: D.08. Pain

Support: Kumamoto University

Title: Valpronic acid attenuated the development of morphine withdrawal syndrome in mice

Authors: T. SHINOZAKI, M. ARAKI, *T. YAMAMOTO
Kumamoto Univ. Hosp., Kumamoto-shi, Japan

Abstract: Background: Valpronic acid has been used for the treatment of epilepsy, bipolar disorder and migrane, but the precise mechanisms of action of valpronic acid is not fully understood. Valpronic acid has many actions, such as the enhancement of GABA-mediated neurotransmission, the involvement of signaling systems like the Wnt/β-catenin and ERK pathways and interference of inositol and arachidonate metabolism. Recently, valpronic acid has been known to have the property of HDAC inhibitor. Recently, a selective HDAC inhibitor, suberoylanilide hydroxamic acid, has been reported to enhance naloxone-induced morphine withdrawal syndrome. On the other hand, other HDAC inhibitor, such as trichostatin A, has been reported to attenuate naloxone-induced morphine withdrawal synderome. In the present study, we examined whether valpronate attenuates the development of naloxone-induced morphine withdrawal syndrome. Methods: Valpronic acid was injected three times daily (900 mg/kg/day) for 5 days. Morphine withdrawal syndrome was induced by ip injection of 3 mg/kg of naloxone after 5 days infusion of 24 mg/kg/day morphine. Level of morphine withdrawal syndrome was evaluated by counting the number of jumping during 10 min after naloxone injection. Results: Valpronic acid attenuated the development of naloxone-induced morphine withdrawal syndrome. Conclusion: HDAC may play an important role in the development of morphine withdrawal syndrome and the inhibition of HDAC by valpronic acid is a useful technique to prevent morphine withdrawal syndrome.

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Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: USU grant RO75QU

Title: Activation of the mu opioid receptor stimulates the binding of RGS4 to the receptor/G protein complex in a GTP-dependent manner

Authors: R. SANTHAPPAN[1], *T. E. COTE[2]
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Abstract: The conditions that cause RGS4 to associate with the mu opioid receptor/G-protein complex in rat brain membranes were examined. Solubilized mu opioid receptors (MOP-r), that retained high affinity, guanine nucleotide-sensitive agonist binding, were immunoprecipitated with antibodies directed against either the N-terminal or the C-terminal of the rat mu opioid receptor. Stimulation of MOP-r with [D-ala^2, N-Me-Phe^4, Gly5-ol]-Enkephalin (DAMGO), a selective MOP-r agonist, caused a 2-fold increase in the amount of the G-proteins that co-immunoprecipitated with MOP-r and also increased by 35% the amount of recombinant RGS4 that associated with the MOP-r/G protein complex. Interestingly, MOP-r activation by DAMGO in the presence of 10 μM GTP further increased the association of RGS4 with the MOP-r/G-protein complex by 72% (unpaired t test, p=0.003). Direct activation of G-proteins with GTPS, a nonhydrolyzable analog of GTP, increased by 2-fold the association of RGS4 with the MOP-r/G-protein complex (unpaired t test, p=0.013). The amount of RGS4 co-immunoprecipitating with MOP-r was determined by comparing the densities of RGS4 bands to the densities of known standard amounts of RGS4 in western blots. The presence of MOP-r in immunoprecipitates was detected by western blotting using anti-MOP-r$_{349\text{-}398}$. Quantification of MOP-r in immunoprecipitated material was determined by saturation [^{3}H]DAMGO binding. It was determined that the ratio of RGS4 to MOP-r was 1 to 1 when the G-proteins associated with MOP-r were fully activated. In Ni^{2+}-resin pull down experiments, it was determined that activation of Goa with GTPS tripled the amount of Goa bound to His_6RGS4 while GTP and GDP were without effect. In SHSY5Y cell homogenates, RGS4 caused a concentration-dependent, noncompetitive attenuation of DAMGO-mediated inhibition of adenylyl cyclase activity but had no effect on GTPS-mediated inhibition of adenylyl cyclase activity. Conclusions: Agonist activation of MOP-r causes Gi/o-type G proteins to bind to MOP-r and causes GTP to activate Gi/o-type G-proteins. GTP-activated G-proteins trigger downstream signaling and also cause RGS4 to bind to the MOP-r/G-protein complex and to cause a noncompetitive attenuation of MOP-r signaling by increasing the rate of GTP hydrolysis by Gi/o-type G-proteins.

Disclosures: R. Santhappan: None. **T.E. Cote:** None.

Poster

244. Opioids and Other Analgesics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 244.06/FF10

Topic: D.08. Pain

Support: National Institute on Drug Abuse DA-025164

University of Minnesota Academic Health Center

3M Fellowship

NIH Training Grant T32 DA07097

Title: Intrathecal pretreatment with AAV5 carrying the gene for human arginine decarboxylase (hADC) inhibits the development of opioid analgesic tolerance

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Abstract: Background: The decarboxylated form of L-arginine (agatmine) has been shown to reduce opioid analgesic tolerance, as well as other manifestations of neuroplasticity. Agmatine inhibits NMDA receptor activation and the production of nitric oxide synthase; consequently the mechanism by which agmatine acts to reduce neuroplasticity likely involves that cascade. Since agmatine has a synthetic pathway an opportunity presents itself to increase expression of the synthetic enzyme (arginine decarboxylase, ADC) and potentially local agmatine levels. Toward this end, we have developed an adeno-associated viral vector (AAV) to provide gene transfer of human ADC following intrathecal delivery. We then compared the development of systemic opioid analgesic tolerance in subjects treated with this vector (AAV5-hADC) or saline controls. Methods: ICR-CD1 male mice were pre-treated with either AAV5-hADC vector or saline eight weeks prior to testing. Baseline tail flick responses were collected using the warm water tail flick immersion test (52.5°C). Opioid tolerance was induced with seven subcutaneous (s.c.) injections of either morphine (3 mg/kg (4 times) and 5 mg/kg (3 times)) or saline over the course of 3 days. On day four, cumulative dose-response curves to s.c. morphine (1, 3, 10, 20 mg/kg) were constructed in each of the four pre-treatment groups (Saline-Saline, Saline-Morphine, AAV5-hADC-saline, AAV5-hADC-morphine.) Relative potency (Tallarida 1987) was calculated for

each dose-response curve and the development of morphine tolerance assessed in each of the two primary treatment groups. Following completion of the experiment, mice were euthanized and spinal cord, dorsal root ganglia, periaqueductal grey, and choroid plexus tissues assessed for mRNA expression. Results: A significant four-fold rightward shift in the morphine dose-response curve was observed in control subjects, as expected. However, in subjects pre-treated with AAV5-hADC, the ED50 values were equivalent between the morphine and saline treatment groups, indicated that morphine tolerance did not develop. Expression of hADC mRNA was confirmed in 15 out of 16 animals in any of the following tissues: Choroid plexus, spinal cord, dorsal root ganglia, periaqueductal gray. Conclusion: We interpret the observed inhibition of morphine analgesic tolerance in the AAV5-hADC treatment group as suggestive of agmatine produced endogenously from the over expression of hADC.

Disclosures: **C.C. Churchill:** None. **S. Schnell:** None. **M. Riedl:** None. **C. Peterson:** None. **K. Kitto:** None. **J. Weinhold:** None. **L. Vulchanova:** None. **C. Fairbanks:** None.

Poster

244. Opioids and Other Analgesics

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Program#/Poster#: 244.07/FF11

Topic: D.08. Pain

Support: CIHR award CERC08

Title: Identification of new opioid receptor variants

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Abstract: Mu-opioid receptor (MOR) is the main target for opioid analgesics. The gene OPRM1 encodes for a number of alternatively spliced variants of the receptor -over 20 alternatively spliced mRNA constructs are known in human, and over 50 in mice. The functional significances of different splice variants are still poorly understood, but there is some evidence for isoform-specific differences in cellular signalling, contributing to certain adverse effects related to use of opioid drugs. Surprisingly, despite high homology, much less splice variants have been described for the other members of the opioid receptor family: delta- and kappa-opioid receptors and opiate receptor-like (DOR, KOR and OPRL, respectively). Identification of new variants of these

receptors, and understanding their signaling pathways could reveal similar diversity as is seen with MOR, and could lead to discovery of novel specific drug targets. Therefore, we aimed to find new isoforms of all four opioid receptors. We first treated cultured Be2C human neuroblastoma cells with 100 nM deltorphin II (DOR selective agonist), 1 uM IBNtxA (MOR1-K selective agonist) or 1 uM morphine (non-selective opioid receptor agonist) for 1, 3, 8 or 24 h to observe changes in mRNA expression of DOR1, MOR1 or MOR1-K (a 6-transmembrane variant of MOR). We also used the spinal cords of DOR exon 1-knockout and wild type mice, chronically treated with morphine or vehicle. Total RNA was extracted from the cells using the guanidinium thiocyanate-phenol-chloroform extraction method, and from the spinal cords using a special column extraction kit for lipid-rich tissues. The quality of the RNA was verified with Agilent Bioanalyzer. The mRNA was processed for determination of the expression levels of the receptors by qPCR (junctions of exons were amplified: 1-2 for MOR1, 13-2 for MOR1-K and 1-2 for DOR1) We observed an up-regulation of all three receptors mRNA in a ligand-specific pattern. The most robust up-regulation was observed for with 100 nM deltorphin II and 1 uM IBNtxA treatments after 1h. Morphine dependent mRNA up-regulation was most prominent in 3 hours. mRNA from these time points were processed for 5'RACE PCR to map possible new 5' splice variants. Multiple PCR-products generated by a gene-specific primers targeting 5' end of selected exons in all four opioid receptor genes were generated, suggesting multiplicity of 5'alternatively spliced isoforms. Hi-seq sequencing approach is applied to map the fine substructure of these alternatively-spliced isoforms.

Disclosures: M.H. Piltonen: None. **L. Diatchenko:** None.

Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: State of Washington Initiative Measure No. 171

Title: Ligand biased differences in morphine and fentanyl antinociception following microinjection into the ventrolateral periaqueductal gray of the rat

Authors: R. L. WESCOM, K. N. CAMPION, K. A. SAVILLE, E. N. BOBECK, *M. M. MORGAN
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Abstract: Mu-opioid (MOP) receptors in the periaqueductal gray (PAG) contribute to the antinociceptive effects of opioids such as morphine and fentanyl. We recently found that relative to morphine, the antinociceptive efficacy of fentanyl is much lower when administered into the ventrolateral PAG as opposed to systemically (Bobeck et al., 2012). This loss of antinociceptive efficacy could be caused by diffusion of fentanyl to sites outside the PAG. Experiment 1 tested this hypothesis by comparing the ability of morphine and fentanyl to produce antinociception when injected in and adjacent to the ventrolateral PAG. It also is possible that morphine and fentanyl antinociception are mediated by differential activation of pre- and postsynaptic MOP receptors in the PAG. MOP receptors are located on both pre- and postsynaptic elements and are coupled to unique signaling pathways at each site. Given that presynaptic receptors have greater control over transmitter release, morphine may produce antinociception by a predominantly presynaptic mechanism and fentanyl via a predominantly postsynaptic mechanism. Experiment 2 tested this hypothesis by determining whether blocking presynaptic Kv+ channels with alpha-dendrotoxin or postsynaptic GIRK channels with tertiapin-Q preferentially attenuates the antinociceptive effect of microinjecting morphine or fentanyl into the ventrolateral PAG. Experiment 1 revealed that morphine and fentanyl were most likely to produce antinociception when microinjected into the ventrolateral PAG as opposed to adjacent sites or into the dorsal raphe nucleus. Although antinociception was evident at injection sites adjacent to the PAG, the present data suggest this antinociception is caused by diffusion into the PAG. Experiment 2 showed that blocking presynaptic Kv+ or postsynaptic GIRK channels by injecting alpha-dendrotoxin or tertiapin-Q into the ventrolateral PAG caused a significant reduction in morphine antinociceptive potency. In contrast, microinjection of alpha-dendrotoxin or tertiapin-Q into the ventrolateral PAG had no consistent effect on fentanyl antinociception. These data demonstrate that the PAG is the primary site for antinociception when morphine or fentanyl are injected in this region. In contrast, morphine and fentanyl differ in their reliance on pre- and postsynaptic potassium channels on antinociception. Morphine antinociception is much more sensitive to inhibition of potassium channel currents than the antinociception produced by fentanyl.

Disclosures: R.L. Wescom: None. **K.N. Campion:** None. **K.A. Saville:** None. **E.N. Bobeck:** None. **M.M. Morgan:** None.

Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: NIH Grant NS70814

NS26363

Title: A possible role of peripheral mu-opioid receptors in DALDA-induced inhibition of ongoing neuropathic pain and persistent inflammatory pain in rats

Authors: *V. TIWARI, S.-Q. HE, F. YANG, Q. XU, R. SHECHTER, A. CARTERET, Y. GUAN, S. RAJA
The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Abstract: Peripherally acting mu-opioid receptor (MOR) agonists, which generally do not cause the central nervous system (CNS) side effects known to centrally penetrating mu-opioids (e.g., morphine), attenuate both mechanical and heat hypersensitivity in rodent models of inflammatory and neuropathic pain. Yet, it is unclear whether they provide analgesic effects for ongoing, persistent pain. Ongoing pain is the most common and bothersome symptom in patients suffering from tissue or nerve injury. However, because most pain behavioral assays in preclinical studies rely heavily on reflex response to evoked sensations, they may not reflect whether a drug is effective in alleviating ongoing pain. Recent studies show that inhibition of ongoing pain in animal models may be examined by a conditioned place preference (CPP) assay. We used a CPP assay to examine the effects of dermorphin [D-Arg2, Lys4] (1-4) amide (DALDA), a potent and highly selective MOR agonist with restrictive penetration into the CNS after subcutaneous (s.c.) administration. We subjected rats to L5 spinal nerve ligation (SNL) to induce ongoing neuropathic pain and tested DALDA at 2-3 weeks after injury. Compared to the preconditioned state, SNL rats spent significantly more time (e.g., preference) in the DALDA-paired chamber after receiving two conditioning sessions of DALDA treatment (45 min/session, 1 session/day; 5 and 10 mg/kg s.c., n=8-12/dose). Importantly, neither dose of DALDA (5 and 10 mg/kg, s.c., n=8) induced CPP in naive rats, suggesting that DALDA-induced CPP in SNL rats is likely due to the reward of pain relief, not from penetration into CNS and direct activation of CNS reward circuitry, an action known to morphine. Further, intraperitoneal (i.p.) pretreatment with methylnaltrexone (5 mg/kg), a peripherally restricted MOR-preferring antagonist, at 10 min before DALDA injection blocked DALDA-induced CPP in SNL rats, suggesting a peripheral mechanism for DALDA-induced relief of ongoing pain. Gabapentin (60 mg/kg, i.p), which we used as a positive control, also induced CPP in SNL rats (n=8), but not in naive rats (n=8). Moreover, DALDA pretreatment (10 mg/kg s.c, 30 min, n=10) significantly inhibited ongoing pain behavior in rats at 15-60 min (phase II) after intraplantar injection of formalin (50 µL, 1%, n=10) and attenuated the subsequent development of mechanical hypersensitivity at 4-6 h after formalin injection. Our studies suggest that peripherally acting mu-opioids may ameliorate ongoing pain associated with neuropathic injury. Further studies are required to examine the underlying neurophysiologic mechanisms.

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Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: Eli Lilly & Company

Title: How do biased u-opioid receptor and non-biased agonists differ on receptor desensitization and downstream signaling?

Authors: ***S. P. ARNERIC**, T. W. STINNETTE
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Abstract: Background: u-opioid receptor (MOR) agonists are the standard of care treatment for severe pain. DeWire et al. (2012) reported reduced GI effects and respiratory depression with TRV130, a biased MOR agonist that couples with G-protein and bypasses beta-arrestin-2 signaling pathways. While it had been reported to have reduced receptor phorsphorylation, data had not been shown regarding effects on functional, acute desensitization to prolonged receptor activation, or its recovery. This study characterized these responses to non-biased (morphine & fentanyl) versus biased (buprenorphine & TRV130) MOR agonists previously shown to lack beta-arrestin-2 engagement using hMOR-1 CHO cells (DiscoveRx™). Results: Different cellular and receptor phosphorylation profiles were observed between the biased and non-biased agonists. Concentration response curves (0.1- 1,000 nM) demonstrated all four agonists inhibited cAMP with EC50= 1-10 nM at time points < 4 hrs; buprenorphine and TRV130's inhibitory G-protein activity were completely lost by 24 hr and failed to recover back to control EC50 levels post a 1-4 hr washout/recovery period (N=3). In contrast, morphine and fentanyl continued to exhibit inhibition at 24 hr, although CRCs where significantly rightward shifted. Both morphine and fentanyl rapidly, and completely, recovered EC50 levels within 1-2 hrs. The biased agonists had significantly lower phosphorylation (pSer375; LSBio #LS-C17026) levels (2-to 5-fold less) when compared to morphine or fentanyl. While all agonists rapidly reduced p-MOR by 1 hr exposure, functional recovery to p-MOR occurred with morphine and fentanyl despite continued ligand exposure, an effect that did not occur with buprenorphine and TRV130. Suprisingly, p-MOR levels elicited by morphine and fentanyl declined during the 4 hr recovery phase, while

cAMP responses were restored. Both responses to TRV130 and buprenorphine were unchanged. MOR activation induces several fold higher levels of AKT and ERK phosphorylation in comparison to p38a and p70S6, although differences between biased and non-biased ligands were not apparent. Conclusions: Our data suggest that the degree of G-protein mediated cAMP response initially tracks with p-MOR375 levels overall, and that loss of cAMP responses (acute desensitization) is more susceptible to agonists with a lower potential to phosphorylate MOR. In contrast, recovery of non-biased ligands requires less pMOR375 to elicit the same G-protein mediated cAMP response. Whether these observations are only relevant to recombinant engineered cells, or correspond to *in vivo* responses, remains to be established.

Disclosures: S.P. Arneric: A. Employment/Salary (full or part-time):; Eli Lilly & Company. **T.W. Stinnette:** A. Employment/Salary (full or part-time):; Eli Lilly & Company.

Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: The Nakatomi Foundation

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Title: Involvement of radixin in the attenuation of morphine analgesia through the small intestinal P-glycoprotein

Authors: *S. TOKUYAMA, T. KOBORI, S. HARADA, K. NAKAMOTO
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Abstract: Aims: Many cancer patients suffer from cancer-related pain around the world. The World Health Organization recommends oral administration of opioids to treat pain from palliative care setting. Hence, the number of patients receiving opioids in conjunction with cancer chemotherapy is expected to increase, giving rise to drug-drug interactions between these agents during absorption process in the small intestine. Note that various anticancer drugs and opioid analgesics are recognized as substrate drugs of P-glycoprotein (P-gp), a drug efflux transporter. Previously, we have reported that repeated oral administration of etoposide (ETP), a

typical substrate of P-gp, attenuates oral morphine analgesia with decreases in serum and brain levels of morphine through increased expression of small intestinal P-gp. We have also proposed that activation of ezrin/radixin/moesin (ERM), scaffold proteins for P-gp, mediated by RhoA/ROCK signaling may have an impact on this mechanism. However, it has yet to be determined which one of three contribute to the alteration in the small intestinal P-gp. Here, the purpose of this study is to determine which ERM contributes the most to the increased expression of P-gp in the small intestine after treatment with ETP. Methods: Each protein expression levels in the plasma membrane fraction of small intestine or localization analysis were conducted by western blotting or immunofluorescence study. The protein-protein interaction between P-gp and each ERM proteins was determined by immunoprecipitation assay. The analgesic effect of morphine (50 mg/kg, p.o.) was evaluated by tail-flick test. Results: On 24 hr after completing repeated treatment with ETP (10 mg/kg/day for 7 days, p.o.), protein expression of radixin but not ezrin or moesin in the small intestinal membrane was significantly increased compared with that of vehicle-treated mice. Additionally, the amount of radixin but not ezrin or moesin co-immunoprecipitated with P-gp was also dramatically increased in the same fraction. The increased expression of radixin and P-gp by ETP treatment was significantly suppressed by concurrently administered rosuvastatin (an inhibitor of RhoA; 5 mg/kg/day for 7 days, p.o.) or fasudil (an inhibitor of ROCK; 5 mg/kg/day for 7 days, p.o.), respectively with inhibitory effect against an attenuation of oral morphine analgesia seen after repeated treatment with ETP. Conclusion: Activation of radixin via RhoA/ROCK signaling by repeated oral treatment with ETP may at least, in part, be involved in an increase in the protein expression of small intestinal P-gp, leading to attenuation of oral morphine analgesia.

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Poster

244. Opioids and Other Analgesics

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Title: β–arrestin–2–biased agonism of delta opioid receptors sensitizes transient receptor potential vanilloid type 1 (TRPV1) in primary sensory neurons

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Abstract: The clinical significance of chronic pain is overwhelming and treating pain and the related loss of productivity in America costs society upwards of $635 billion annually. Morphine and other agonists acting at the mu opioid receptor (MOR) remain first line drugs for the treatment of moderate to severe chronic pain. However, prolonged use of MOR agonists can lead to opioid-induced hyperalgesia (OIH), characterized by exaggerated pain sensitivity. Recent data from our lab demonstrate that chronic activation of peripheral MOR with β-arrestin2-biased agonists sensitizes TRPV1, producing behavioral symptoms of OIH. Delta opioid receptors (DOR) are gaining considerable attention as promising new targets in the treatment of chronic pain. DOR agonists demonstrate antinociceptive properties and are reported to have fewer side effects than traditional opioids; however, no studies have evaluated whether or not agonists at DOR are capable of producing OIH following chronic administration. In the present study, immunocytochemical analyses demonstrate DOR colocalization with TRPV1 in peptidergic and non-peptidergic rat peripheral sensory neurons. Additional experiments in heterologous expression systems show that treatment with DOR agonist SNC80, not ARM390, reduce β-arrestin2 association with TRPV1 and recruit β-arrestin2 to DOR. Peripheral sensory neurons from rat trigeminal ganglia (TGs) were nucleofected with YFP-tagged DOR or YFP control vector and were assessed for TRPV1 activation following stimulation by capsaicin. DOR-YFP and YFP had no effect on basal TRPV1 response. However, pretreatment with β-arrestin2-biased DOR agonist SNC80, not ARM390, significantly sensitizes TRPV1. Interestingly, this sensitization is species-dependent, as cells expressing human or rat DOR display significantly greater enhancement than those expressing mouse DOR. In addition to cellular sensitization of TRPV1, chronic administration of SNC80 *in vivo* produces behavioral signs of OIH in rats, but not mice. This supports the concept that DOR-TRPV1 cross-talk is species-dependent. Together these data suggest that species-dependent β-arrestin2 recruitment to DOR, away from TRPV1, may play a peripheral role in OIH.

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Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

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DA024865

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Title: The prototypical kappa opioid receptor (KOR) antagonist, norBNI, uniquely regulates KOR function in peripheral sensory neurons

Authors: *R. J. JAMSHIDI, B. A. MCGUIRE, L. C. SULLIVAN, T. A. CHAVERA, W. P. CLARKE, K. A. BERG
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Abstract: KOR couples to a variety of intracellular signaling cascades, including inhibition of adenylyl cyclase (AC), activation of Extracellular Signal-Regulated Kinase (ERK), and c-Jun N-terminal Kinase (JNK). Previous studies with norbinaltorphimine (norBNI) show that this selective KOR "antagonist" is an "agonist" for activation of JNK. Activation of JNK by norBNI results in a long-term reduction in KOR function in HEK cells and in brain following a single administration. Similarly, we have found that local administration of norBNI leads to long-lasting, JNK-mediated, reduction of KOR function in peripheral sensory (pain-sensing) neurons. Using a rodent behavioral model of nociception, we found that peripheral KOR-mediated antinociception in the ipsilateral paw was abolished 2 and 7 days following a single intraplantar (i.pl.) injection of norBNI. The KOR responsiveness of the contralateral paw was unaffected. The long-term inhibition of KOR-mediated antinociception was completely abolished by the JNK inhibitor, SP600125, injected i.pl., prior to norBNI. Long-term inhibition of KOR function by norBNI also occurred when norBNI was applied directly to adult rat peripheral sensory neurons in culture (*ex vivo*). Treatment of neuronal cultures with norBNI for 1h, followed by washing, abolished KOR-mediated inhibition of AC activity (in a JNK-sensitive manner), however KOR-mediated activation of ERK was unaffected. Since JNK is a well-known activator of transcription factors, ultimately leading to protein synthesis, we sought to determine if protein synthesis was required for the long-term effects of norBNI. In hindpaws treated (i.pl.) with the protein synthesis inhibitor, cycloheximide (CHX), prior to norBNI administration (i.pl.), the long-term effect of norBNI on peripheral KOR-mediated antinociception was completely abolished. Similarly, the inhibitory effect of norBNI on KOR-mediated inhibition of AC activity in peripheral sensory neuron cultures was abolished completely with CHX pretreatment. Additionally, inhibiting protein translation with rapamycin, an mTOR inhibitor, also completely abolished the long-term effects of norBNI *in vivo* and *ex vivo*. Taken together, these results

suggest that activation of JNK by norBNI leads to increased protein synthesis of an unknown protein that subsequently leads to prolonged reduction in some, but not all, KOR functional responses in peripheral sensory neurons. Moreover, these data provide strong evidence that norBNI is a protean ligand which has powerful capacity to regulate peripheral KOR function.

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Poster

244. Opioids and Other Analgesics

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Support: Seed grant Program, College of liberal Arts (CLLA), TAMU

Title: Differential effects of various opioids on the development of allodynia and abuse in the context of pain

Authors: ***M. A. EMERY**, M. L. S. BATES, P. J. WELLMAN, S. EITAN
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Abstract: The use of opioids has been associated with altered responses of D2-like dopamine receptors (D2DRs) that contribute to the pathology of addiction and other mental illnesses. Our recent studies demonstrate that this effect of opioids on the response of D2DRs is markedly pronounced during adolescence. In addition, our recent studies demonstrate that the effects of opioids on D2DRs' responses are ligand-selective. Specifically, in a rodent model of recreational use, buprenorphine, methadone, hydrocodone, oxycodone, and morphine differentially alter the responses of the dopaminergic system in adolescents. Burn injuries are very common in the pediatric population, representing the fifth most common cause of non-fatal childhood injuries. Burn injuries have greater than 98% survival rate among people below age 20, but they are known to be extremely painful. In addition to acute pain, 18% of burn survivors experience persistent pain long after healing was complete. The need to use opioids to manage severe pain in burn victims is ultimately unavoidable, but currently results in a problematic increase in prescription opioid abuse. Additionally, there is evidence that morphine may paradoxically result in hyperalgesia/allodynia following chronic use for pain. Thus, this study examined the effect of hydrocodone, oxycodone, and morphine on the development of allodynia symptoms and

subsequent alteration of D2DRs responses and opioid reward in a rodent model of burn injury. A burn or sham injury was induced on the dorsal portion of the hindpaw of adolescent mice. The resulting pain was treated orally with various opioids administered twice daily for 14 days. During these 14 days, mice were tested for the development of mechanical and cold allodynia. Each time, mice were tested for baseline pain sensitivity followed by the antinociceptive response to the various opioid. Subsequently, mice were tested for the response to the D2/D3 receptor agonist quinpirole, or the acquisition of morphine conditioned place preference (CPP). Our results demonstrate a complex interaction between the specific opioid administered and the experience of burn pain. Specifically, the development of allodynia and the subsequent alteration in the response to a D2/D3 agonist and morphine CPP are both ligand-selective (i.e. dependent on the specific opioids used) and altered by the experience of pain. These findings suggest that the use of various opioids for pain management in burn victims carry differential risks for the development of hyperallodynia, altering the neurochemistry of adolescents' brain, especially the dopaminergic system, and the development of opioid addiction.

Disclosures: **M.A. Emery:** None. **M.L.S. Bates:** None. **P.J. Wellman:** A. Employment/Salary (full or part-time):; Texas A&M University. **S. Eitan:** A. Employment/Salary (full or part-time):; Texas A&M University.

Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: R21 NS079897

Title: Increased response to glutamate in small dorsal root ganglia neuron of morphine tolerant rats

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Abstract: The hyperalgesic effects of opiates are one of most intriguing aspect of these widely prescribed drugs. Our lab has focused on changes that occur in primary sensory neurons of rats rendered tolerant to morphine. We conducted patch clamp recordings on neurons in intact dorsal

root ganglia (DRG) and found that 5 days after twice-daily injection of an escalating dose of morphine (10 to 40 mg/kg/s.c.), small DRG neurons consistently showed increased excitability. The rheobase was reduced from 267.7± 29.8 pA to 203.7 ± 13.6 pA ($p < 0.05$, $n = 30$). The membrane threshold was reduced from -13.3 ± 1.7 mV to -20.3 ± 1.1 mV ($p < 0.001$, $n = 25$). While these results are consistent with previous reports, we also found that after morphine injection, small diameter primary sensory neurons showed a greatly increased response to puff application of 1mM glutamate, 686.5 ± 127.5 pA vs. 227.4 ± 25.7 pA in controls. Though this result suggest an enhancement of glutamate receptor function, analysis of individual glutamate receptors did not reveal any increase in receptor response to selective agonist application. Responses to puff application of KA (100 μM), AMPA (100 μM), and DHPG (group I mGluR specific agonist, 1 mM) on small sensory neurons were similar in morphine treated and control rats. Unexpectedly, there was a marked decrease response to NMDA (100 μM) in the small sensory neurons of morphine treated rats (33.4 ± 7.9 pA in morphine group vs. 554.0 ± 174.1 pA in control group, $n = 12$, $p < 0.01$). To control for opiate withdrawal, we included morphine (5 μM) in the perfusate bathing the DRGs during the recordings. These results suggest that the increased response of small DRG neurons to glutamate is involved in morphine-induced hyperalgesia but the mechanism remains to be determined.

Disclosures: **K. Gong:** None. **P. Ohara:** None. **L. Jasmin:** None.

Poster

244. Opioids and Other Analgesics

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Title: Curcumin attenuates thermal hyperalgesia in a rat full thickness thermal injury model

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Abstract: Introduction: The nature of recovery and healing from severe burn injuries involve intensely painful procedures including wound debridement, dressing changes, and strenuous physical and occupational therapy. In fact, procedural pain is the most common grievance

reported by the burn population. Although opioids are available to treat severe pain, a multitude of side effects including development of tolerance accompany routine use of opioids for severe pain. In order to reduce the amount of opioids necessary for burn patients, alternative analgesics with reduced side effect profiles deserve exploration. Curcumin, the active compound in turmeric, which has been utilized in ancient medicine for centuries, has potential to be utilized as an analgesic or as an adjuvant that has potential for use in burn pain management. Objective: To assess the effect of curcumin on inflammatory signaling *in vitro*, and to asses curcumin's effect on mechanical allodynia and thermal hyperalgesia in a full thickness thermal injury model *in vivo*, and to measure curcumin's effect on calcitonin gene-related peptide (CGRP)in human dental pulp. Methods: *In vitro* assays were performed on heat shocked human keratinocyte-derived cells and phosphorylation levels of NF-kB and p38 MAPK were assessed. Utilizing a rat model of full thickness thermal injury model in Sprague Dawley male rats, curcumin was injected into the thermally injured hind paw for 5 consecutive days post thermal injury and both thermal hyperalgesia and mechanical allodynia was assessed. Finally, human dental pulp was collected and CGRP levels were assessed after inflammatory stimulation either with or without pretreatment with curcumin. Results: Curcumin treatment suppressed phosphorylation levels of NF-kB and p38 MAPK in heat shocked keratinocytes-derived cells. Curcumin also, when delivered locally, attenuates thermal hyperalgesia in a full thickness thermal injury rat model. Conclusions: Curcumin has been shown to alter gene expression, modulate several signaling pathways, and may interact directly or indirectly with target molecules to produce anti-inflammatory effects as well as alter thermal hyperalgesia and mechanical allodynia. Therefore curcumin has potential to aid in reducing the amount of opioids necessary for burn procedures in burn patients.*The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.*

Disclosures: **M.M. Salas:** None. **L.N. Petz:** None. **M. Fowler:** None. **J. Clifford:** None.

Poster

244. Opioids and Other Analgesics

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Title: Local anaesthesia reduces the acute inflammatory edema and early elevation of glutaminase in dorsal root ganglion neurons during adjuvant induced arthritis

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Abstract: Glutamate, released from peripheral afferents, is implicated as a sensitizer of peripheral nociceptive terminals during inflammation. Glutamate is synthesized from glutamine in peripheral terminals via the enzyme phosphate-activated glutaminase. In rat adjuvant induced arthritis (AIA) model, an early elevation of glutaminase was observed in the ipsilateral dorsal root ganglion (DRG) neurons during acute (24h) phase of inflammation. We hypothesize that electrical activity mediates, in part, this increase of DRG glutaminase in response to acute peripheral inflammation. In this study, unilateral AIA was induced with a single subcutaneous injection of complete Freud's adjuvant (CFA) into the rat hindpaw. Sciatic nerve blockade was performed by concurrent injection of 1% lidocaine with epinephrine into the sciatic nerve at the mid-thigh level. Paw thickness was measured as an index of the severity of inflammation. At 24 h of AIA, rats were anesthetized and transcardially perfused with fixative. Ipsilateral L4 DRGs were collected and GLS was localized in DRG neuronal cell bodies with immunofluorescence microscopy. Mean grey intensity of GLS was evaluated with image analysis software Image J. Local anesthetic blockade of neuronal conduction in sciatic nerve partially alleviated the acute hindpaw edema and suppressed the elevation of glutaminase at 24 h post CFA injection. As no changes were observed in DRG of the non-inflamed side within 24 h, the elevation of glutaminase at this time point was systemically mediated. The partial relief of edema and suppressed glutaminase expression indicate that increased neuronal electrical activity may be one of the mechanisms that drive the increase of glutaminase expression in the DRG in response to peripheral inflammation. Our result supports the notion that increased nerve activity at the inflamed nerve terminal contributes to the early alteration of glutamate metabolism in the DRG neuronal cell body during acute inflammation. Further studies are needed to evaluate the effect of sciatic nerve blockade during the chronic phase of AIA.

Disclosures: Z. Zhang: None. **B. Bolt:** None. **S. Das:** None. **K. Miller:** None. **M. Anderson:** None.

Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: CNPq

CAPES

Title: Antinociceptive properties of the mastoparan peptide Agelaia-MP I from social wasps

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Abstract: The current analgesic therapy is based on the sequential prescription of pain killers, which has as last resource drugs that act on opioid receptors, such as morphine, known to be very effective but also to produce undesirable systemic side effects, tolerance and addiction. Therefore, the search for new drugs with alternative targets became necessary in order to minimize side effects and enhance the efficacy of treatment. The wasps, arthropods of the order Hymenoptera, contain a great diversity of bioactive toxins in their venom that have antimicrobial, anticonvulsant, anxiolytic and antinociceptive properties. Mastoparans are an abundant class of peptides in the venom of wasps which has shown potential as new antimicrobial drugs and are excellent tools for the study of G protein coupled receptors. The main objective of this work is to study the antinociceptive activity of the mastoparan peptide Agelaia - MP I and the mechanisms involved. Agelaia-MP I (MW 1565 Da) was isolated from an active fraction of the venom of the social wasp Parachartergus fraternus, sequenced by De novo MS/MS using MALDI-TOF/TOF and identified as a mastoparan identical to the previously described from other wasp specie, Agelaia pallipes pallipes. The peptide was synthesized and then tested in the bioassays. Agelaia-MP I presented a dose-dependent antinociceptive activity in mice injected i.c.v. at concentrations of 3.2, 4.8 and 6.4 mM in two different models: hot plate and tail flick. The largest dose reported maximum antinociceptive effect for up to four hours and kept the nociception reduced three days after i.c.v. injection. The antinociceptive effect of the peptide in the hot plate model was about 2.5 times stronger than in the tail flick experiment, indicating a possible action in the pain ascending pathway. Further experiments with isolated sciatic frog nerve in sucrose gap assay showed that Agelaia-MP I induced partial (80%) and reversible blockade of action potential amplitude at 1mM. Since voltage dependent sodium channels (Nav) are also an important target for pain treatment, additional patch clamp experiments with hNav 1.1 to 1.7 will be performed to determine if the peptide is a sodium channel blocker. Taken together, these results revealed the great potential of the study of compounds isolated from wasp venoms with activity in the central nervous system. In addition, the antinociceptive effect described here is a novel activity for the mastoparan class of peptides.
Acknowledgements: CNPq and CAPES. Mice experiments were authorized by the Ethical

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Poster

244. Opioids and Other Analgesics

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Program#/Poster#: 244.19/FF23

Topic: D.08. Pain

Support: KAKENHI #26460695

Title: Thermal hyperalgesia is offset by top-down inhibition in offset analgesia

Authors: E. IKEDA, H. KOBINATA, S. ZHANG, T. LI, K. MAKITA, *J. KURATA
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Abstract: Offset analgesia (OA) is defined as a disproportionate decrease of perceived pain intensity after a brief temporary increase in pain stimulus, and is considered mediated by the descending pain inhibitory system. The extent of OA might potentially be influenced by the other pain modulatory mechanisms at the spinal cord or the other neural systems. Here we examined whether the extent of OA be influenced by hyperalgesia or adaptation to pain using thermal stimulation and continuous recording of perceived pain intensity. We also examined the effects of different ramp rates on adaptation to pain. [Methods] After IRB approval and informed consent, we recruited 14 healthy volunteers including 8 men and 6 women with the age range from 23 to 51 years old. We used a Peltier-type thermal stimulator (PATHWAY, Medoc, Israel) and a digital visual analogue scale (VAS) recorder (CoVAS, Medoc, Israel). We applied a painful heat stimulus via a 27 mm-diameter probe on the ventral surface of the left forearm and simultaneously recorded perceived pain intensity continuously. The baseline temperature of the probe was set at 32°C and stimulus intensity calibrated for each individual at the 60/100 mm of VAS (Tv6). "OA5" and "OA15" blocks consisted of a three-temperature stimulus train of 30s: T1 = Tv6 for 5 s, T2 = Tv6+1°C for 5 and 15 s, and T3 = Tv6 for 20 s, respectively, at a ramp rate of 56°C/s. A "constant stimulus (CS)" block consisted of a constant Tv6 stimulus of 30 s at a ramp rate of 56°C/s (Fast) or 6°C/s (Slow). The OA5, OA15, Fast CS, and Slow CS blocks, 3 for each, were given at a pseudorandom order. Statistical analysis was performed with analysis of

variance at a significance threshold of $P < 0.05$. [Results] The peak VAS (48.7 ± 20.0 vs. 68.0 ± 18.0; $p = 0.01$) and its latency (4.4 ± 0.7 vs. 9.7 ± 3.5; $p = 0.01$) were significantly higher and longer in OA15 than in OA5. That is, a 15-s thermal stimulus caused hyperalgesia followed by possible adaptation 10 s after the beginning. We observed a comparable decrease in perceived pain during T3 in both OA5 and OA15 blocks (65.1 ± 16.2 vs. 66.2 ± 22.3, respectively; $p = 0.88$). On the other hand, between Fast CS and Slow CS, there were no differences in either the peak VAS, its latency, and the extent of adaptation (23.8 ± 3.6 vs. 31.9 ± 4.8, respectively; $p = 0.19$). [Discussion] Although we observed hyperalgesia followed by 15 s of continuous thermal stimulation at the VAS of 6, it did not affect the extent of OA. It implied that descending pain inhibition by OA might have offset "wind-up" hyperalgesia at the dorsal horn of the spinal cord. We also found that a ramp rate at faster than 6°C/s did not affect adaptation to pain.

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Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Title: Epinephrine dose not worsen histological damage induced by lidocaine neurotoxicity in the rats

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Abstract: Backgound: Epinephrine has a risk to increase the local anesthetics neurotoxicity in spite of the popular use in patients undergoing spinal anesthesia. The strong vasoconstrictive property of epinephrine possibly reduces dural, and spinal blood flow and decreases clearance of local anesthetics from the spinal cord, resulting in a prolongation of stagnant time of the anesthetics in the subarachnoid space. Therefore, we examined whether additional epinephrine deteriorates histological damage and neurofunctional impairment induced by lidocaine neurotxicity in rats. Methods: Thirty rats were randomly received 40μl/g from 1ml solution which included 7.5%lidociane or 5%lidocaine dissolved in 10%glucose with or without 0.1mg, or 0.5mg epinephrine. Another 10 rats were received 0.1mg or 0.5mg epinephrine dissolved in 1ml of 10% glucose. Seven days after the injection, L2 spinal cord with both anterior and

posterior roots and dorsal ganglion as well as cauda equina were excised for light and electron microscopic examination. The neurological function of hind limbs was evaluated by walking behavior, and the responses to the radiant heat stimulation. Results: Histological abnormality was observed in the posterior root only in rats received 7.5%lidocaine both with and without epinephrine. The lesion was almost restricted in posterior root just entrance into the spinal cord. The incidence of the lesion at 7.5%lidocaine was not significantly different between the rats with epinephrine (25%) and those without epinephrine (33%). Other areas and other groups did not show any histological abnornality. There was no significant difference in sensory threshold among the groups. Disturbance of hindlimb movement prolonged in rats receiving 5% and 7.5% lidocaine with epinephrine (3hours), which was significantly longer than rats receiving 5% and 7.5% lidocaine without epinephrine (1hour). All the rats received intrathecal 0.5mg epinephrine alone could walk normally within 15minutes. Conclusion: Both 5% and 7.5% lidocaine with epinephrine prolonged anesthetic effect compared to those additional epinephrine, its incidence and primary lesion is the same to our previous study on lidocaine. Moreover, the incidence of the lesion in 7.5% lidocaine with epinephrine was not significant different from 7.5% lidocaine without epinephrine. Apparent sensory impairment did not occur in all groups. Thus, intrathecal epinephrine did not deteriorate histological damage and functional impairment even if the prolonging effect on the action of intrathecal lidocaine, suggesting that the use of epinephrine is not associated with augmentation of local anesthetic neurotoxicity.

Disclosures: **T. Takenami:** None. **Y. Nara:** None. **H. Okamoto:** None.

Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Title: Antineuropathic effect of 7-hydroxy-3,4-dihydrocadalin in streptozotocin-induced diabetic rats

Authors: *H. I. ROCHA-GONZALEZ[1], M. RAMÍREZ-AGUILAR[2], V. GRANADOS-SOTO[3], J. RESYES-GARCÍA[1], J. TORRES-LÓPEZ[4], J. HUERTA-CRUZ[4], A. NAVARRETE[5]
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Sede sur, México, D.F., Mexico; [4]Lab. de Mecanismos del dolor, Univ. Juárez Autónoma de Tabasco, Villahermosa, Tabasco, Mexico; [5]Facultad de Química, UNAM, México, D.F., Mexico

Abstract: Neuropathy is the most common and debilitating complication of diabetes and results in hyperalgesia and allodynia. Hyperglycemia clearly plays a key role in the development and progression of diabetic neuropathy. Current therapeutic approaches are only partially successful and they are only thought to reduce the pain associated with peripheral neuropathy. Some natural products offer combined antioxidant, anti-inflammatory and antineuropathic properties that may help to treat in a more integrative manner this condition. The purpose of this study was to investigate the antineuropathic effect of 7-hydroxy-3,4-dihidrocadalin in streptozotocin-induced diabetic rats and mice; as well as, the possible mechanism of action involved in this effect. Rats and mice were injected with 50 or 200 mg/Kg streptozotocin, respectively, to produce hyperglycemia. The formalin test and von Frey filaments were used to assess the nociceptive activity. Rota-rod was utilized to measure motor activity and malondialdehyde assay to determine anti-oxidative properties. After 3 weeks of diabetes induction, chemical hyperalgesia was observed in the streptozotocin-injected rats. Oral acute administration of 7-hydroxy-3,4-dihidrocadalin (0.3-30 mg/kg) decreased in a dose-dependent manner the formalin-evoked hyperalgesia in diabetic rats. In addition, methiothepin (5-HT_1 receptor antagonist, 1 mg/kg, i.p.) and ODQ (guanylate cyclase inhibitor, 2 mg/kg, i.p.), but not naltrexone (opioid receptor antagonist, 1 mg/kg, s.c.),prevented 7-hydroxy-3,4-dihidrocadalin-induced antihyperalgesic effect. The anti-hyperalgesic effect of 7-hydroxy-3,4-dihidrocadalin was similar to that produced by pregabalin (10 mg/kg, p.o.). Furthermore, oral acute administration of 7-hydroxy-3,4-dihidrocadalin (30 mg/kg) reduced streptozotocin-induced changes in malondialdehyde concentration from plasma samples. Unlike pregabalin, 7-hydroxy-3,4-dihidrocadalin did not affect motor activity. Six weeks after diabetes induction, tactile allodynia was observed in the streptozotocin-injected rats. At this time, oral administration of 7-hydroxy-3,4-dihidrocadalin (30 mg/kg) or pregabalin (10 mg/kg) reduced in a similar way tactile allodynia in diabetic rats. Data suggests that 7-hydroxy-3,4-dihydrocadalin has therapeutic potential for diabetic neuropathy treatment. This effect seems to involve activation of 5-HT_1 receptors and inhibition of guanylate cyclase enzyme, as well as antioxidant properties.

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Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: NSERC Grant, 341472-07

Title: Remembering dynamic changes in acute pain: Effects of pain intensity and memory delays

Authors: *M. KHOSHNEJAD[1], M. FORTIN[2], G. DUNCAN[1], P. RAINVILLE[1]
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Abstract: This psychophysical study investigated the effects of pain intensity and post-stimulus delays on the short-term memory of dynamic changes in acute pain. The intensity of pain induced by 8-sec noxious contact-heat stimuli of varying temperatures (47.5, 48, 48.5, 49 °C) was rated continuously during the stimulus or after a delay of 6, 10 or 14 sec, using an electronic visual analog scale in ten healthy volunteers. Principal component analysis (PCA) was applied to the raw time courses, consistent with our previous work (Khoshnejad et al. Pain, 2013). Three components explained about 90% of the total variance in perceptual temporal profiles across all trials and subjects with the first, second, and third components accounting for 53%, 31% and 7% of the overall variance, respectively. ANOVA showed main effects of temperature on component 1 ($p=0.006$) and of memory on components 1 and 2 ($p's<0.001$). However, there was no significant difference between the 3 memory delays ($p's>0.5$) and no interaction between memory and temperature ($p's=0.99$). Reconstruction of the mean curves from the first two components shows an underestimation of both time and overall pain felt in memory; however, this effect is comparable across the four temperatures tested and the three memory delays. These effects were confirmed using standard parameters extracted from the raw time-courses: area under the curve (AUC), maximum pain (MP), and total time (TT). ANOVA showed main effects of temperature on AUC and MP and of memory on AUC and TT ($p's<0.01$), but no interaction ($p's=0.99$). These results confirm a systematic distortion of pain magnitude and temporal information in short-term memory, with an overall preservation of the maximum pain felt (*i.e.*, no main effect of, or interaction with, memory on MP). These effects appear inconsistent with a simple decay of a sensory memory trace and may reflect the conversion of the dynamic pain experience into a more stable but distorted template underlying memory encoding, storage and recall.

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Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Support: NIDA grant 016644

Title: Gonadal hormone modulation of antinociceptive tolerance to delta-9-tetrahydrocannibinol

Authors: *R. M. CRAFT[1], A. A. WAKLEY[2]
[2]Psychology, [1]Washington State Univ., Pullman, WA

Abstract: We have previously shown that cannabinoid agonists, including delta-9-tetrahydrocannabinol (THC), are more potent and in some cases more efficacious in female than in male rats, in terms of their antinociceptive effects (Tseng & Craft, Eur J Pharmacol 430:41, 2001). Moreover, females develop greater antinociceptive tolerance than males when given THC chronically (Wakley et al., submitted). In the present study, we determined whether activational effects of gonadal hormones are responsible for sex differences in the development of tolerance to THC. Adult Sprague-Dawley rats were sham-gonadectomized or gonadectomized (GDX); females were implanted s.c. with estradiol-filled (1-mm capsule/rat) or blank capsules, and males were implanted with testosterone-filled (10-mm capsule/100 g body weight) or blank capsules. Additionally, oil or progesterone (500 micrograms/rat) was administered s.c. every 5 days starting on the 4th day post-surgery. On Day 14 post-surgery, THC was administered cumulatively and rats were tested for antinociception on the warm water tail withdrawal and paw pressure tests (pre-chronic test). Vehicle or THC was then administered twice-daily for 9 days, and the next day THC dose-effect curves were redetermined (post-chronic test). At the pre-chronic test, THC's antinociceptive potency was significantly greater in sham-GDX females than in sham-GDX males on the tail withdrawal test ($p=0.001$) but not on the paw pressure test ($p=0.16$). Gonadectomy did not significantly affect THC's antinociceptive potency on either test (sham-GDX vs. GDX, within sex); however, progesterone decreased THC potency in GDX females, on the paw pressure test ($p=0.006$). Antinociceptive tolerance developed to THC in all groups treated twice-daily with THC, on both tests. Sham-GDX females developed more tolerance than sham-GDX males ($p=0.015$) on the tail withdrawal but not paw pressure test. Overall, estradiol increased females' THC sensitivity ($p<0.001$). However, neither testosterone in males nor estradiol or progesterone in females significantly influenced the magnitude of tolerance development in GDX rats. These results suggest that sex differences in the development of antinociceptive tolerance to THC are not due to activational effects of gonadal hormones.

Disclosures: R.M. Craft: None. **A.A. Wakley:** None.

Poster

244. Opioids and Other Analgesics

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Topic: D.08. Pain

Title: Characterization of 2-261, a novel positive allosteric modulator of GABAA receptor β2/3 subunit, in experimental neuropathic pain

Authors: *C. QU[1], J. Y. XIE[1], M. H. OSSIPOV[1], T. B. JOHNSTONE[2], K. GEE[2], F. PORRECA[1]
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Abstract: Loss of inhibition due to attenuation of GABA tone has been suggested to play an important role in the development and maintenance of chronic neuropathic pain. Potentiation of GABAA receptor function via direct activation by agonists or positive allosteric modulation has been proposed as a potential mechanism for new analgesics. Previous drug discovery efforts have been hampered by severe central nervous system side effects related to modulation of GABAA receptors, including sedation, ataxia, amnesia, and addiction/withdrawal. Compound 2-261 is a novel β2/3 subunit-selective GABAA positive allosteric modulator (PAM). We have previously shown that spinal nerve ligation (SNL) produces an aversive state that reflects spontaneous (i.e., stimulus-independent) pain and that can be captured using conditioned place preference (CPP), likely reflecting negative reinforcement. In addition, relief of spontaneous pain may be reflected by increased dopamine efflux in the nucleus accumbens (NAc). Here, male Sprague-Dawley rats received L5/L6 SNL or sham surgery and intrathecal (i.th.) catheterization and were allowed to recover for 2 weeks. Spinal administration of 2-261 (0.2 μg in 5 μl, i.th.) reversed evoked tactile and thermal hypersensitivity in nerve-injured rats. It also produced robust conditioned place preference in nerve-injured, but not in sham-operated, rats. Additionally, spinal 2-261 significantly increased dopamine efflux in the NAc selectively in nerve-injured rats, likely reflecting that the reward of pain relief. Spinal 2-261 didn't produce any effects on CPP or NAc DA efflux in sham-operated rats, indicating that this compound delivered by this route is not intrinsically rewarding. Importantly, no motor side effects were observed in 2-261-treated rats. The data show that spinal 2-261 alleviates nerve injury-induced evoked and spontaneous pain and increases NAc DA release selectively in injured rats. Therefore, GABAA β2/3 subtype-selective PAMs represent a promising new mechanism for the development of novel analgesics

without producing undesirable side effects in the central nervous system and/or intrinsic rewarding effects.

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Poster

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AFIP

Title: The oral administration of trans-caryophyllene attenuates mechanical hypernociception in chronic pain induced by sciatic nerve injury and sleep restriction

Authors: *L. G. FREIRE[1], G. R. MOLSKA[2], A. A. F. Z. LOPES[1], D. SUCHECKI[1]
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Abstract: The bidirectional relationship between sleep and pain has been the topic of interest in recent investigations, since patients with pain disorders sleep poorly and poor sleepers exhibit higher pain sensitivity. Likewise, animals with peripheral nerve injury, submitted to sleep restriction or sleep deprivation, show reduced pain threshold. Usually, pain treatment involves chronic administration of anti-inflammatory compounds, which usually produces numerous side effects. For this reason, natural products may represent an important contribution for the development of new therapeutic strategies. Trans-caryophyllene (TC) is the major compound of essential oil of several medicinal plants, such as Cannabis sativa, with pronounced antinociceptive activity; therefore the aim of this study was to evaluate whether sleep restriction (SR) led to worsening of mechanical hypernociception induced by chronic contrition injury of the sciatic nerve (CCI) and whether TC was capable to prevent hypernociception and/or to reverse SR effect. Male Wistar rats (3 month-old, n = 5) were submitted to CCI or sham surgery and after recovery (2 to 3 days), they were distributed in two main groups: 1) control, non sleep-restricted (CTL) and 2) SR for 15 days, 18 h/day (from 4 pm to 10 am). These groups were treated either with vehicle (corn oil, groups CTL+VEH, CCI+VEH, SR+VEH, CCI+SR+VEH)

or TC (20 mg/kg; groups CCI+TC, SR+TC, CCI+SR+TC). Thus, comparison of data was done with the following groups: CTL, Non sleep-restricted Sham, CCI+VEH, SR+VEH, CCI+SR+VEH, CCI+TC, SR+TC, CCI+SR+TC. TC antihypernociceptive potential effect was assessed by von Frey test before surgery (baseline measurement), 5 days (before onset of SR), and 8, 14 and 21 days after surgery. CCI and SR alone or in combination resulted in reduced pain threshold compared to CTL group at all time-points; reduction of pain threshold in SR+VEH group was observed after onset of restriction period ($p < 0,005$). CCI+SR+VEH group showed a trend for higher pain sensitivity compared with CCI+VEH and SR+VEH groups. Treatment with TC for 21 consecutive days significantly inhibited mechanical hypernociception in CCI ($p < 0.03$), SR ($p < 0.05$), and CCI+SR animals ($p < 0.004$) compared with their respective non-restricted groups. These findings confirm the antinociceptive effect of trans-caryophyllene in experimental neuropathic pain associated with sleep restriction. The evidence for biological activity of trans-caryophyllene encourages further studies, particularly regarding its mechanisms of action.

Disclosures: L.G. Freire: None. **G.R. Molska:** None. **A.A.F.Z. Lopes:** None. **D. Suchecki:** None.

Poster

245. Somatosensory Transduction

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 245.01/FF30

Topic: D.08. Pain

Support: NIH Grant NS077330

Title: Store-operated calcium channels play an important role in neuropathic pain via the ERK signaling pathway

Authors: *H. HU, X. GAO, R. GAO, Y. TIAN, J. E. BARRETT
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Abstract: Store-operated calcium channels (SOCs) are calcium-selective cation channels and are implicated in various functions of many types of cells. Our previous study has shown that YM-58483, a potent SOC channel inhibitor, strongly attenuates spare nerve injury (SNI)-induced pain hypersensitivity, suggesting a potential role of SOCs in neuropathic pain. We recently demonstrated that SOCs are expressed in dorsal horn neurons and identified STIM1, STIM2 and

Orai1 as key components of SOCs that mediate the SOC entry and SOC currents in dorsal horn neurons. However, the functional consequence of SOC activation remains elusive. In the present study, we demonstrated that activation of SOC entry (SOCE) by thapsigargin (TG) leaded to phosphorylation of extracellular signal-regulated kinase (ERK). Inhibition of SOCE by YM-58483 or $GdCl_3$ blocked TG-induced ERK activation. SNI induced robust ERK activation, which was significantly reduced by YM-58483. Knockdown of STIM1, STIM2 or Orai1 decreased SNI-induced ERK activation. Importantly, knockdown of STIM1, STIM2 or Orai1 attenuated spare SNI-induced pain hypersensitivity. Our findings reveal that the SOC signaling plays an important role in neuropathic pain via the ERK pathway.

Disclosures: H. Hu: None. **X. Gao:** None. **R. Gao:** None. **Y. Tian:** None. **J.E. Barrett:** None.

Poster

245. Somatosensory Transduction

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 245.02/FF31

Topic: D.08. Pain

Support: NIH Grant NS077330

Title: Activation of NK1 receptors leads to store-operated calcium entry in mouse spinal cord dorsal horn neurons

Authors: *J. XIA, R. GAO, Y. TIAN, J. E. BARRETT, H. HU
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Abstract: We have previously demonstrated that store-operated calcium channels (SOCs) are expressed in mouse spinal cord dorsal horn neurons and inhibition of SOC entry (SOCE) by YM-58483 (an inhibitor of SOCs) attenuates pain hypersensitivity, indicating that SOCs may be involved in pain processing. It is well known that substance P (SP) play an important role in pain transmission through the stimulation of neurokinin 1 (NK1) receptor, which results in calcium release from ER calcium stores. Previous studies demonstrated that reduction of the ER calcium can activate SOCE. We therefore hypothesized that activation of NK1 receptors leads to SOCE. To test this hypothesis, we performed Ca^{2+} imaging, siRNA knockdown and evaluated pain sensitivity using a behavioral assay. We found that application of 100 nM SP induced a transient calcium response in the absence of extracellular Ca^{2+}, representing the calcium released from intracellular Ca^{2+} stores; the subsequent addition of 2 mM $CaCl_2$ caused sustained responses. To

determine whether the SP-induced $[Ca^{2+}]_i$ changes were mediated by NK1 receptors, we pretreated neurons with 1 μM RP 67580, a potent and selective NK1 receptor antagonist. SP-induced calcium release and calcium entry were both blocked by RP 67580. To determine whether this Ca^{2+} entry was through SOCs, we pretreated neurons with 3 μM YM-58483 or 1 μM $GdCl_3$, and found that SP-induced Ca^{2+} release was not affected by YM-58483 or $GdCl_3$; however, Ca^{2+} influx was blocked by both YM-58483 and $GdCl_3$. To confirm that this effect was mediated by Orai1, a key component of SOCs, neurons were transfected with the specific Orai1 siRNA. Knockdown of Orai1 abolished SP-induced Ca^{2+} entry, but had no effect on Ca^{2+} release. Finally, behavioral data showed that intrathecal injection of 0.25 nmol SP induced robust spontaneous nociceptive behavior, which was drastically reduced by YM-58483 in a dose-dependent manner. Taken together, our results demonstrate that SOCs are involved in NK1 receptor-mediated Ca^{2+} signaling in spinal cord dorsal horn neurons. These findings provide a potential mechanism underlying YM-58483-induced central analgesia.

Disclosures: **J. Xia:** None. **R. Gao:** None. **Y. Tian:** None. **J.E. Barrett:** None. **H. Hu:** None.

Poster

245. Somatosensory Transduction

Location: Halls A-C

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS-042867

James S. McDonnell Foundation Grant 220020293

GWU Luther Rice Grant for Undergraduate Research

Title: A quantitative analysis of Meissner's corpuscles in the fingertips of humans, chimpanzees and monkeys

Authors: ***A. VERENDEEV**[1], C. THOMAS[1], S. C. MCFARLIN[1], W. D. HOPKINS[2,3], K. A. PHILLIPS[4], C. C. SHERWOOD[1]
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Abstract: Meissner's corpuscles (MCs) are tactile mechanoreceptors found in the hairless skin of mammals, including fingertips. They are characterized by sensitivity to light touch and therefore might be associated with the evolution of manipulation abilities of the hands in primates. We examined MCs in different primate species, including common marmoset (*Callithrix jacchus*, n=5), baboon (*Papio anubis*, n=2), rhesus macaque (*Macaca mulatta*, n=3), chimpanzee (*Pan troglodytes*, n=1) and human (n=8). Fingertips of the first, second, and fourth digits were collected from both hands of specimens, dissected and histologically stained using hematoxylin and eosin. The density (counts of MCs per mm^2 of dermis) and the size (width of MCs) were quantified. Overall, there were no differences in MCs density or size among the digits or between the hands (all *p*s nonsignificant). When averaged across hands and digits for each individual, one-way ANOVA revealed significant species differences in both the density ($F(4,14)=4.027$; $p=0.022$) and the size ($F(4,14=22.696$; $p<0.001$) of MCs. We found that apes (i.e., humans and chimpanzees) had a higher density of MCs per mm^2 of epidermal length than the monkeys ($t(17)=3.431$; $p=0.003$). However, there were no difference in MCs sizes between apes and monkeys ($t(17)=1.443$; $p=0.172$). We further correlated the density and size of MCs to an index of dexterity as reported previously (Iwaniuk et al., 1999). Spearman correlation coefficient analysis revealed a significant positive relationship between dexterity and MCs density ($\rho=0.616$; $p=0.005$) as well as MCs size ($\rho=0.552$; $p=0.014$), such that more dexterous species had both higher densities and larger MCs.

Disclosures: A. Verendeev: None. **C. Thomas:** None. **S.C. McFarlin:** None. **W.D. Hopkins:** None. **K.A. Phillips:** None. **C.C. Sherwood:** None.

Poster

245. Somatosensory Transduction

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Topic: D.09. Tactile/Somatosensory

Support: Nat Ocean Service Grant NA10SEC4810008

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Thermo Scientific, gifted antibodies

National Marine Sanctuaries, tissue collection

Woods Hole Oceanographic Institution, tissue samples

Title: Somatosensory innervation in the skin of humpback whales (Megaptera novaeangliae)

Authors: *S. A. ELDRIDGE[1,2], F. MORTAZAVI[2], V. HERRERA[3], F. L. RICE[4], D. KETTEN[5], D. L. ROSENE[2]
[1]Biol. Dept., Univ. of Massachusetts Dartmouth, Dartmouth, MA; [2]Dept. of Anat. and Neurobio., [3]Boston Univ. Sch. of Med., Boston, MA; [4]Integrated Tissue Dynamics, Albany, NY; [5]Biol. Dept., Woods Hole Oceanographic Inst., Woods Hole, MA

Abstract: Underwater physical cues provide critical information to cetaceans (whales, dolphins and porpoise) for finding prey, navigating migrations, assessing dive depth, and interpreting and triangulating communication signals. Auditory and visual systems of marine mammals have been studied, however, little is known about somatosensory reception in cetaceans. Here, we describe innervation in skin biopsies from the flanks of humpback whales (*Megaptera novaeangliae*) (NMFS permit 15240). Immunohistochemistry (IHC) was optimized to visualize antibodies specific for highly conserved molecules of axons, neural support cells, myelination, and the low-threshold mechanoreceptors (LTMRs) that transduce stretch, indentation and vibration into action potentials. Results were compared with the prototypical, glabrous skin of mice, macaque monkeys, and humans. The exceptionally thick cetacean epidermis is densely interdigitated with long pencillate dermal papillae, creating an extensive epidermal-dermal interface. IHC revealed axons immunoreactive to α-brain sodium channel I (BNaC) running along this boundary. In prototypic mammalian skin, BNaC is specific to LTMRs of dorsal root ganglia neurons. Other LTMRs, marked by α-calcitonin gene-related peptide (CGRP), formed clusters of fine-caliber neurofilaments and cells in the epidermal stratum basale, along the lateral wall of dermal papillae. In prototypic glabrous skin, this formation is found at the deep end of epidermal pegs, and is the tactile Merkel cell-neurite complex (MCNC), a slowly-adapting ending associated with heavily myelinated axons. In whale skin, the orthogonal orientation and lateralized location suggests a novel MCNC. Colocalization among antibodies to neurofilament-H (sensory fibers), PGP 9.5 (neurons and glia), the glial (Schwann cell) marker S100, BNaC, and CGRP differentiated fiber types in the subepidermal neural plexus and dermal papillae. Axons had a particularly large caliber compared to that in other species, consistent with the higher conduction velocity necessary to traverse distances up to 5m to the spinal cord. Another remarkable feature, not reported in any other mammal, is that axons in nerve fascicles appeared suspended within a large non-cellular space. Molecular homology of cutaneous innervation in whales and prototypic mammals suggests that whale skin contains a wide variety of unique innervation patterns and structural adaptations. These results will guide future work assessing how whale integument may be tuned to amplify signals or filter noise, protect axons from the pressures of deep dives, and triangulate signal sources in an aquatic environment.

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Poster

245. Somatosensory Transduction

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 245.05/GG2

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant (AR059397)

Title: Ion channels/receptors expressed along group IV sensory afferent fibers within rat skeletal muscle

Authors: *R. RAMACHANDRA[1], K. S. ELMSLIE[2]
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Abstract: Exercise Pressor reflex (EPR) is activated by mechanical distortion and the metabolic by-products of exercising skeletal muscle, which stimulates receptors and ion channels to generate action potentials in thinly myelinated group III and unmyelinated group IV. This activity helps to increase cardiac output in response to exercise. The EPR can be inappropriately activated in disease states, such as peripheral vascular disease, leading to increased risk of myocardial infarction. We are investigating the receptors and ion channels expressed by group IV muscle afferent neuronal that could be involved in EPR. In this study, we utilize immunohistochemistry to identify receptors and ion channels expressed in the sensory afferent nerve fibers within rat skeletal muscles (gastrocnemius and soleus). Group IV afferent fibers were identified by using an antibody to label peripherin. Some of these fibers were co-labeled with antibodies targeting P2X3 (ATP) and α7 nACh (choline) receptors. We are also investigating the voltage-gated sodium (NaV) channels that are expressed along these afferent fibers. We have found that α7 nACh receptors are co- labeled with P2X3 on group IV nerve terminals that provides support for the idea that choline could be an activator of the EPR. The visualization of these receptors and channels along the afferent fibers compliments data from electrophysiological and whole-animal studies to provide a more complete understanding of their role in generating the EPR.

Disclosures: R. Ramachandra: None. **K.S. Elmslie:** None.

Poster

245. Somatosensory Transduction

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 245.06/GG3

Topic: D.09. Tactile/Somatosensory

Support: NIDCR IRP

Title: Encoding temperature valence

Authors: L. POGORZALA, M. ISAACSON, H. SOLINSKI, *M. HOON
NIDCR, BETHESDA, MD

Abstract: Temperature changes trigger characteristic sensations of cooling, or warming, but how these thermal percepts are produced is still not fully understood. We wanted to study how the feeling of temperature, or the emotive valence generated by temperature, is formed. We devised a new method to investigate how peripheral sensory neurons and molecular receptors prompt these responses. Previously, behavioral tests have utilized passive assays such as the two plate preference and reflex response tests. However, these tests are incapable of determining the affective component in the animal being tested; they do not define the quality of the stimuli being sensed. Therefore, we developed an operant assay which allowed examination of an animal's ability to cognitively recognize temperature change. We established a novel apparatus capable of generating a rapid change in temperature in the skin of mice and linked this to a training regime in which mice learn to recognize specific thermal cues and quantitatively display a decision about the quality of thermal sensation they sense. A simple go/no go paradigm allowed water restricted mice to be trained to form a strong association between a specific temperature change and a water reward. Subsequently, trained animals were tested with novel thermal cues. This method allowed us to 1) examine whether thermal salience is retained in different temperature ranges (i.e. are temperature changes generalized? For example, does cooling from 20-15°C cause a similar sensation to that produced by cooling from 40-35°C?) 2) Determine how thermal acuity changes across a range of temperature. Lastly, we exploited this new approach to evaluate the effect, of the selective perturbation of different subsets of peripheral neurons, and of the elimination of receptors expressed in these neurons.

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Poster

245. Somatosensory Transduction

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Topic: D.09. Tactile/Somatosensory

Support: NIH/NINDS R01 NS073119

NIH/NIGMS T32 GM007367

NIH/NIAMS P30AR044535

Title: Elucidating mechanisms of touch-receptor plasticity during target-organ remodeling

Authors: *E. A. LUMPKIN[1], K. L. MARSHALL[2], B. A. JENKINS[3], Y. BABA[1], Y. WANG[4], D. R. LESNIAK[4], G. J. GERLING[4]
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Abstract: Touch is encoded by a diverse array of receptors that generate unique firing patterns to inform the brain about mechanical stimuli. These receptors are embedded in the skin, which is a dynamic sensory organ that undergoes continual remodeling, including epidermal renewal and cyclical hair growth. This raises a key question: what are the mechanisms by which touch receptors maintain reliable firing during normal skin remodeling? We use Merkel cell-neurite complexes in mice to address this question. Merkel cells localize to skin areas specialized for high tactile acuity such as whisker follicles, fingertips, and touch domes in hairy skin. Merkel cells are contacted by slowly adapting type I (SAI) afferents, which together form gentle touch receptors that encode information about object features and static pressure. In mice, hair-growth cycles occur in synchronous waves, providing an excellent model system to study neuronal remodeling during skin structural changes. Mouse skin mechanical measurements demonstrated that skin becomes thicker and less stiff during hair growth. As skin is pivotal in transferring force to tactile end organs, we hypothesized that touch-evoked firing will change over the hair cycle unless homeostatic mechanisms, such as neuronal remodeling, compensate. To test this hypothesis, we investigated SAI-afferent structure across hair-cycle phases. During hair growth, we found the number of Merkel cells increased by over 50% and the new Merkel cells were incorporated into SAI end organs, suggesting they are functional transduction units. Neuronal branching complexity increased, correspondingly, with 40% more terminal branches in growth phases and higher branching orders. We postulate that this remodeling offsets the skin changes to

maintain consistent signaling. Collectively, these data indicate that tactile afferents remodel during skin changes. To identify molecular cues that govern neuronal remodeling, we focused on Bone Morphogenetic Protein (BMP) pathway signaling because 1) it governs the transition between resting and growth hair-cycle phases in the skin, and 2) overexpression of BMPs causes a decrease in cutaneous innervation density. Thus, we hypothesize that BMP signaling plays a role in afferent remodeling during hair cycling. To test this, we have outlined the timing of hair cycle-induced afferent remodeling and assessed expression of BMP receptors the skin. Together, our findings reveal mechanisms that govern homeostatic touch receptor remodeling in mammalian skin.

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Poster

245. Somatosensory Transduction

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Program#/Poster#: 245.08/GG5

Topic: D.09. Tactile/Somatosensory

Title: Cellular and behavioral characterization of ASIC4 in knockout mouse

Authors: *S.-H. LIN[1], C.-C. CHEN[2]
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Abstract: Acid-sensing ion channel 4 (ASIC4) is the most recent member of the degenerin/epithelial sodium channel (DEG/ENaC) family and is reported to express mainly in the nervous system. Like all other DEG/ENaC subunits, ASIC4 has a structure characterized by two transmembrane domains, one extracellular loop and intracellular N- and C-terminal domains. However, heterologous expression study indicated that ASIC4 cannot form an ion channel by itself and thus its function is proposed to be a modulator for other ASICs in the nervous system. Since information about ASIC4 is limited in the literature, we generated a knockout mouse line by inserting the CreERT2 coding sequences into the ATG site of Exon1 in the mouse Accn4 gene. ASIC4 homozygous knockouts were viable, fertile, and displayed no obvious defects in appearance. Q-PCR study suggested that in adult wildtype animal, ASIC4 transcript is expressed broadly in the central nervous system, most abundantly in the pituitary gland and barely in the dorsal root ganglion. All these transcripts are eliminated and no evidence of ASIC1a and/or ASIC3 mRNA compensation in the knockout mice. After crossing with CAG-

Td-Tomato reporter mice, we examined the induction of Cre recombination activity in adult ASIC4 homozygous animals by daily intraperitoneal injection of tamoxifen (3mg/day) for 7 days. Td-tomato signals were detected in many CNS regions, including the pituitary gland, olfactory bulb, hippocampus, amygdala, hypothalamus, cerebellum, cortex and the spinal cord. We next screened known behavioural phenotypes that were reported to be effected in the ASIC1a knockout mice. To our surprise, results indicated that ASIC4 knockout behaved normally in the fear conditioning task, their pain threshold for thermal and mechanical stimuli were normal, and the severity for experimental autoimmune encephalomyelitis (EAE) and kainic acid-induced seizure were the same to wildtype control. We concluded that ASIC4 might not be participated in the proposed function to modulate other ASICs in the nervous system.

Disclosures: S. Lin: None. **C. Chen:** None.

Poster

245. Somatosensory Transduction

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Program#/Poster#: 245.09/GG6

Topic: D.09. Tactile/Somatosensory

Support: SFB665

Title: Vibration detection thresholds in a human cohort with congenital hearing deficits

Authors: *R. MOSHOURAB[1], V. BEGAY[1], J. WALCHER[1], C. WETZEL[1], M. GROSS[2], G. LEWIN[1]
[1]Neurosci., Max-Delbrück Centrum, Berlin, Germany; [2]Audiol. and Phoniatrics, Charité - Universitätsmedizin, Berlin, Germany

Abstract: Mechanosensory senses might share common genes that play a role in the transduction of mechanical energy, such as sound and touch. A recent study revealed that mutations in the USH2A gene, which causes deafness in Usher's syndrome, diminishes tactile sensation 1. We hypothesized that distinct components of tactile sensation, vibration, pressure, temperature, and pain, that pertain to specific mechanoreceptors classes are affected in subjects with congenital hearing deficits. A cohort with congenital deafness (n = 36; 17 females and 19 males; ages 14-21) from a school for the deaf were screened with an extensive battery of tests for deficits in tactile sensation of the dominant hand. The testing protocol determined the vibration detection threshold (VDT) at 10Hz and 125Hz, tactile acuity (using tactile acuity cube), thermal

sensation thresholds, and mechanical detection (von Frey hairs) and pain thresholds (pinprick test). Vibrations were applied below the nail of little finger, and a two alternative forced choice - where amplitude decreased in a logarithmic pattern after 6 correct choices and increased after 2 incorrect choices - was implemented. Data were compared to an age-matched healthy cohort (n=27). Elevated tactile acuity and vibration detection thresholds were observed in deaf subjects. Median values for tactile acuity threshold were significantly higher in the deaf cohort (1.675 mm compared to 1.425 mm in controls, P=0.024). We observe a strong effect on VDT, especially at 10Hz (mean threshold 8.01 μm in deaf individuals, compared to healthy age-matched controls, 4.07 μm, P=0.0003). In a considerable proportion of our deaf subjects 42% (15/36) thresholds found to be above 7.87 μm. Pain thresholds to pinprick in the deaf cohort were not significantly affected. Here we provide evidence of common genetic elements involved in auditory and cutaneous mechanotransduction. Next generation sequencing methods will be used in order to identify gene variants that control both touch and hearing. 1.Frenzel, H. et al. A Genetic Basis for Mechanosensory Traits in Humans. PLoS Biol. 10, e1001318 (2012).

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Poster

245. Somatosensory Transduction

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Topic: D.08. Pain

Support: NSFC31340048

NSFC31270082

MRC/G1002183

MR/K021303/1

Title: Role of somatic/perisomatic membrane potential of nociceptive neurons in peripheral nociceptive transmission

Authors: ***X. DU**[1], H. HAO[1], S. GIGOUT[2], D. HUANG[1], Y. YANG[1], C. WANG[1], D. SUNDT[3], D. JAFFE[3], H. ZHANG[1], N. GAMPER[1,2]

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Abstract: Peripheral somatosensory neurons (including pain-sensing neurons, the nociceptors) are pseudo unipolar neurons that convey versatile information about body's environment to the CNS. It is generally accepted that in healthy organism peripheral nerves conduct action potentials from their respective sites of origin (peripheral nerve endings) to the superficial laminae of dorsal spinal cord without interruption and that sensory neuron somata do not contribute significantly to such conduction. Yet, growing evidence suggest that somatic/perisomatic compartment of sensory neurons can influence peripheral sensory transmission, particularly in some chronic pain conditions. Resting membrane potential (E_{rest}) is an important parameter regulating neuronal excitability, however, our understanding of how E_{rest} is regulated in sensory neuron somata or how changes in somatic E_{rest} affect peripheral sensory transmission is insufficient. In this study we evaluated the influence over E_{rest} of several major ion channels expressed in nociceptive neurons. Thus, we characterized contributions of Kv, K_{Na}, ATP-sensitive (K_{ATP}), Kv7 (M-type), and two-pore (K2P) K^+ channels; hyperpolarization-activated cyclic nucleotide-gated channels (HCN), T-type Ca^{2+} channels and TTX-sensitive and TTX-resistant voltage-gated Na^+ channels to E_{rest}. The strongest and most prevalent effect on E_{rest} was achieved by modulating M channels while targeting 4-aminopiridine-sensitive Kv channels, K2P, $K_{ATP,}$ HCN, Na^+ and Ca^{2+} channels also produced some effects. Next, we investigated how modulation of somatic/perisomatic ion channels affects peripheral nociceptive transmission *in vivo*. Acute focal application of M and K_{ATP} channel enhancers and HCN blocker to L5 DRG via the implanted DRG cannula *in vivo* significantly alleviated pain induced by hindpaw injection of bradykinin. Finally, we developed a mathematical model of mammalian C-fibre nocicieptor which supported our finding that hyperpolarization of a somatic/perisomatic compartment of nociceptive neuron can fail propagation of an action potential past the axonal bifurcation. Our study identifies ion channels that set somatic E_{rest} of nociceptive neurons and provides evidence for a robust filtering role for somatic/perisomatic compartment of peripheral nociceptive neuron. Keywords: M channel/Two-pore K+ channel/ Kv channels/HCN/ion channels/DRG/nociception/pain

Disclosures: X. Du: None. **H. Hao:** None. **S. Gigout:** None. **D. Huang:** None. **Y. Yang:** None. **C. wang:** None. **D. Sundt:** None. **D. Jaffe:** None. **H. Zhang:** None. **N. Gamper:** None.

Poster

245. Somatosensory Transduction

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Program#/Poster#: 245.11/GG8

Topic: D.08. Pain

Support: DA031259

AHA13GRNT17130021

Title: Bidirectional modulation of heteromeric acid-sensing ion channel 1a/3 channels by zinc

Authors: *X. CHU[1], X. ZHA[2], Q. JIANG[1]
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Abstract: Acid-sensing ion channels 1a and 3 subunits are all expressed in sensory neurons, where they are though to play critical roles in pain perception associated with tissue acidosis. Our previous studies have shown that both homomeric ASIC1a and 3 channels are inhibited by physiological concentrations of zinc. ASIC1a and 3 can form functional channels in heterogonous system, which is believed to be expressed in neurons. Here, we found that heteromeric ASIC1a/3 channels are regulated by physiological concentration of zinc with dual effects. Different from homomeric ASIC1a and ASIC3 in response to zinc, co-application of zinc dose-dependently potentiates both the peak amplitude and sustained component of heteromeric ASIC1a/3 channel currents, pretreatment with zinc between 3 to 100 μM exert the same potentiation as co-application. However, pretreatment with zinc induced the significant inhibition of heteromeric ASIC1a/3 channels when concentration of zinc is over 100 μM. The potentiation of heteromeric ASIC1a/3 channels by zinc is pH-dependent, as zinc shifts the pH-dependences of ASIC1a/3 current from a pH_{50} of 6.5 to 6.9; while the inhibition of ASIC1a/3 currents by zinc is pH-independent. The inhibition of ASIC1a/3 currents by pre-applied zinc was independent of pH activation, steady-state desensitization, voltage, or extracellular Ca^{2+}. Further, we showed that the effect of zinc is dependent on the extracellular histidine residue. Systemic mutation of histidine residues in extracellular domain of ASIC1a subunit didn't affect the effect of zinc on heteromeric ASIC1a/3 channels. However, mutating histidine residues, located in the extracellular domain of the ASIC3 subunit abolished the zinc effects on heteromeric ASIC1a/3 channels. These findings suggest that histidines (located in the ASIC3) in the extracellular domain of heteromeric ASIC1a/3 subunit is critical for zinc-mediated effect and provide the basis for future mechanistic studies addressing the functional importance of heteromeric ASIC1a/3 channels in pain modulation.

Disclosures: X. Chu: None. **X. Zha:** None. **Q. Jiang:** None.

Poster

245. Somatosensory Transduction

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Support: NIH Grant R44 DA026363-02

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Title: The novel T-type Cav3 channel biologic probe ML218 produces state-dependent inhibition of T-currents and mitigation of neuropathic and inflammatory pain in rodents

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Abstract: The T-type Cav3 channel (T-channel) plays a key role in pain signaling. The T-channel is involved in at least two key stages of pain pathways: first, in sensory neurons of the dorsal root ganglion (DRG) and second at the thalamic pain relay. Chronic nerve constriction injury and diabetic neuropathy cause upregulation of Cav3.2 subtype protein and augment of its-mediated T currents in the DRG neurons of rats. Conversely, gene knockout, antisense knockdown, or silencing of the Cav3.2 isoform produces pain relief in both neuropathic and inflammatory pain in rodents. To discover a potent and selective T-channel biologic probe that can be available without intellectual property restriction for all research, we completed the T-type Cav3 biologic probe discovery project. We first validated a fluorometric HTS method and then conducted a primary HTS, and followed by a “hit-to-probe” optimization. The lead has been designated as the T-channel probe ML218. The present study was undertaken to character the probe *in vitro* and investigate its efficacy *in vivo*. Using western blot with Cav3.1 and 3.2 antibodies we confirmed that DRG neurons exclusively express Cav3.2, but not Cav3.1 proteins. Under voltage-clamp, ML218 (100 nM) selectively blocks the T-current with approximately 100-fold selectivity over high-voltage activated Ca+, Na+ and K+ currents in the DRG neurons.

ML218 produced a voltage and frequency-dependent inhibition of the T-current. Under current-clamp, ML218 (1 μM) reversibly blocked T-channel triggered bursts without effects on resting potentials and membrane input resistance of the thalamic neurons. ML218 (50 μM) had no effects on NMDA-mediated EPSPs. One hour after administration of ML218 (30 mg/kg, ip), the plasma ML218 concentration was 976±177 nM in rats and 2,430±230 nM in mice. At this dose, acute treatment with ML218 mitigated chronic pain induced by spared nerve injury, streptozotocin-induced diabetic neuropathy and reserpine-induced chronic pain in rats. The 30 mg/kg dose ML218 also reduced hyperalgesia in the Complete Freund's adjuvant (CFA)-induced inflammatory pain in mice; while sparing normal nociception. At 60 and 100 mg/kg, ML218 did not cause significant changes in active/inactive cycle and reduction in locomotion and rearing of mice during 24 hours homecage monitoring using the SmartCage system. These results demonstrate that ML218 is a useful probe for investigation of physiologic and pathophysiologic roles of the T-channel, and can form a basis to develop a novel therapeutic with selective and state-dependent inhibition of the T-channel to treat chronic neuropathic pain.

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Poster

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SFB-TR128 B6 Meuth/Budde/Pape

Max-Planck-Research Award 2007

Title: Control of activity mode and sensory signal processing in the thalamus: The novel role of KCNQ channels

Authors: *M. CERINA[1], H. J. SZKUDLAREK[2], P. COULON[2], T. KANYSHKOVA[2], P. MEUTH[2], K. GOEBEL[3], T. SEIDENBECHER[2], S. G. MEUTH[1], H. C. PAPE[2], T. BUDDE[2]
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Abstract: KCNQ channels are slow voltage activated K+ channels. They represent the molecular substrate of the M current (IM), which operates below action potential threshold and limits neuronal excitability. We detected mRNA and protein expression of these channels in the ventrobasal thalamic complex (VB). To determine the contribution of KCNQ channels to thalamic activity modes and to analyse their possible role in somatosensory and noxious stimulus processing, VB neurons were characterized *in vitro*, in silico and *in vivo*. Whole-cell recordings were performed in mouse VB slices. Channel properties were modulated by using the specific KCNQ channel opener retigabine and inhibitor XE991. The consequences of IM activation were investigated in a TC neuron computer model and in mice using hot plate tests. In voltage-clamp, KCNQ channels generated a slow K+ outward current which was sensitive to retigabine and XE991. In current-clamp, retigabine reduced tonic firing and promoted the burst-like firing mode. The same effect was produced by adding an IM component to the TC neuron model. During hot plate testing, intrathalamic injection of retigabine and XE991 significantly increased and decreased the latency to the occurrence of pain behaviour, respectively. These findings indicate that IM limits TC neurons' excitability. Moreover, membrane hyperpolarization induced by KCNQ channel activation represents a new mechanism for the facilitation of LTS-mediated burst firing. Given the analgesic effect induced by retigabine injection and the known anti-nociceptive effect of thalamic burst firing during noxious stimulation, KCNQ channels may represent a novel target to control pain sensation on thalamic level.

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Poster

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Support: IZKF Münster Bud3/010/10

DFG BU1019/9-2

DFG BU1019/11-1

Title: Somatosensory signal processing in ventrobasal thalamocortical neurons- contribution of KCNQ channels

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Abstract: Nearly all sensory stimuli are conveyed from the periphery to respective cortical processing areas via the thalamus, where initial information processing occurs. Thalamocortical relay (TC) neurons which represent the majority of cells in the thalamus are determining its relaying and gating functions and are characterized by unique electrophysiological properties. Relay of information by the TC neuron is represented by tonic firing of Na+/K+-mediated action potentials where individual action potentials are nearly equally spaced; while gating of the information transfer is reflected by burst firing mediated by a T-type Ca2+ current where few individual action potentials are grouped together and these groups are separated by long quiescence periods. Recent studies indicate that TC neurons express K+ channels belonging to the KCNQ channel family (Kv7.2-Kv7.5). Opening of neuronal KCNQ channels induces a slow outward K+ current activating below the action potential threshold. This current has been termed M-current and typically results in spike frequency adaptation, a feature not observed in TC cells. For that reason involvement of KCNQ channels to firing mode regulation of TC neurons is thought to be of minor consequence and involvement of these channels in thalamic functions is continuously neglected. In the present study we provide evidence that KCNQ channels are functionally expressed in ventrobasal thalamic complex (VB) of mice and evaluate their physiological role. Extracellular single-unit recordings of TC neurons in VB of behaving animals showed that local pharmacological activation of KCNQ channels with retigabine increased the number of burst-associated spikes that were fired at higher frequency. Moreover, when animals were tested in a hot plate paradigm, local thalamic infusion of retigabine increased the latency to the occurrence of aversive behavior while blocking activity of KCNQ channels with XE991 decreased the latency. Simultaneous unit recordings showed that 35% of the VB neurons increased their activity during the hot plate test. Local application of retigabine reduced the number of responsive units to 25%, while XE991 increased it to 50%. On average, the peak activity of responsive units under control conditions occurred ~7 s after exposure to the hot plate. Retigabine increased this to ~14 s and XE991 decreased it to ~3 s. These findings indicate that KCNQ channels contribute to sensory information processing in VB. Their activation in freely

behaving mice mediates an increase in the latency to pain related behavior in response to thermal noxious stimuli paralleled by a decrease of responsive VB units and an increase of burst firing.

Disclosures: **H.J. Szkudlarek:** None. **M. Cerina:** None. **P. Coulon:** None. **P. Meuth:** None. **P. Blaesse:** None. **T. Kanyshkova:** None. **K. Göbel:** None. **T. Seidenbecher:** None. **S.G. Meuth:** None. **H. Pape:** None. **T. Budde:** None.

Poster

245. Somatosensory Transduction

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Program#/Poster#: 245.15/GG12

Topic: C.13. Sensory Disorders

Title: TRP channel mediated cross-talk between axotomized and intact neurons in dorsal root ganglia revealed by probing activity with an ion channel photoswitch compound

Authors: *C. HEROLD, R. H. KRAMER
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Abstract: Transient Receptor Potential (TRP) ion channels play a major role in detecting sensory information and pain. TRP channels have also been shown to function as a key integrators of many pain related signals and TRP channel activation is implicated in the emergence of pain hypersensitivity and chronic pain. Interestingly, some TRP channels exhibit an unusual activation state identified by dynamic changes in ion selectivity after strong and prolonged stimulation. However, the physiological implications of this activation state remain completely unknown. We hypothesized that this activation contributes to physiological changes after peripheral axotomy and is involved in the communication between axotomized and non-axotomized cell bodies in the dorsal root ganglia. Since studying this activation with conventional methods in intact tissues remains hardly feasible, we developed an assay using a reporter molecule to indirectly read out dynamic selectivity over time in intact dorsal root ganglia tissue. In detail, we designed the photoswitch molecule QAQ which constitutes a light sensitive intracellular blocker of voltage-gated sodium, potassium and calcium channels. Block can be regulated by rapid and reversible photoisomerization of the central azobenzene group from trans to cis configuration upon 380 or 500nm light illumination, respectively. QAQ blocks ion channels in its trans configuration and switch to cis relieves the block. Given its double-charged character, QAQ is membrane-impermeable but it can enter cells using certain TRP channels as a conduit. In conclusion, QAQ functions as an activity dependent local anesthetic

inhibiting neuronal excitability in a light-sensitive manner after selective cell entry. Therefore, conferred light sensitivity functions as an indicator of TRP channel activity and reversible silencing of active pain neurons enables a new approach to study nociception. In addition, integrated QAQ entry over time amplifies even small changes in channel activity invisible to other techniques. Using this assay, we could demonstrate that the Spared Nerve Injury (SNI) mouse model for chronic pain leads to elevated TRP channel activity 24 hours after surgery. In contrast, activity levels decrease again in a 7 day post SNI time frame. More interestingly, spared, intact sensory neurons also show increased TRP channel activity. This cross activation is limited to slow conducting C fibers and TRPV1 expression is not essential for this effect. In summary, these findings might have implications for the regulations of neuronal plasticity and dynamic adaptation in the pain system.

Disclosures: C. Herold: None. **R.H. Kramer:** None.

Poster

245. Somatosensory Transduction

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Program#/Poster#: 245.16/GG13

Topic: C.13. Sensory Disorders

Support: This research was supported by the NIDCR Intramural Research Program

Title: Neuropeptides in Sensory Neurons

Authors: *S. K. MISHRA, J. HUANG, M. A. HOON
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Abstract: Recently there has been a renaissance in the study of neuropeptides in sensory systems as modulators and transmitters of somatosensory stimuli. We showed previously that the small polypeptide Nppb is expressed in peripheral sensory neurons and is critical for responses to itch-inducing compounds. This raised the issue of the role of a second peptide that had been reported to be expressed in DRG-neurons and had also been thought to be a primary transmitter for itch-behavior, GRP. Here we present evidence for a lack of GRP expression in peripheral sensory neurons while there are high levels of GRP expression in the dorsal horn of the spinal cord. These results indicate that GRP is not acting as a peripheral transmitter, but functions downstream of the DRG. A previous report also suggested that Nppb played a role as an analgesic at the level of the spinal cord. In order to evaluate the latter study we examined the

behavioral responses of mice lacking Nppb to painful stimuli. We found no evidence of deficits to noxious heat or mechanical stimuli either in naïve mutant mice or following intraplantar injection of CFA in Nppb knockout mice. Together these results argue against Nppb being important in pain signaling pathways and instead strongly suggest Nppb is a highly selective peripheral neuromodulator of itch sensation.

Disclosures: S.K. Mishra: None. **J. Huang:** None. **M.A. Hoon:** None.

Poster

245. Somatosensory Transduction

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Topic: C.13. Sensory Disorders

Title: KCNS1 as a biomarker for increased pain perception in patients with musculoskeletal pain

Authors: *A. FRENCH[1], J. BISHOP[2], S. ATKINSON[2], V. MAY[2], G. LIEBERMAN[2], M. NAYLOR[2]
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Abstract: Objectives: The primary goal of this research is to determine whether an allele within the gene encoding for the potassium channel alpha subunit KCNS1 is associated with increased pain perception in patients with chronic musculoskeletal pain. Previous research demonstrated that the potassium channel encoded by this gene plays a role in neuronal excitability and that expression in sensory neurons is substantially down-regulated in patients with neuropathic pain with Val substitution. Specifically those with homogenous Val/Val missense single nucleotide polymorphism (SNP) at the rs734784 KCNS1 allele experience increased pain sensitivity. Given this, we aim to: (1) determine whether the distribution of KCNS1 genotypes differs in our sample of chronic musculoskeletal pain patients compared to the general population, (2) identify physical and psychological characteristics of patients with musculoskeletal pain by KCNS1 genotype, and (3) to investigate whether clinical response to Cognitive Behavioral Therapy (CBT) for coping with chronic pain differs based on KCNS1 genotype. We hypothesize that patients with the Val/Val SNP will report greater pain sensitivity and show greater improvement post-CBT. Methods: 201 adult male and female patients with chronic musculoskeletal pain were recruited to participate in this study. All participants underwent clinical evaluations and saliva samples were collected for DNA analysis. Clinical evaluations included a battery of behavioral inventories. After consenting patients were randomly assigned to either eleven weeks of group

CBT or an attention control group. Ongoing data analysis includes creation of statistical models to determine the interaction of characteristics impacting pain experience. Results: Preliminary analysis of these data revealed that the frequency of Val/Val substitution in our sample closely represents that of the general population (20.4% vs. 20.5%). In addition, at baseline, patients homogenous for the Val/Val substitution had lower pain levels (p=.02), higher SF-36 mental component scores (p=.04) and reported reduced catastrophizing (p=.006) compared to patients with no Val substitution. Conclusion: These findings demonstrate that genetic variations in the KCNS1 allele correlate with pain perception. Contrary to our hypothesis, musculoskeletal pain patients with homogenous Val/Val mutations report lower average pain than those with no Val substitution, suggesting KCNS1 plays a different role in pain perception than in neuropathic pain. Work is underway to assess whether differences in genotype can be correlated with treatment outcomes.

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Poster

246. Neocortical Plasticity

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Topic: B.08. Synaptic Plasticity

Support: PAPIIT IN 212013

Title: Conditioned taste aversion prevents the long-lasting BDNF-induced enhancement of synaptic transmission in the insular cortex: A metaplastic effect

Authors: A. RIVERA-OLVERA, *M. L. ESCOBAR
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Abstract: Metaplasticity is a homeostatic process by which the capacity of synapses to express plastic changes is itself subject to variation depending on previous experience. In particular, training in several behavioral tasks modifies the possibility to induce long-term potentiation (LTP). Recently, we have reported that prior training in conditioned taste aversion (CTA) prevents the subsequent induction of long-term potentiation generated by high frequency stimulation in the projection from the basolateral nucleus of the amygdala (Bla) to the insular cortex (IC). One key regulator of long-term synaptic modifications related to learning and

memory maintenance is brain-derived neurotrophic factor (BDNF). Our previous studies have demonstrated that acute microinfusion of BDNF on the IC induces a lasting potentiation of synaptic efficacy at the Bla-IC projection. The aim of the present study was to analyze whether CTA training modifies the ability to induce subsequent BDNF-induced potentiation of synaptic transmission in the Bla-IC projection *in vivo*. Thus, CTA trained rats received intracortical microinfusion of BDNF in order to induce lasting potentiation 48 h after the aversion test. Our results show that CTA training prevents the induction of *in vivo* BDNF-LTP in the Bla-IC projection. These findings reveal that CTA training produces a change in the ability to induce subsequent *in vivo* BDNF-LTP in the IC, suggesting that changes in the ability to induce subsequent synaptic plasticity in the neocortex contribute to the formation and persistence of aversive memories.

Disclosures: A. Rivera-Olvera: None. **M.L. Escobar:** None.

Poster

246. Neocortical Plasticity

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Program#/Poster#: 246.02/GG16

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant EY018119

Title: Alterations in neurotrophin expression associated with adult experience-dependent plasticity

Authors: *S. A. MARIK, C. D. GILBERT
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Abstract: Plasticity in the adult cortex can be induced by alterations in sensory experience. The representation of the whiskers in the mouse somatosensory cortex can be altered by whisker plucking, which results in remapping of the areas receiving input from the remaining whiskers. This remapping is associated with a parallel process of axonal outgrowth and pruning with a net increase in density of horizontal excitatory axons projecting into the deprived cortex. To determine the molecular mechanisms underlying the axonal changes, we examined the temporal alterations in BDNF expression following whisker plucking. After one day of whisker plucking there is upregulation of BDNF expression throughout the barrel cortex, which then, after two days, becomes more localized to the deprived cortex. One the other hand, the BDNF precursor,

proBDNF, is expressed in the somatosensory cortex before plucking, with particularly high levels in layer V pyramidal somata, and this expression decreases following whisker plucking. The decrease in proBDNF may be related to the elevation in BDNF, with the former being constitutively expressed and then converted into the latter after removal of sensory input. The balance between BDNF and proBDNF may account for the push pull nature of axonal changes involved in experience-dependent plasticity.

Disclosures: S.A. Marik: None. **C.D. Gilbert:** None.

Poster

246. Neocortical Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: A*STAR SICS Intramural Funding

Title: Epigenetic regulation of BDNF affects parvalbumin interneuron maturation during experience-dependent plasticity of the mouse barrel cortex

Authors: *D. X. KOH[1,3,5,2], S. LO[5,2,4], G. J. AUGUSTINE[5,2], J. C. SNG[1,4]
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Abstract: During early development, interactions between sensory experience and innate genetic programs are crucial for sculpting neuronal circuits. We used the mouse primary somatosensory cortex (S1) to investigate the molecular mechanisms involved in gene regulation during experience-dependent plasticity. Differences in cortical function and gene regulation were compared between barrel cortices in the same animal that received differential sensory input as a result of unilateral whisker deprivation. 30 days after whisker deprivation at birth, we found that inhibitory transmission between layer II/III parvalbumin interneurons and pyramidal neurons was decreased. We hypothesize that this results from epigenetic mechanisms that regulate genes responsible for maturation of inhibitory circuits. Histone acetylation, an epigenetic mark for gene transcription, is kept in equilibrium by histone acetyltransferases and histone deacetylases (HDACs). We observed that HDAC1 expression decreased during the course of S1 development.

However whisker deprivation caused HDAC1 expression to remain relatively high (34% increase). This was associated with (1) decreased acetylation of histones, (2) increased binding of HDAC1 to promoter I of brain-derived neurotrophic factor (Bdnf), and (3) reduced transcription of the Bdnf gene. Whisker deprivation also delayed maturation of layer II/III inhibitory circuits in S1, evident by a 34% decrease in parvalbumin expression within interneurons and a 17% reduction in perineuronal nets, which have been implicated in maturation and stabilization of inhibitory synapses. Our results are consistent with the hypothesis that HDAC1 activity modulates BDNF expression required for the maturation of parvalbumin interneurons within layer II/III inhibitory circuits.

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Poster

246. Neocortical Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: Ministry of Science and Higher Education Grant 6420/B/P01/2011/40 to E.S.

FENS abstract slot

Title: Post-training changes in the numerical density of CB1 and PV immunoreactive puncta in barrel cortex following fear conditioning in the mouse

Authors: *E. SIUCINSKA, W. BRUTKOWSKI, T. BERNAS
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Abstract: In the cerebral cortex immunostaining for the type 1 cannabinoid receptor (CB1) was found in axon terminals, all of which contained GABA and formed symmetric synapses. Parvalbumin (PV) neurons include chandelier and basket cells. Parvalbumin immunopositive chandelier cells synapse on the axon initial segments and basket cells regulate the cell soma of pyramidal cells, regulating output and integration across cortical areas. Previous ultrastructural studies using stereological counting techniques show a 70% increase in the density of inhibitory synapses on spines of neurons located in layer IV barrels that represent the stimulated vibrissae, have suggested synaptic remodeling occurred at least 24hr after fear conditioning. The present study estimates the mean CB1 (Abcam 1:500) and PV- (Sigma 1:12,000) immunoreactive puncta

(CB1-IR, PV-IR) numerical density (Nv) in a region of the mouse the primary somatosensory cortex known to be involved in plastic changes induced 24h following three days fear conditioning. In all experiments precise location of layer IV cells were identified using Hoechst 33258 staining of tangential sections. A confocal microscopy stereological technique, the "disector", was used in the CB1-IR and PV-IR puncta analyses. In barrels belonging to the row of vibrissae stimulated during fear conditioning, the average density of CB1-IR puncta and PV-IR axon terminals in the hollows increased by approximately 25% as compared with the hollow of row on the contralateral control side. The finding suggest that both, endocannabinoid receptor 1 and PV-IR axon terminals may be a key part of processes involved in conditioning-dependent plasticity of the primary somatosensory cortex. The observed increase of PV-IR neuropil may be related to controlling the effectiveness of perisomatic inhibition, or metabolic activation of the population of PV-IR neurons.

Disclosures: **E. Siucinska:** None. **W. Brutkowski:** None. **T. Bernas:** None.

Poster

246. Neocortical Plasticity

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Topic: B.02. Ligand-Gated Ion Channels

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P50MH100024 (to R.L.H.)

R01MH051106 (to D.J.L.)

Title: Visualization of NMDA receptor-dependent AMPA receptor synaptic plasticity *in vivo*

Authors: ***Y. ZHANG**[1], R. H. CUDMORE[1], D.-T. LIN[1,2], D. J. LINDEN[1], R. L. HUGANIR[1]
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Abstract: Regulation of AMPA receptor (AMPAR) membrane trafficking plays a critical role in synaptic plasticity and learning and memory1. However, how AMPAR trafficking occurs *in vivo* remains elusive. Using *in vivo* two-photon microscopy in the somatosensory barrel cortex, we found that acute whisker stimulation leads to significant increases in the expression of surface AMPA receptor GluA1 subunit (sGluA1) in both synaptic spines and dendritic shafts and only small changes in spine size and no changes in spine turnover. Interestingly, initial spine

properties bias changes in spine sGluA1 content and spine size. Changes in spine sGluA1 are positively correlated with changes in dendritic shaft sGluA1, while changes in spine sGluA1 between neighboring spines are negatively correlated. The sensory stimulated increase in spine sGluA1 is NMDA receptor dependent and long lasting similar to major forms of synaptic plasticity in the brain. Our findings shed light on the complexity of AMPAR membrane trafficking and experience-dependent remodeling of synaptic strength *in vivo*.

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Poster

246. Neocortical Plasticity

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Topic: B.08. Synaptic Plasticity

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EU FP7 Future Emergent Technologies grant 243914

Title: Synapse-specific plasticity and calcium-permeable AMPA receptors expression in the neocortical layer-5 microcircuit

Authors: *T. LALANNE[1], J. OYRER[2], R. P. COSTA[3], A. J. CHUNG[1], M. FARRANT[2], P. J. SJÖSTRÖM[1]
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Abstract: Although they play critical roles in the neocortical circuitry functions and development, little is known about the plasticity of inhibitory neurons, mainly because they are

difficult to identify. We examined plasticity at excitatory pyramidal cell (PC) synapses onto basket (BCs) and Martinotti cells (MCs), two major inhibitory cell types in layer-5 of visual cortex. In acute slices from postnatal day 12-21 mice, neurons were identified by morphology, and spiking pattern when possible. With a 50-Hz induction protocol that consistently potentiated PC-PC synapses (after/before ± SEM = 120% ± 7%, n = 10, $p < 0.05$, Student's t-test), we observed non-Hebbian depression of both PC-BC (74% ± 7%, n = 7, $p < 0.05$) and PC-MC connections (54% ± 10%, n = 5, $p < 0.05$). In hippocampus, calcium-permeable (cp-) AMPARs underlie non-Hebbian plasticity at excitatory synapses onto some inhibitory neuron types. We thus set out to determine the presence of cp-AMPARs at these three synapse types. With intracellular loading of spermine to specifically block cp-AMPARs at positive voltages, spontaneous release onto BCs showed inward rectification (rectification index RI+60mV/-60mV = 0.40 ± 0.03, n = 6, versus 1.32 ± 0.15, n = 4, for controls without spermine, $p < 0.001$). Similar results were obtained for evoked currents in PC-BC pairs (RI+40/-40 = 0.104 ± 0.04, $p < 0.001$, n = 6), suggesting the presence of cp-AMPARs. In agreement, the cp-AMPAR blocker NASPM reduced both spontaneous (61 ± 5%, n = 5, $p < 0.01$) and evoked currents in BCs (44% ± 4%, n = 5, $p < 0.001$). In contrast, PC-MC connections did not rectify (RI+40/-40 = 1.41 ± 0.15, p = 0.12, n = 4), suggesting the absence of cp-AMPARs. To study the postsynaptic side in isolation, we uncaged NPEC-AMPA. Uncaging-evoked currents were reduced by NASPM in BCs (61% ± 5%, n = 7, $p < 0.001$) but not MCs or PCs (99% ± 10%, n = 4, p = 0.96 for MCs). In addition, uncaging-evoked currents rectified in BCs (RI+40/-40 = 0.10 ± 0.09, $p < 0.001$, n = 8) but not in MCs (RI+40/-40 = 0.97 ± 0.1, p = 0.8, n = 6). Finally, simulations using a computer network model tuned to our Naspm wash-in data suggested that cp-AMPARs impact early BC but not late MC feedback inhibition onto PCs. In conclusion, although both PC-BC and PC-MC synapses show non-Hebbian plasticity cp-AMPARs are specifically expressed at PC-BC connections. This implies that cp-AMPARs do not alone determine non-Hebbian plasticity. However, synapse-specific cp-AMPAR expression may govern early BC and late MC feedback inhibition. Such synapse-specific plasticity may critically influence information processing in cortical networks.

Disclosures: **T. Lalanne:** None. **J. Oyrer:** None. **R.P. Costa:** None. **A.J. Chung:** None. **M. Farrant:** None. **P.J. Sjöström:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.07/GG21

Topic: D.09. Tactile/Somatosensory

Support: MRC Grant G0901299

Title: Development of sub- and suprathreshold plasticity in RS and IB cells in layer V of the mouse barrel cortex *in vivo*

Authors: *S. GREENHILL, K. FOX
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Abstract: We have previously shown that, in layer V of the rat barrel cortex, regular spiking (RS) and intrinsic bursting (IB) cells respond differently to whisker deprivation; RS cells show depression of responses to deprived whisker stimulation and an increase in spike timing fidelity for short latency responses to spared whisker stimulation. IB cells show no depression but do show generalised potentiation of responses to spared whisker stimulation (Jacob et al, Neuron 73, 391-404 (2012)). We tested whether these results generalised to the mouse barrel cortex. Sharp electrode intracellular recordings were made from acutely anaesthetised preparations of both naive mice and mice whose D-row whiskers had been unilaterally clipped for either 3 or 10 days. The D1-D3 barrels were localised before recording using intrinsic optical imaging at 700nm to guide the electrode recordings. Upon penetration and electrophysiological classification of a layer V pyramidal cell, the receptive field and whisker evoked activity of the cell was automatically mapped using a 3x3 piezo matrix stimulator. Cells were classified as RS or IB depending on their response to depolarising current. In wild-type mice (C57BL/6J), RS cells in layer V showed a depression in firing rates (to 71% of control) in response to both principal (deprived) and surround (spared) whisker stimulation after 3 and 10 days D-row spared deprivation. However, subthreshold responses generally displayed increased amplitude with longer latencies, suggesting a complex relationship between subthreshold activity and firing rate. Additionally, the rate of spontaneous firing was reduced in RS cells recorded from deprived animals. In contrast, IB cells displayed spike-rate potentiation (to 212% of control) across all vibrissae types, but only after 10 days of deprivation. Subthreshold IB responses were of larger amplitude and shorter latency after both 3 and 10 days deprivation. These data suggest that in common with rat layer V, RS and IB cells show different plasticity responses to whisker deprivation but in contrast to the input-specific responses observed in rat layer V, mouse layer V neurones show plasticity that is input agnostic. Current studies are underway to identify the molecular substrates underlying these different forms of plasticity.

Disclosures: **S. Greenhill:** None. **K. Fox:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.08/GG22

Topic: B.08. Synaptic Plasticity

Support: NIH grant EY-12782

Title: Role of experience in plasticity outcomes of spatially separate synaptic pathways onto individual neurons in mouse visual cortex

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Abstract: In the neocortex, individual cells of like type can undergo heterogeneous plasticity responses from depression (LTD) to potentiation (LTP), or no change (NC) in response to a common fixed time delay synaptic conditioning protocol. However it is not known whether all synapses onto a common cell have the same plasticity outcome. Nor is it known what role visual experience plays in shaping the distribution of differential plasticity outcomes. Thus, we evaluated the synaptic plasticity responses of separable sets of synaptic inputs onto common layer 2/3 pyramidal neurons in primary visual cortex in response to simultaneous stimulation of distinct sets of afferents in acute brain slices from visually intact mice and from mice reared with binocular deprivation from before the natural time of eye-opening. The two stimulation sites in layer 4 were spatially isolated by occlusion testing followed by alternative activation of each pathway to evoke a post-synaptic potential (PSP) every 10 seconds in an interleaved fashion. After a ten minute baseline period, the activation of both pathways was simultaneously paired with direct post-synaptic activation that preceded the synaptic stimulation by 10 milliseconds resulting in 3-7 post-synaptic spikes over ten minutes at 0.1 Hz followed by reversion to the interleaved stimulation for 30 minutes. The ratio of the evoked PSP post-/pre- conditioning was calculated for each pathway by taking the 5 minute average peak amplitude over 25-30 minutes post-conditioning compared to 5 minute average before the onset of conditioning. Our results from 132 pathways onto 71 neurons validate in the mouse cortex our previous findings from other rodent species demonstrating heterogeneous plasticity outcomes ranging from LTD to LTP for individual cells with a median post/pre ratio of 0.84 in visually intact mice- both naïve and anesthesia controls. Visually deprived mice did not show a significant difference in the change of synaptic strength of individual pathways with a mean 0.89 post/pre ratio for 63 pathways onto 31 neurons (Rank Sum, $p=0.42$). However, the relationship between two pathways onto a common cell differed depending on the level of visual experience with a weak correlation between two pathways in visually deprived mice (median $R= 0.49$, $p= 0.005$, $n= 30$ cells) while no such relationship existed in sighted mice (median $R = 0.023$, $p= 0.9$, $n= 31$ cells) with a significant

difference between correlation coefficients (t-test, p=0.05) as measured by a linear fit to 10,000 random permutations of ordinate and abscissa designation of post/pre ratios of two pathways onto a common cell for each treatment group (Monte Carlo Permutation Analysis).

Disclosures: O.M. Fitch: None. **M.J. Friedlander:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.09/GG23

Topic: B.08. Synaptic Plasticity

Support: MRC Grant G0901299

Title: The role of CaMKII-alpha in experience-dependent structural plasticity of Layer2/3 pyramidal dendritic spines in mouse barrel cortex

Authors: *G. SEATON, K. D. FOX
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Abstract: Most functional plasticity studies to date have focused on Layer2/3 neurons, while structural plasticity remains largely uncharacterised in these layers. It is not clear from the literature on visual and somatosensory cortex whether Layer2/3 neurons show significant levels of structural plasticity in response to sensory deprivation, whilst it is clear that they show substantial CaMKII-dependent functional plasticity (Glazewski et al 2000 Nature neuroscience 3 (9), 911-918). To address this issue we investigated experience-dependent structural plasticity of the basal dendritic spines of Layer2/3 pyramidal neurons in the barrel cortex. C57Bl6-Jax mice were injected intracranially with AAV-Cre(CaMKII)-GFP and AAV-Flex-GFP, to target sparse expression of GFP (under the control of Cre-recombinase and the CaMKII-alpha promoter) in a population of Layer2/3 pyramidal neurons. Using 2-photon laser-scanning microscopy, chronically intracranial windowed GFP mice were then imaged to quantify basal turnover and the proportion of persistent spines (i.e. spines that survive for 8 days or more). Mice were then subjected to sensory (chessboard whisker) deprivation and the imaging paradigm timed so that spines were quantified and characterised 24 hours post deprivation, with subsequent 4 day imaging time points extending up to two weeks post deprivation. To investigate the role of CaMKII-alpha in the structural component of experience-dependent plasticity in the barrel cortex, we imaged double virus injected CaMKII- Threonine-286-Alanine (CaMKII-T286A)

mutant mice, and characterised spine density, morphology, turnover and persistence before and after whisker deprivation. Initial observations suggest that CaMKII-T286A mice have a 2 fold higher basal turnover rate, of 4%, compared to WT control of 2%. 24 hours post deprivation, CaMKII-T286 mice show an increased percentage of new spines compared to WT control, however new spines in WT appear to persist for longer, some lasting over two weeks after formation. Structural morphology and clustering dynamics also appear to be altered compared to WT controls. These data, for the first time, suggest a role for CaMKII-alpha in the initial phases and subsequent maintenance, of structural changes in the basal dendritic spines of Layer2/3 neurons, and therefore support the hypothesis that structural plasticity underlies the functional experience-dependent plasticity observed in the mouse barrel cortex.

Disclosures: G. Seaton: None. **K.D. Fox:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.10/GG24

Topic: D.09. Tactile/Somatosensory

Support: CNRM

Title: Sensory training reorganizes intracortical connections differentially across modalities after a mild TBI

Authors: *S. L. JULIANO[1], E. M. SHINDELL[2], S. SCHWERIN[2]
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Abstract: Intracortical connections expand over a lengthy period following a controlled cortical impact (CCI) in a mouse model of traumatic brain injury (TBI). To investigate the effects of introducing a training paradigm on cortical architecture, we studied the outcome of a situation where mice with and without CCI were trained on a task involving sensory discrimination with their whiskers. The injury directly affects the barrel cortex, but is flanked on either side by intact sensory and motor cortices. Animals recovered for 2 days and then trained to distinguish between rough and smooth textures for 1, 4 or 12 weeks, to determine if the length of training influenced neuroplastic changes. The mouse used its whiskers to choose a coarse versus smooth texture, cross a gap and receive a food reward. This task was difficult for the mice and they were not all able to learn. Following training, the mouse brains were studied by injecting fluorescent

dextrans into select slices through both barrel and motor cortex. The slices were maintained in a chamber perfused with oxygenated aCSF for three hours post injection. To detect and quantify the labeled intracortical connections we examined microscope images using a Hessian-based edge detection filter in Image J. In particular we measured the density of labeled pixels and determined the distance and dispersion of the apical dendrites, descending axons and medial and lateral projections. We previously reported that in the absence of training, longer recovery durations result in greater expansion in barrel cortex than shorter recovery periods and that few changes were observed in motor cortex. The training task after TBI resulted in an expansion of processes in both sensory and motor cortices, compared to control animals or animals with TBI alone. Specifically, we observed a greater expansion in processes extending AWAY from the injury regardless of cortex injected. In addition, the animals that learned the task showed more expansion in sensory cortex compared with the expansions in motor cortex. In summary, these results have implications for rehabilitation regarding the timing and modality of training and the resulting effects on different cortical regions.

Disclosures: S.L. Juliano: None. **E.M. Shindell:** None. **S. Schwerin:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.11/GG25

Topic: B.08. Synaptic Plasticity

Support: NIDCD DC009635

NIDCD DC012557

Title: Inhibitory and excitatory spike-timing-dependent plasticity in the auditory cortex

Authors: *J. A. D'AMOUR, R. C. FROEMKE
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Abstract: Inhibition must be carefully calibrated with excitation in neural circuits. This excitatory-inhibitory balance promotes information flow through neural networks while limiting over-excitability and spurious plasticity. However, this means that changes to excitatory synapses should be tracked by corresponding changes to specific inhibitory synapses to preserve overall excitability and excitatory-inhibitory balance. How inhibitory synapses are modified in

parallel with excitatory synapses has remained unclear. Here we study spike-timing-dependent plasticity (STDP) of inhibitory synapses onto layer 5 neurons in slices of young mouse (P12-24) auditory cortex, together with concomitant STDP of excitatory synapses onto the same neurons. Repetitive pairing of extracellular stimulation with single postsynaptic action potentials induced substantial long-term potentiation (LTP) of inhibitory inputs irrespective of the precise temporal order as long as spikes occurred within ~10 msec (162.2±15.3% n=34 for short positive spike timing intervals, and 133.7±15.8% n=26 for short negative intervals), similar to the prediction of a recent theoretical study (Vogels et al., Science 2011). This was in contrast to excitatory inputs onto these neurons, which displayed a conventional asymmetrical time window for STDP induction (132±6.2% n=46 for short positive intervals, and 81.9±2.8% n= 30 for negative intervals). Inhibitory plasticity was linked to excitatory changes, as NMDA receptors were required for modifications of both excitatory and inhibitory synapses. Irrespective of the temporal order of spike pairing, these combined synaptic modifications acted together to normalize the excitatory-inhibitory ratio of events paired together with postsynaptic action potential generation. Moreover, after excitatory and inhibitory STDP, subthreshold events could become suprathreshold, and the temporal integration window between the onset of excitation and inhibition became shorter, potentially improving spike timing precision. These findings demonstrate that cortical inhibitory synapses are highly plastic, and more importantly, indicate that inhibitory plasticity requires interactions with functionally-related excitatory synapses in order to properly regulate excitatory-inhibitory balance.

Disclosures: J.A. D'Amour: None. **R.C. Froemke:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.12/GG26

Topic: D.09. Tactile/Somatosensory

Support: 0133/P01/2010/70

Title: Interhemispheric interactions contribution in experience dependent plasticity

Authors: *J. A. JABLONKA[1], P. URBAN[2], M. KAZMIERCZAK[3], Z. BORZYMOWSKA[4], E. KUBLIK[4]
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Warsaw, Poland; [3]Rutgers Univ. Cell Biol. & Neurosci., Piscataway, NJ; [4]Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Rodent whisker representation in the somatosensory cortex has a clear somatotopic organization in a form of barrel-like neuronal densities in layer IV forming so-called barrel field (BF) and arranged in a pattern strictly corresponding to the organization of whiskers on the snout. We used this model to study experience-dependent plasticity induced by partial sensory deprivation i.e. unilateral clipping of all but row B whiskers. The cortical representation of the sparred row B was investigated electrophysiologically and by 14C-2-deoxy-D-glucose (2DG) brain mapping. We compared evoked field potentials (EP) and metabolic activity changes (2DG incorporation) evoked in both hemispheres by a unilateral and bilateral stimulations of row B whiskers in deprived and undeprived rats. It was shown with 2DG mapping that cortical representation of the spared whiskers was enlarged after one month of everyday whisker trimming. Bilateral stimulation of row B whiskers induced 2DG incorporation in the cortical area over sparred row B representation that was wider by 44% ($p < 0.05$) compared to the control side and control undeprived animals. Interestingly, after unilateral whiskers stimulation the spared to control difference of row B representations was only 20% ($p<0.05$). Preliminary electrophysiological data shows that the amplitude of potentials evoked by the stimulation of spared row B tend to be lower than in control animals, both in contra- and ipsilateral hemisphere. However, the responses recorded in deprived hemisphere after stimulation of row B from ipsilateral (undeprived) whisker pad tend to be enlarged - they can be as large as those induced by contralateral whiskers stimulation at the depth of 500um. Such activation profile supports the hypothesis that the interhemispheric influences from contralateral side may play important role in inducing the enlargement of the 2DG representation of spared row B whiskers in input-deprived barrel field.

Disclosures: J.A. Jablonka: None. **P. Urban:** None. **M. Kazmierczak:** None. **E. Kublik:** None. **Z. Borzymowska:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.13/GG27

Topic: D.09. Tactile/Somatosensory

Title: Altered microRNAs expression in cortical plasticity after sensory stimulation

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Abstract: In rodents, sensory experience alters the whisker representation in layer IV of the barrel cortex. Excitatory and inhibitory interneurons, together with the astrocytic network, modify the functional representation in an orchestral manner. Our group showed that continuous whisker stimulation in adult mice induces depression of neuronal responses and insertion of new inhibitory synapses on spines. This form of cortical plasticity is controlled by several gene regulatory mechanisms including the activation of genetic programs controlling the expression of microRNAs. The transitory and localized expression of these small, non-coding RNAs in dendrites and their capacity to respond in an activity-dependent manner make brain-specific miRNAs ideal candidates for the fine tuning of gene expression associated with neural plasticity. To investigate the involvement of miRNAs in cortical plasticity, we selected four microRNAs known to be implicated in other forms of synaptic plasticity: miR-125b, miR-132, miR-137 and miR-138. After unilateral stimulation of three whiskers (C1−3) in the adult mouse, we compared the expression level of these miRNAs in stimulated and adjacent non-stimulated barrels using *in situ* hybridization with digoxigenin-labeled Locked Nucleic Acid probes. Whisker stimulation increases the expression, of miR-132 after 3hours of stimulation ($p=0.02$; $n=6$) and miR-137 ($p=0.03$; $n=6$; 24 hrs of stim.), whereas it reduces the level of miR-125b ($p=0.002$; $n=6$; 9 hrs of stim.). No significant difference was detected for miR-138. In addition to this quantitative comparison, we combined microRNA *in situ* hybridization and immunolabeling for various neuronal markers that were chosen for the localization in both excitatory and inhibitory circuits as well as in astrocytes. Analysis of three dimensional confocal acquisitions showed a colocalization of miR-125b with GAD65/67 and parvalbumin; miR-132 with GAD65/67 and Vglut2, miR-138 with parvalbumin, Vglut1 and PSD95, and miR-137 with Vglut1, GS and S100ß. Stimulation alters the degree of colocalization in the stimulated barrel. For example, double labeling for miR-138 and PSD95 is significantly increased in the stimulated barrel as compared to the level of colocalization in the neighboring non- stimulated barrel ($p=0.012$; $n=3$). Using increased neuronal activity in the whisker-to-barrel pathway, our results indicate that microRNAs have a potential role in sensory activity-dependent cortical plasticity in the adult mouse by acting specifically within the different cellular components of the adult neocortical circuit.

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Poster

246. Neocortical Plasticity

Location: Halls A-C

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Topic: B.02. Ligand-Gated Ion Channels

Support: EU FP7 Future Emergent Technologies grant #243914 “Brain-i-nets” (P. Jesper Sjöström)

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CIHR OG 126137 (P. Jesper Sjöström)

NSERC DG 418546-2 (P. Jesper Sjöström)

Title: Control of vesicle release by cortical presynaptic NMDA receptors

Authors: *T. ABRAHAMSSON[1], R. P. COSTA[2], K. A. BUCHANAN[3], D. ELGAR[3], A. V. BLACKMAN[3], J. OYRER[3], A. A. TUDOR-JONES[3], M. C. W. VAN ROSSUM[2], P. J. SJÖSTRÖM[1,3]
[1]Neurol. and Neurosurg., The Res. Inst. of the McGill Univ. He, Montreal, QC, Canada; [2]Inst. for Adaptive and Neural Computation, Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Dept of Neurosci., Univ. Col. London, London, United Kingdom

Abstract: Presynaptic NMDARs (preNMDARs) with unclear functional role have been found at several central synapse types. They have often been implicated in increasing the probability of release, but precisely how has remained unknown. We compared evoked and spontaneous release onto layer-5 pyramidal cells (PCs) using whole-cell recordings in acute slices of juvenile mouse visual cortex. AP5 blockade of preNMDARs downregulated responses evoked at firing rates >8 Hz (69% ± 3%, n = 16, p < 0.001) but not <8 Hz (97% ± 2%, n = 19, p = 0.14). Even though spontaneous release rates were <8 Hz (2.4 ± 0.5 Hz, n = 16, p < 0.001), AP5 reduced miniEPSC frequency (66% ± 3%, n = 16 vs. controls 98% ± 2%, n = 4, p < 0.001), seemingly in contradiction. Quantal amplitude was not affected, as miniEPSPC amplitude remained unaltered (96% ± 1% vs. 98% ± 0.4%, p = 0.11). Two-photon imaging revealed that AP5 reduced basal calcium levels in a subset of PC boutons (67% ± 5%, n = 5), which could potentially specifically reduce probability of spontaneous release while leaving evoked release unaffected. PreNMDARs could affect evoked release in several different ways, e.g. by altering probability of evoked release directly, by increasing the readily releasable pool (RRP) size, or by upregulating RRP replenishment rate. We used Schneggenburger-Neher’s approach to examine the RRP, depleting it with 14-pulse trains at 30 Hz every 80 seconds, and then comparing preNMDAR blockade to external [Ca2+] reduction. The GluN2B-specific NMDAR blocker Ro25-6981 reduced both RRP size (-36 ± 7%, n = 11 vs. controls -7.1% ± 5%, n = 11, p < 0.05) and replenishment rate (-

31% ± 7% vs. 18% ± 7%, $p < 0.001$). However, although lowering [Ca2+] decreased the RRP size (-27% ± 4%, $n = 9$, $p < 0.05$ vs. controls), replenishment rates were unaffected (7.8% ± 7%, $p = 0.33$). PreNMDAR upregulation of RRP replenishment rates is thus dissociated from general calcium influx. We next extended the Tsodyks-Markram short-term plasticity model to include preNMDARs and tuned it to control and Ro25-6981 conditions. The model corroborated that replenishment rates were downregulated in preNMDAR blockade, and it also captured the 8-Hz frequency threshold. In conclusion, preNMDARs may elevate the probability of spontaneous release directly by increasing basal calcium levels in boutons, presumably by flickering open at resting membrane potential. However, preNMDARs may upregulate probability of evoked release indirectly as a consequence of increasing RRP replenishment rates. In this view, one functional role of preNMDARs may be to maintain neurotransmission in the face of high-frequency firing.

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Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.15/GG29

Topic: B.08. Synaptic Plasticity

Title: Imbalanced synaptic plasticity rules lead to energy efficient connectivity

Authors: J. SACRAMENTO[1], *M. C. VAN ROSSUM[2]
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Abstract: It is believed that energy efficiency is an important constraint in brain evolution. As synaptic transmission dominates energy consumption, energy can be saved by ensuring that as few as possible synapses are active. It is therefore likely that the formation of sparse codes and sparse connectivity is a fundamental objective of synaptic plasticity. In this work we study how sparse connectivity can result from a synaptic learning rule of excitatory synapses. Information is maximised when potentiation and depression are balanced according to the mean presynaptic activity level and the resulting fraction of zero-weight synapses is around 50%. However, an imbalance towards depression increases the fraction of zero-weight synapses without

significantly affecting discrimination performance. We show that imbalanced plasticity corresponds to imposing a regularising constraint on the L1-norm of the synaptic weight vector, a procedure that is well-known to induce sparseness. Imbalanced plasticity is biophysically plausible and leads to more efficient synaptic configurations than a previously suggested approach that prunes synapses after learning. Our framework gives a novel interpretation to the high fraction of silent synapses found in brain regions like the cerebellum.

Disclosures: J. Sacramento: None. **M.C. Van Rossum:** None.

Poster

246. Neocortical Plasticity

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Topic: B.08. Synaptic Plasticity

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BBSRC,

HFSP

MRC

Royal Society

Title: Distinct mechanisms of spike timing-dependent LTD at vertical and horizontal inputs onto L2/3 pyramidal neurons in mouse barrel cortex

Authors: *A. RODRIGUEZ-MORENO[1], A. BANERJEE[2], A. GONZALEZ-RUEDA[3], C. SAMPAIO-BAPTISTA[2], O. PAULSEN[3]
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Abstract: Spike timing-dependent plasticity (STDP) is an attractive candidate to mediate the synaptic changes that support circuit plasticity in sensory cortices during development. STDP is prevalent at excitatory synapses, but it is not known whether the underlying mechanisms are universal, or whether distinct mechanisms underpin STDP at different synapses. Here, we set out to compare and contrast STDP at vertical layer 4 and horizontal layer 2/3 inputs onto

postsynaptic layer 2/3 neurons in the mouse barrel cortex. We find that both vertical and horizontal inputs show STDP, but that they display different time windows for induction of timing-dependent long-term depression (t-LTD). Moreover, whereas t-LTD at vertical inputs requires presynaptic NMDA receptors and is expressed presynaptically, using paired recordings we find that t-LTD at horizontal inputs requires postsynaptic NMDA receptors and is expressed postsynaptically. These results demonstrate that similar forms of plasticity on the same postsynaptic neuron can be mediated by distinct mechanisms, and suggest that these forms of plasticity may enable these two types of cortical synapses to support different functions.

Disclosures: A. Rodriguez-Moreno: None. **A. Banerjee:** None. **A. Gonzalez-Rueda:** None. **C. Sampaio-Baptista:** None. **O. Paulsen:** None.

Poster

246. Neocortical Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH R01 EY019277

NIH F31 NS086241

Title: Microglial P2Y12 signaling is critical for visual plasticity

Authors: *G. O. SIPE, A. K. MAJEWSKA
Neurobio. & Anat., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Synaptic plasticity is critical for neurodevelopment and proper function of the adult nervous system. Studies show that microglia play critical roles in neurodevelopment, but mechanisms driving these roles are poorly understood. We explored purinergic signaling as a potential mediator between microglia and neurons during synaptic plasticity. Purinergic signaling has been implicated in microglial behavior, but studies focused on inflammatory roles. Non-inflamed microglia highly and selectively express the purinergic receptor, P2Y12, which functions in microglial chemotaxis. We posited that purinergic signaling contributes to the microglial motility underlying synapse surveillance and may be critical for microglial roles in synaptic refinement. We used P2Y12 knock-out (KO) mice or the P2Y12 antagonist, clopidogrel in wildtype (WT) mice, to disrupt P2Y12 signaling and examined microglia morphology,

microglia motility and functional synaptic plasticity in the visual cortex. We used immunohistochemistry for the microglia specific ionized calcium-binding adapter (Iba-1) protein to stain microglia in fixed sections and confocal imaging to quantify microglial process complexity. We also used 2-photon microscopy to monitor microglial motility in P2Y12 KO mice by crossing them with CX3CR1-GFP knock-in mice. Finally, we used intrinsic optical signaling to measure ocular dominance plasticity in P2Y12 disrupted mice. Our evidence suggests that P2Y12 disruption prevents ocular dominance shifts indicative of synaptic plasticity. P2Y12 disruption also decreases microglial process complexity, without affecting basal microglial process motility. We are now investigating how P2Y12 disruption prevents ocular dominance plasticity by examining changes in microglial-synapse interactions, synaptic turnover, and microglial-synapse phagocytosis. Our studies will explore new non-pathological roles for microglial purinergic signaling in neurodevelopment.

Disclosures: G.O. Sipe: None. **A.K. Majewska:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.18/GG32

Topic: B.08. Synaptic Plasticity

Support: NIH grant HD67218

Title: Motor skill learning induced AMPA receptor synaptic translocation in the fmr1 KO mouse

Authors: A. SURESH, *A. DUNAEVSKY
Developmental Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Fragile X syndrome (FXS) is the most common inherited form of intellectual disability. Patients with FXS exhibit a range of neurological deficits including motor skill deficits. Previously, we found that fmr1 KO mice have an impairment in motor skill learning in a forelimb reaching task and reduced learning-induced synaptic plasticity in the forelimb area of the primary motor cortex (M1). Specifically, fmr1 KO mice do not exhibit learning induced increases in dendritic spines as seen in the trained hemisphere of wild type mice and show a delay in synaptic insertion of AMPAR subunit GluA1 in the forelimb region of the primary motor cortex. Here we investigated the spatiotemporal expression of AMPAR in order to understand the relationship between AMPAR insertion and dendritic spine formation and

stability. We performed *in utero* electroporation of SEP-AMPAR subunits and the morphological marker tdTomato to label layer 2/3 neurons in M1 of fmr1 KO mice. We then performed repeated *in vivo* two photon imaging of dendritic spines and AMPAR subunits following motor skill training in adolescent mice. Our preliminary data suggest that as in dendrites of layer 5 neurons, motor learning results in a rapid increase in dendritic spines on layer 2/3 neurons in the trained hemisphere. Moreover, an increase in synaptic GluA2 is observed in wild type mice but not in the fmr1KO mice. These studies will help elucidate the relationship between structural and functional synaptic plasticity with learning.

Disclosures: A. Suresh: None. **A. Dunaevsky:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.19/GG33

Topic: B.08. Synaptic Plasticity

Support: NINDS/NIH NS075136 (J.B.D.)

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National Natural Science Foundation of China No. 81071022,91132726 (T.X.)

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Title: Dynamic synaptic re-organization in the motor cortex in parkinson's disease

Authors: T. XU[1,2], L. GUO[1,2], J.-I. KIM[3,4], H. XIONG[1,2], *Y.-W. WU[3,4], Y. CUI[1,2], J. B. DING[3,4]
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Abstract: Parkinson's disease (PD) affects seven to ten million people worldwide. Human and animal studies have shown that the parkinsonism results from degeneration of midbrain dopaminergic neurons. The majority of research to date has focused on synaptic adaptations in the striatum, a region that receives the highest density of dopaminergic innervation. Cortical

areas have received very little attention, even though the motor cortex, the command center that governs precise fine motor control, also receives rich projections from midbrain dopamine neurons. While it is known that PD patients' motor learning and motor memory are highly impaired, there remain key gaps in our understanding of the cause of these deficits. In particular, we know very little about how dopamine regulates motor cortex physiology and how dendritic spine dynamics are altered by the loss of its dopaminergic innervation. To investigate the process of dopamine depletion-induced synaptic remodeling in the intact motor cortex, we repeatedly imaged the same apical dendrites of layer V pyramidal neurons. Neurons in the forelimb area of the motor cortex and barrel cortex were identified by the expression of yellow fluorescent protein (Thy1-YFP-H line) and monitored using trans-cranial two-photon laser scanning microscopy. We found dramatic increases in both spine formation and spine elimination in two different PD mouse models (MPTP injection, which lesions dopamine neurons; and Reserpine injection, which depletes dopamine). The spine remodeling is region-specific, as the barrel cortex did not show changes in either spine formation or elimination. This rewiring in the motor cortex can be partially rescued by injection of L-Dopa (a drug commonly used to treat PD patients), suggesting this phenomenon is dopamine-dependent. In addition, spine elimination and formation are selectively regulated by D1 and D2 dopamine receptor signaling, respectively. Dendritic spines are the postsynaptic structure where excitatory synapses are located, and changes in spine morphology and dynamism serve as good indicators of synaptic plasticity. Together, our study reveals unique roles of D1 and D2 dopamine receptor signaling in regulating spine dynamism and functional plasticity, and this abnormal spine turnover in the motor cortex may underlie the learning and motor memory deficits seen in PD.

Disclosures: **T. Xu:** None. **L. Guo:** None. **J. Kim:** None. **H. Xiong:** None. **Y. Wu:** None. **Y. Cui:** None. **J.B. Ding:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

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Program#/Poster#: 246.20/GG34

Topic: B.08. Synaptic Plasticity

Support: NIH Grant EY014439

Title: Deprivation-induced strengthening of pre- and postsynaptic inhibitory transmission in layer 4 of visual cortex during the critical period

Authors: *M. NAHMANI, G. G. TURRIGIANO
Dept. of Biol., Brandeis Univ., WALTHAM, MA

Abstract: Inhibition from fast-spiking (FS) interneurons plays a crucial role in shaping cortical response properties and gating developmental periods of activity dependent plasticity, yet the expression mechanisms underlying FS inhibitory plasticity remain largely unexplored. In layer 4 of visual cortex (V1), monocular deprivation (MD) induces either depression or potentiation of FS to star pyramidal neuron (FS→SP) synapses, depending on the age of onset (Maffei et al., 2004; Maffei et al., 2006). This reversal in the sign (- to +) of plasticity occurs on the cusp of the canonical critical period (CP). To investigate the expression locus behind this switch in sign of inhibitory plasticity, mice underwent MD during the pre-CP (eye-opening to postnatal day (p)17) or CP (p22-p25), and FS→SP synaptic strength within layer 4 was assessed using confocal and immuno-electron microscopy, as well as optogenetic activation of FS cells to probe quantal amplitude at FS→SP synapses. Brief MD prior to p17 or p25 did not alter the density of FS→SP contacts. However at the ultrastructural level, FS→SP synapses in deprived hemispheres during the CP, but not the pre-CP or in GAD_{65}KO mice, had larger synapses and increased docked vesicle density compared to synapses from the non-deprived control hemispheres. Moreover, FS→SP evoked miniature inhibitory postsynaptic currents increased in deprived hemispheres when MD was initiated during the CP, accompanied by an increase in the density of postsynaptic $GABA_A$ receptors at FS→SP synapses. These coordinated changes in FS→SP synaptic strength define an expression pathway modulating excitatory output during CP plasticity in visual cortex. Ongoing experiments aim to compliment these findings by exploring the contribution of FS inhibition to visual responsiveness in freely behaving mice.

Disclosures: M. Nahmani: None. **G.G. Turrigiano:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.21/GG35

Topic: B.08. Synaptic Plasticity

Support: TWU Department of Biology

Research Enhancement

Closing the GAP

Undergraduate Microgrant programs

Title: Cortical neuronal morphology and alteration of Neurexin distribution by Rho GTPases

Authors: *S. K. VALAPPIL[1], H. L. HYNDS[2]
[2]Biol., [1]Texas Womans Univ., DENTON, TX

Abstract: Rho GTPases are a family of small G-proteins that act as a molecular switches by cycling between guanosine triphosphate (GTP)-bound active forms and guanosine diphosphate (GDP)-bound inactive forms. Rho GTPases transduce intracellular signals and regulate a variety of cellular processes. Rho GTPase signaling is best known for their regulation of actin dynamics and thereby regulate neurite outgrowth and networking. At spines forming active synapse, the actin network provides a pushing force on membrane to form spine head and generates increased contact area. Though the roles of Rho GTPases in cellular cytoskeleton is vastly studied, their role in synaptic organization and plasticity is not as completely explored. The intracellular domains of presynaptic membrane protein Neurexin interacts with PDZ-domains of scaffolding proteins and is paralleled by assembly of actin filaments. Hence we speculate that Rho GTPases have a direct or indirect regulatory effect on neurexin distribution and function. To investigate this, we assessed how altering Rho GTPase activity affects the morphology of neurons from neonatal rat cortices, as well as the localization of Neurexin during synaptogenesis. We employed pharmacological activators and inhibitors of Rho GTPase signaling. Using immunocytochemistry and qualitative analysis, we demonstrated morphological changes and distribution of Neurexin in cortical neurons cultured for 7 days *in vitro*. We found that RhoA activation leads to formation of prominent single neural process with few to no minor processes, whereas selective inhibition of Rho-associated protein kinase leads to the formation of extensive process elaboration and fasciculation. Localization of MAP2 towards the cell body and Tau in distal processes was conspicuous in both treated and untreated cells. Neurexin was distributed evenly in cell bodies and neural processes. This finding is supported by the presence of intercellular connections observed both on cell bodies and dendritic extremities. Exploring the specialized neural structures at synapse and understanding synthesis, transport, assembly and interactions of Neurexin is imperative to address neuropsychological disorders such as autism spectrum disorder (ASD), schizophrenia, epilepsy, intellectual disorders (IDs) and Alzheimer's disease.

Disclosures: S.K. Valappil: None. **H.L. Hynds:** None.

Poster

246. Neocortical Plasticity

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.22/GG36

Topic: B.08. Synaptic Plasticity

Support: Supported by the Start-up grant from UCSD, CA, USA (to P.V.B.)

Title: Immunohistochemical and morphometric characterization of neuropeptide Y positive neurons in the human neocortex during regional chronic ischemia

Authors: *V. AKULININ[1], P. V. BELICHENKO[2], A. V. MYTSIK[1], A. V. SERGEEV[1], S. S. STEPANOV[1]
[1]Histology, Cell Biol. and Embryology, Omsk State Med. Acad., Omsk, Russian Federation; [2]Dept. of Neurosciences, Sch. of Medicine, Univ. of California, San Diego, La Jolla, CA

Abstract: Neuropeptide Y (NPY) is densely localized in the central nervous system. NPY and its receptors take a part in the modulation of different neuronal functions: learning and memory, neuroprotection, mood regulation, and food intake, and they also have anticonvulsant effect. The aim of this study was to define changes in expression of neuropeptide Y (NPY) in human brain during ischemia. Human neocortex biopsy tissues were obtained from patients during operational removal of the brain tumor performed at the Omsk State Medical Academy with the approval of the Ethical Committee. Specimens from 15 different ages, ranging from 23 to 62-year-old specimens were ischemic group. The brain biopsy of 5 patients undergoing non-tumor surgery served as control samples. Layers II-III of neocortical Brodmann's areas 10, 17, and 21 were investigated using indirect immunohistochemical method with antibodies against neuropeptide Y (NPY; Sigma Chemicals, St. Louis, USA; diluted 1:8000) for recognizing NPY positive neurons. Morphometric analysis of neurons was performed using ImageJ 1.46 program with Mann-Whitney test for statistical evaluation. NPY positive cells were identified as basket cells, Martinotti cells and neuroglioform cells and they formed large fiber networks. In control cases, the numerical density of NPY positive neurons was 5.8 ± 1.2 per 1 mm2, without significant differences between areas 10, 17, 21 and they formed a dense network of processes (the distance between the transverse sections labeled dendrites was 8.6 ± 2.2 microns). In chronic ischemia cases, the area of NPY positive immunofluorescence (labeled cell bodies and their processes) was significantly increased: 1.95 fold in area 10, 2.17 fold in area 21, and 2.15 fold in area 17 compared to controls ($p < 0.001$). The abundance of the NPY+ cells in the ischemic group was slightly reduced; and fiber density with varicosity formation were significantly elevated. In conclusion, these differences were mainly due to increases in the amount of NPY positive dendrites but not neurons, indicating new formation, growth and branching dendrites of NPY positive neurons. The present study demonstrates that the NPY neurons display resistance and even hyperplasia during regional chronic ischemia. Supported by the Start-up grant from UCSD, CA, USA (to P.V.B.)

Disclosures: **V. Akulinin:** None. **P.V. Belichenko:** None. **A.V. Mytsik:** None. **A.V. Sergeev:** None. **S.S. Stepanov:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.23/HH1

Topic: B.08. Synaptic Plasticity

Title: Chronic *in vivo* imaging of synaptic reorganization in visual cortex after traumatic brain injury

Authors: ***E. WITKOWSKI**, G. DEWALT, A. FOSTER, W. ELDRED, I. DAVISON
Biol., Boston Univ., Boston, MA

Abstract: Traumatic brain injury (TBI) impairs the cognitive, emotional, and physical abilities of millions of Americans every year. Despite dramatic increases in prevalence in recent years, the neurobiological mechanisms underlying TBI remain poorly understood. This is particularly true for mild TBI, where cellular and subcellular damage is difficult to detect by human neuroimaging methods but can still cause pronounced impairment. With high-resolution chronic *in vivo* imaging in a rodent model of TBI, we visualized the extent and time course of fine-scale structural changes in cortical networks following injury. To test how mild TBI affects the stability of neural circuits in mouse visual cortex, we examined structural correlates of excitatory synaptic connectivity before and after mild TBI. Imaging of dendritic spines on layer V pyramidal cells revealed a transient phase of elevated turnover lasting approximately 3 days after injury, consisting of balanced loss and formation of spines. In contrast, dendritic branching and spine density remained stable, indicating that mild TBI induced reorganization of the existing cortical network rather than large-scale changes in dendritic anatomy or number of excitatory synapses. High-resolution chronic imaging approaches promise to help establish how trauma affects the structure and function of neural circuits over different phases of injury and recovery.

Disclosures: **E. Witkowski:** None. **G. DeWalt:** None. **A. Foster:** None. **W. Eldred:** None. **I. Davison:** None.

Poster

246. Neocortical Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 246.24/HH2

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS16446

NIH Grant NS067017

Title: Dorsal column lesions in monkeys differentially affect widespread activity and response properties of putative inhibitory and excitatory neurons in area 3b

Authors: *J. L. REED, H.-X. QI, J. H. KAAS
Psychology, Vanderbilt Univ., NASHVILLE, TN

Abstract: In our primate model of spinal cord injury, unilateral lesions of the dorsal column (DC) disrupt sensory input from one hand, impairing sensation and motor control. Over time, primary somatosensory cortex (area 3b) reactivates and hand use improves. We hypothesize that response properties of area 3b neurons are consistent with partial recoveries of hand use, and we suggest that response abnormalities may relate to lesion severity. We evaluated 3b neurons in 10 monkeys (Aotus, Saimiri) with or without DC lesions by implanting a 100-electrode array in the hand representation. In 5 monkeys, unilateral DC lesions were made, and 5-13 weeks later the array was implanted contralateral to lesion. Tactile stimuli indented skin at individual locations on the digits and palm to activate area 3b in anesthetized monkeys. Here we expand our previous reports by assessing widespread spatiotemporal activity in area 3b of fast-spiking units (FSUs) and regular-spiking units (RSUs), corresponding to putative inhibitory and excitatory neurons. In monkeys with and without lesions, some neurons show widespread activation to suprathreshold stimuli, firing in response to discrete stimulation on more than one hand location. Paradoxically, minimal receptive field (mRF) sizes of 3b neurons are restricted, while mRFs after DC lesion tend to be large and disorganized. We examined whether neurons fired at consistent latencies to multiple sites and how neuron type and lesion extent affected these responses. In normal monkeys, 50% of FSUs and 38% of RSUs responded to tactile stimulation. In monkeys with DC lesions, 53% of FSUs and 25% of RSUs recorded were responsive. When lesions were nearly complete (~1% DC sparing), activity of RSUs was more spatially restricted and latencies were longer. After partial lesions (~20-50% sparing), response latencies resembled those of normal RSUs, but activity was less widespread. FSUs generally maintained widespread activation similar to normal after partial lesions, but nearly complete lesions resulted in restricted activity

and longer latencies. Thus, we found that hand use recovered even after loss of DC input from one hand restricted 3b activation and delayed latencies. Spatially restricted FSU activity after an extensive DC lesion may reflect a mechanism that reduces suppression. However, RSU activity was even more spatially restricted; suggesting that restricted activity of FSUs and RSUs is a consequence of limited input. Long latencies are likely indicative of alternative pathways providing tactile input to 3b. Thus, treatments to promote neuron activation and function of secondary pathways may augment other interventions for spinal cord injury.

Disclosures: J.L. Reed: None. **H. Qi:** None. **J.H. Kaas:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.01/HH3

Topic: D.13. Motor Neurons and Muscle

Support: CIHR Grant 37765

Barbara Turnbull Foundation

Title: Amplification of synaptic currents without bistability in motoneurons: A tale of two channels modulated by monoamines

Authors: *P. ROSE[1], R. MARATTA[1], K. FENRICH[2]
[1]Queens Univ., Kingston, ON, Canada; [2]Fac. of Rehabil. Med., Univ. of Alberta, Edmonton, AB, Canada

Abstract: CaV1.3 channels on the dendrites of motoneurons are critical components of the mechanisms responsible for amplification of weak synaptic currents and bistability. It is widely assumed that these two functions are inseparable. However, not all motoneurons exhibit bistability, but all motoneurons require amplification of synaptic currents to generate a high frequency discharge. Our goal was to test the hypothesis that the combination of CaV1.3 and CaV1.3 dependent K+ channels can amplify synaptic currents without causing bistability. To test this hypothesis we constructed anatomically realistic compartmental models equipped with CaV1.3 and CaV1.3 dependent K+ channels. These channels were confined to dendritic zones, approximately 100 um long, 250-450 um from the soma on small motoneurons and 700-900 um

from the soma on large motoneurons). In keeping with experimental observations (Lee and Heckman 1998), the densities of these channels were tuned to match experimentally observed differences in the thresholds for activating and deactivating CaV1.3 channels in small and large motoneurons in response to slow triangular voltage commands delivered to the soma. The densities of the CaV1.3 channels determined activation thresholds. The densities of the CaV1.3 dependent K+ channels controlled deactivation thresholds. Amplification and bistability were measured by recording the current delivered to the soma by a 1 second long activation of uniformly distributed excitatory synapses. Depending on the number of synapses activated, the synaptic current was amplified 2 to 7 fold. At the termination of the synaptic activity, the current declined in a series of staircase-like steps lasting 3-4 seconds before reaching a sustained current of 1.4 to 7.1 nA. Reducing the conductance of CaV1.3 channels by 50% or increasing the conductance of CaV1.3 dependent K+ channels by 60% reduced the duration of the staircase-like steps and abolished the sustained currents. In contrast, synaptic amplification was not lost. In large motoneurons, the duration of the stair-case decline was shorter and the sustained currents were smaller (2.6 to 3.4 nA). Synaptic currents were amplified 3 fold. A 25% reduction in the conductance of CaV1.3 channels or increasing the conductance of CaV1.3 dependent K+ channels by 36% eliminated the sustained currents and substantially reduced the duration of the staircase decline. Once again, synaptic amplification was retained. These results suggest that decreasing the modulation of CaV1.3 and CaV1.3 dependent K+ channels by monoamines provides a means of amplifying synaptic currents without causing bistability.

Disclosures: P. Rose: None. **R. Maratta:** None. **K. Fenrich:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

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Program#/Poster#: 247.02/HH4

Topic: D.13. Motor Neurons and Muscle

Support: FAPESP

Title: Effect of cannabidiol treatment associated with CB1 and CB2 receptors antagonists after neonatal rat sciatic nerve axotomy

Authors: *M. PEREZ[1], L. P. CARTAROZZI[1], F. S. GUIMARÃES[2], E. A. DEL BEL[2], A. L. R. OLIVEIRA[1]
[1]Unicamp, Campinas, Brazil; [2]Univ. of São Paulo, Ribeirão Preto, Brazil

Abstract: The endocannabinoid system is composed by cannabinoid receptors (CB), endogenous ligands (anandamide and 2-Arachidonoylglycerol) and enzymes that synthesize and degrade such molecules. Endocannabinoids and the respective receptors are found in several tissues, including the nervous system. Ligands to CB1 and CB2 act on demand both in response to physiological stimuli or in pathological conditions and can play neuromodulatory actions. Recent studies showed increased levels of endocannabinoids and also cannabinoid receptors after nervous system lesion, suggesting a role on neuroprotective responses. Several exogenous cannabinoids can interact with the endocannabinoid system, thus acting on the nervous system. Among them, the cannabidiol (CBD) arises as a viable drug to treat nerve injuries due to its antioxidant and neuroprotective properties. Thus, the present study aimed to investigate the neuroprotective potential of CBD following acute neonatal peripheral nerve lesion. For that, we used two days old Wistar rats, which were divided into the following experimental groups: sciatic nerve axotomy (crushing at mid-thigh) and CBD treatment (CBD group), axotomy and vehicle treatment (VE group), axotomy + CBD + AM251 treatment (AM251 group - CB1 inhibitor) and axotomy + CBD + AM630 treatment (AM630 group - CB2 inhibitor). Spinal motoneuron survival was evaluated in Nissl stained sections of the lumbar spinal cord, five days following injury. Such analysis showed that CBD treatment was ~40% more effective when compared to the vehicle group. CB1 and CB2 blockage led to partial increase in neuronal degeneration, even after CBD treatment, indicating that neuroprotection is at some extent triggered by ligand/receptor interaction. Glial reaction was evaluated by immunohistochemistry using antibodies against GFAP (glial fibrillary acidic protein - astrogliosis marker) and IBA-1 (Ionized calcium binding adaptor molecule 1 - microgliosis detection). CBD treatment reduced both astroglial (40%) and microglial (62%) reaction at lamina IX, in the surroundings of lesioned motoneurons. Importantly, CB1 but not CB2 blockage completely reversed the positive effects of CBD. Overall, the present results show that CBD is neuroprotective and reduces glial reaction following neonatal axotomy. Such effects possibly require CB1 receptor and downstream signaling to be effective.

Disclosures: **M. Perez:** None. **L.P. Cartarozzi:** None. **F.S. Guimarães:** None. **E.A. Del Bel:** None. **A.L.R. Oliveira:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.03/HH5

Topic: D.13. Motor Neurons and Muscle

Support: 5T32NZ077984-02

Title: Reduction of survival of motor neuron protein in the enteric nervous system impairs neuromuscular transmission to smooth muscle and gastrointestinal function

Authors: *S. E. GOMBASH, K. C. WILLIAMS, J. A. FITZGERALD, C. C. IYER, V. L. MCGOVERN, I. S. GRANTS, F. L. CHRISTOFI, C. C. COWLEY, A. H. M. BURGHES, K. D. FOUST
Ohio State Univ., Columbus, OH

Abstract: All cells require a basal level of the ubiquitous survival motor neuron (SMN) protein for embryonic development. However, motor neurons in the spinal cord specifically require increased SMN levels for proper maturation of neuromuscular signaling. Like the central nervous system, the enteric nervous system (ENS) is rich in motor neurons, but the requirement for SMN within the ENS has not been investigated. The ENS is found in the walls of the gastrointestinal (GI) tract and is estimated to have as many neurons as the spinal cord. Among its various functions, the ENS controls peristalsis that allows for the passage of materials through the gut. In the present study we investigated whether basal SMN levels are sufficient for proper ENS physiology and function. To accomplish this, Nestin-cre mice were crossed to tdTomato reporter mice to characterize recombination within the ENS. Reporter gene expression was detected in enteric neurons and glial cells within the myenteric plexus, but was absent in both smooth and skeletal muscle and motor neurons within the CNS. Nestin-cre mice were then crossed to mice with a floxed Smn exon 7 allele on a SMNΔ7 background (SMN2+/+;SMNΔ7+/+;SmnF7/-), thereby reducing Smn in targeted cells. Because CNS motor neurons are not subject to recombination, Nestin-F7 mice are long-lived and ambulatory. Neurotransmission to smooth muscle in the distal colon was examined in Nestin-F7 mice using *ex vivo* organ bath assays. Electrical field stimulation of ENS neurons resulted in opposite responses in control (SMN2+/+; SMNΔ7+/+; SmnF7/+) and experimental colons showing defective neuromuscular transmission. The addition of tetrodotoxin to the bath verified that alterations in muscle response were neuronally driven. To determine whether these changes resulted in functional deficits, Nestin-F7 and control littermates were assayed in tests of gastric emptying, constipation, and colonic motility. Gastric emptying and intestinal transit were significantly slower in experimental mice compared to control littermates. In assays of constipation, Nestin-F7 mice produced significantly less stool with reduced moisture content that corresponded to reduced colonic motility in the bead latency test. Collectively, these data show that basal levels of Smn within the ENS impairs neuromuscular signaling within the GI tract.

Disclosures: S.E. Gombash: None. **K.C. Williams:** None. **J.A. Fitzgerald:** None. **C.C. Iyer:** None. **V.L. McGovern:** None. **I.S. Grants:** None. **F.L. Christofi:** None. **C.C. Cowley:** None. **A.H.M. Burghes:** None. **K.D. Foust:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Program#/Poster#: 247.04/HH6

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant NS072454

Title: Direct cutaneous modulation of motoneuron activity: Evidence for a monosynaptic circuit connecting cutaneous afferents with spinal motoneurons

Authors: *P. M. SONNER, M. A. DALLMAN, D. R. LADLE
Wright State Univ., Dayton, OH

Abstract: Sensory information from mechanoreceptors and nociceptors is processed along interneuron pathways prior to modulating motoneuron (MN) output. Here we report a novel pathway in which a subset of cutaneous Saphenous (Saph) nerve sensory afferents monosynaptically connects with spinal MNs. To assess this circuit, an isolated spinal cord preparation was utilized in C57BL6 mice at birth (P0-P2) and one-week postnatal (P7/P8). The peripheral Saph nerve was stimulated at 0.2Hz and the resultant change in membrane potential was recorded from MN axons exiting from the ventral root of lumbar segment 3. A monosynaptic response was observed in all P7/P8 preparations (n=4), upon Saph nerve stimulation. Interestingly, monosynaptic latency responses in P0-P2 preparations were not observed (n=4). The stimulation strength required to elicit these responses were similar to those required to activate low-threshold proprioceptors, and below that for C-fiber activation. A possible explanation for this phenomenon could be that small numbers of proprioceptive afferents are misrouted in the Saph nerve during development. To test whether the monosynaptic MN connections arising from the Saph nerve were proprioceptive in origin, experiments were conducted in genetically altered mice. We first utilized an Er81 KO mouse, in which proprioceptive afferents fail to project into the ventral horn largely eliminating their monosynaptic connections with MNs (Arber et al., 2000). Interestingly, stimulation of the Saph nerve in Er81 mutants still elicited monosynaptic responses in MNs, similar to that observed in control mice. Secondly, we utilized another mouse model (Parvalbumin-Cre; Munc18-1 flox/flox), in which synaptic transmission of proprioceptive axons are blocked, but axonal projections into the ventral horn remain intact, resulting in responses upon Saph nerve stimulation that were very similar to those observed in Er81 mutants and in control animals.

Lastly, we performed anatomical tracing experiments, in which central axons of afferents projecting in the Saph nerve are retrogradely labeled with fluorescent dextran. We show that a limited number of Saph nerve afferents project into the ventral horn and are in close proximity to MNs. In addition, these axons do not express parvalbumin (a protein found in proprioceptive sensory neurons), again suggesting these afferents are unlikely to be proprioceptive. Taken together these data strongly suggest that the subset of Saph afferents that monosynaptically contact spinal MNs are non-proprioceptive and may represent a novel pathway by which cutaneous sensory information can directly modulate MN firing activity.

Disclosures: P.M. Sonner: None. **M.A. Dallman:** None. **D.R. Ladle:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Postdoctoral Fellowship from the Vicerrectoría de Investigación, Pontificia Universidad Católica de Chile

Title: Stereological and immunohistochemical analysis of inhibitory and excitatory innervation of the cranial motor nuclei that control extraocular muscles in the mouse

Authors: *N. M. DOIG, V. LUCO, J. CORTÉS, A. OÑATE, P. HENNY
Lab. of Neuroanatomy, Pontificia Univ. Católica De Chile, Santiago, Chile

Abstract: The extraocular muscles are responsible for movements of the eyes and are innervated by motoneurons of the oculomotor (III), trochlear (IV) and abducens (VI) cranial nuclei. Extraocular muscles have distinctive patterns of activity during sleep, relative to other groups of cranial muscles, in that they become increasingly active during the rapid eye movement (REM) stage of sleep. The aim of this study is to examine excitatory and inhibitory innervation of the cranial motor nuclei which control eye movement using immunohistochemical and stereological methodology in wild-type adult mice, as this specific pattern of activity during REM sleep could be related to the ratio of inhibitory to excitatory innervation. In order to achieve this we

examined specific markers for inhibitory presynaptic terminals: the vesicular inhibitory amino acid transporter (VIAAT) and the glycine transporter subtype 2 (GlyT2) as well as excitatory presynaptic terminals using the vesicular glutamate transporter subtypes 1 (VGluT1) and 2 (VGluT2). These markers were revealed for light microscopy using a DAB-Nickel reaction and the sections are then counter-stained using Nissl. Using the optical fractionator method, we can estimate numbers of terminals within the IIIrd, IVth and VIth nuclei. Using this data we can assess the total number of excitatory and inhibitory terminals, as well as their relative ratio of innervation. Preliminary data shows the relative densities of terminals in the abducens (VI) nucleus are mainly excitatory with 76% positive for a glutamatergic marker (VGluT1 or VGluT2; n=2) and 24% positive for a marker of inhibitory terminals (VIAAT; n=3). Current analysis is underway to establish the number and proportion of terminals in the IIIrd and IVth nuclei. We are extending our analyses to examine the innervation of these nuclei by neuromodulators using antibodies for the vesicular monoamine transporter subtype 2 (VMAT2) which labels dopaminergic, serotonergic, noradrenergic, and histaminergic terminals; and also for orexinergic terminals (orexin A). The results from this study will provide detailed information about innervation to the motor nuclei which control the activity of extraocular muscles in wild-type mice. This data will then be able to be compared to similar data from other cranial motor nuclei which show different levels of activity during sleep-wake states. Comparison with non-oculomotor cranial motor groups will also be relevant in relation to neurodegenerative diseases that affect motoneuron survival, such as amyotrophic lateral sclerosis, in which oculomotor neuronal groups have been documented to be spared from early degeneration.

Disclosures: **N.M. Doig:** None. **V. Luco:** None. **J. Cortés:** None. **A. Oñate:** None. **P. Henny:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.06/HH8

Topic: D.13. Motor Neurons and Muscle

Support: German Science Foundation CRC 870

German Science Foundation Research Training Group 1091

Graduate School of Systemic Neurosciences Munich

Title: Task-specific activation of extraocular motoneurons in Xenopus laevis

Authors: J. M. SCHULLER[1], A. G. KNORR[2], *S. GLASAUER[2], H. STRAKA[1]
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Abstract: During vertebrate locomotion, visuo-vestibular reflexes and intrinsic efference copy-driven extraocular motor discharge minimize retinal image slip through compensatory eye movements. The presence of different subsets of extraocular motoneurons with a wide range of morpho-physiological properties is essential to generate spatio-temporally adequate eye movements. The horizontal optokinetic reflex with its alternating slow following and fast resetting phases offers an ideal motor behavior to study the potential recruitment of task-specific neuronal units. Here, we studied the optokinetic reflex, functional organization of extraocular motoneurons and influence of active locomotion during optokinetic stimulation in semi-intact preparations of larval Xenopus laevis with an intact visual system and an isolated brainstem/spinal cord. Eye movements, evoked by velocity step and sinusoidal optokinetic stimulation (0.2-50°/s; 0.032-1 Hz) with a vertically striped drum, were captured with a camera at a rate of 50 Hz and quantified by computerized video analysis. Typical responses of pre-metamorphic Xenopus tadpoles during optokinetic step stimulation consisted of slow phases with a horizontal ocular motor range of ~50° and an average gain of ~0.45 at a constant stimulus velocity of 0.5°/s, interrupted by resetting fast phases with velocities of ~300°/s. Simultaneous motion recordings of one eye and multiple-unit discharge of the lateral or medial rectus nerve on the other side during optokinetic stimulation and spontaneous fictive locomotion allowed evaluating the interaction between intrinsically generated motor commands and optokinetic sensory feedback signals. Single spike analysis and determination of activation thresholds, response properties and discharge regularity confirmed a differential recruitment of individual motoneurons and task-specific contribution during extraocular motor behaviors. Preferential activation of individual motor units was observed for slow and fast phase components during the optokinetic reflex as well as for locomotion-related spino-extraocular coupling. While the latter behavior recruited phasic motor units with large spikes, optokinetic slow phases were mediated by low threshold, persistently discharging units with small spikes. The task-specificity of motor units demonstrated only limited integration of intrinsic locomotor efference copies and image motion-derived visual signals at the level of individual motoneurons and rather suggests that part of the effective motor behavior results from a separate activation of muscle fibers by the respective sets of extraocular motoneurons.

Disclosures: J.M. Schuller: None. **S. Glasauer:** None. **H. Straka:** None. **A.G. Knorr:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Program#/Poster#: 247.07/HH9

Topic: D.13. Motor Neurons and Muscle

Support: German Science Foundation CRC 870

Graduate School of Systemic Neurosciences Munich

Title: Vestibulo-ocular reflexes are mediated by functionally distinct subgroups of extraocular motoneurons

Authors: H. DIETRICH[1], S. GLASAUER[2], *H. STRAKA[1]
[1]LMU Munich - Biocenter Martinsried, Planegg, Germany; [2]LMU Munich, Dept. Neurology, Klinikum Grosshadern, Munich, Germany

Abstract: Vestibulo-ocular and optokinetic reflexes ensure spatio-temporally precise gaze stabilization during passive or self-generated head and body motion. Effective conjugate eye movements in the horizontal plane are generated by synergistic contractions of the lateral and medial rectus eye muscles, innervated by abducens and oculomotor motoneurons, respectively. Task-specific motoneuronal subtypes integrate visuo-vestibular inputs into reactive motor commands that allow a range of dynamically different eye movements from slow, tonic to fast, twitch-like contractions of functionally co-adapted extraocular muscle fiber types. Here, we studied this neuronal diversity in a morpho-physiological approach including the synaptic pharmacology of abducens motoneurons in Xenopus laevis tadpoles. Experiments were performed on isolated semi-intact preparations, which allow in-vitro manipulations within the intact neuronal vestibulo-ocular circuitry from the sensory periphery to the ocular motor plant. Multiple-unit extracellular recordings of the lateral rectus nerve close to its target muscle insertion site during natural stimulation of the vestibular endorgans and subsequent spike shape analysis confirmed the presence of two major subgroups of abducens motoneurons. During horizontal sinusoidal rotation, one group of motoneurons exhibited responses with amplitudes and phase relations independent of stimulus frequency, thus precisely encoding angular head velocity. A second group of motoneurons displayed responses with magnitudes and dynamics that systematically varied with stimulus velocity and frequency, thereby additionally transmitting information about the peak acceleration of head motion. Common to both subgroups were motor units with a range of activation thresholds for rotational stimuli, generating a continuum of tonic to phasic response patterns, respectively. Pharmacological blockade of excitatory neurotransmission of vestibular neurons onto abducens motoneurons by focal application of NMDA and AMPA-receptor antagonists into the abducens nucleus revealed a differential expression of glutamate receptor subtypes that supports a dual motoneuronal organization. In

addition, blocking glycinergic transmission by focal injections of strychnine suppresses modulated inhibitory inputs from the ipsilateral vestibular nucleus. These findings confirm the notion of separate functional subgroups of extraocular motoneurons that differ from each other in several pharma-physiological properties and act in concert to ensure image stabilization over a large range of motion dynamics.

Disclosures: H. Dietrich: None. **S. Glasauer:** None. **H. Straka:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.08/HH10

Topic: D.13. Motor Neurons and Muscle

Support: Quest Diagnostics

Title: A method to detect silent carriers of spinal muscular atrophy by analysis of human sperm

Authors: M. C. EVANS, M. C. GALLEN, N. J. ROBICHAUD, D. A. HILL, C. D. BRAASTAD, *J. J. HIGGINS, Jr.
Quest Diagnostics, Worcester, MA

Abstract: Spinal muscular atrophy (SMA) is the second most common fatal autosomal recessive disorder after cystic fibrosis, affecting approximately 1 in 6,000 to 10,000 live births. The disorder is characterized by hypotonia, proximal muscle weakness, and respiratory distress due to degeneration of motor neurons in the spinal cord. SMA is caused by mutations in the survival motor neuron 1 (*SMN1*) gene. A nearly identical homolog of the *SMN1* gene, *SMN2*, lies in an inverted orientation in cis- with *SMN1* on chromosome 5q. The molecular diagnosis of SMA is accomplished through the detection of homozygous deletions of *SMN1*. The presence of *SMN2* and the varying numbers of both *SMN1* and *SMN2* complicate molecular testing, but the sequence differences between the two genes allow them to be distinguished. More than 95% of SMA patients have a homozygous deletion of *SMN1* exon 7. The occurrence of two copies of the *SMN1* gene on one allele and zero copies on the other (2+0 genotype or silent carrier) is recognized as a source of false-negative carrier testing results. This genotype is present in about 4% of the carrier population but varies considerably between different ethnic groups. To identify null alleles for *SMN1, w*e used haploid human sperm cells in an end-point limiting dilution assay. The results show the ability to detect sperm that are null for *SMN1* copies. This study addresses a

key limitation in SMA carrier screening and is a step toward using diploid cells to identify silent carriers of SMA.

Disclosures: **M.C. Evans:** A. Employment/Salary (full or part-time):; Quest Diagnostics. **M.C. Gallen:** A. Employment/Salary (full or part-time):; Quest Diagnostics. **N.J. Robichaud:** A. Employment/Salary (full or part-time):; Quest Diagnostics. **D.A. Hill:** A. Employment/Salary (full or part-time):; Quest Diagnostics. **C.D. Braastad:** A. Employment/Salary (full or part-time):; Quest Diagnostics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Quest Diagnostics. **J.J. Higgins:** A. Employment/Salary (full or part-time):; Quest Diagnostics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Quest Diagnostics.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.09/HH11

Topic: D.13. Motor Neurons and Muscle

Title: Active intrinsic properties differ between murine cranial and spinal motoneurons

Authors: ***M. A. TADROS**[1], A. J. FUGLEVAND[2], A. M. BRICHTA[1], R. J. CALLISTER[1]
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Abstract: Motoneurons differ in the motor behaviours they control and their vulnerability to disease. For example, cranial motoneurons such as hypoglossal motoneurons (HMs) are involved in licking, suckling, swallowing, respiration, and vocalization. In contrast spinal motoneurons (SMs) innervating the limbs are involved in locomotor and postural function, tasks requiring comparatively higher loads and lower speed movements. Correspondingly, the contractile speed of tongue muscle is typically faster than that of limb muscle. Nevertheless, little is known about the relative properties of these two motoneuron pools. In this study, we used whole-cell patch clamp recording to compare the electrophysiological properties of HMs and SMs in age-matched mice. Transverse slices (300 μm thick) were obtained from the brainstem or lumbosacral spinal cord of C57Bl/6 mice (P7-10). Whole-cell recordings were made from visualized motoneurons at 23^{o}C using a KCH_3SO_4-based internal solution. Passive membrane properties were remarkably similar in HMs and SMs (n = 28 and 26, respectively). No differences were observed in input resistance, cell capacitance, and resting membrane potential. In addition, action potential (AP)

properties such as rheobase, voltage threshold, spike amplitude, and afterhyperpolarization did not differ between the two populations. In contrast, AP half-width was smaller in HMs (0.95 ± 0.03 vs. 1.49 ± 0.08 ms) and they discharged at higher frequencies in response to square step (1 s duration, 50 pA increments, 450 pA above rheobase; 31.3 ± 1.3 vs. 24.5 ± 2.5 Hz; n = 27 and 15) and triangular ramp current injection (0.3 nA/s, maximum frequency 42.2 ± 4.0 vs. 26.9 ± 2.4 Hz, n = 17 and 10). Therefore, while HMs and SMs have similar passive properties, their discharge in response to similar levels of depolarizing current differs markedly. This suggests each population possess differing suites of ion channels that allow them to discharge at rates that match the different mechanical properties of the muscle fibers underlying their distinct motor functions.

Disclosures: **M.A. Tadros:** None. **A.J. Fuglevand:** None. **A.M. Brichta:** None. **R.J. Callister:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

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Program#/Poster#: 247.10/HH12

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant R01-NS26539

NIH Fellowship F32-NS083099

Title: Abnormal migration and functional architecture in zebrafish facial motor neurons

Authors: *K. L. MCARTHUR, J. R. FETCHO
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Abstract: Developing neurons often arise in one location in the brain and then migrate to another. Normal migration supports proper circuit formation; thus, abnormal migration can be associated with abnormal neural activity and behavior. However, studies of cellular migratory mechanisms have described genetic mutations that disrupt migration but spare behavior - such that mutant animals with unexpected neuronal positioning survive and behave grossly like their wild type (WT) counterparts. This raises the possibility that the developing brain can, under some circumstances, compensate for unexpected cell locations in order to maintain normal function. In recent work with zebrafish larvae, we have revealed the functional properties of WT

facial branchiomotor neurons (FBMNs), which undergo a dramatic caudal migration in the hindbrain early in development. We now present preliminary evidence that at least some of the FBMN functional architecture is preserved in two lines of mutant zebrafish, where these cells fail to migrate caudally. Both calcium imaging with GCaMP5 and whole-cell recordings indicate that mutant FBMN activity is at least qualitatively similar to WT FBMN activity. Although further studies are needed to rigorously determine if more subtle differences exist, this result is not altogether surprising. FBMNs are critical for feeding and respiration, and mutant zebrafish are still viable - gross disruption of FBMN activity would likely kill the fish. Further, using transgenic expression of the photoconvertible protein Dendra, we see that mutant FBMNs adopt a dorsoventral age topography similar to what we have found in WT fish. Thus, FBMNs can arrange themselves by age regardless of which rhombomere they ultimately occupy. However, dye-filled mutant FBMN dendritic arbors ramify in different rhombomeres - though their position relative to the soma generally matches what we have seen in the WT. Therefore, it seems that mutant FBMNs can join functional circuits in ways that preserve gross functional activity, despite the fact that their dendrites are in the wrong place along the rostrocaudal axis. Perhaps key ascending and descending inputs from respiratory and feeding-related centers are able to find target FBMNs at any position along the rostrocaudal hindbrain axis, as long as age order is maintained. This work builds on current knowledge of the fundamental principles guiding circuit formation and has exciting implications for our understanding of how neuronal populations can evolve to shift their location without completely disrupting behavior.

Disclosures: K.L. McArthur: None. **J.R. Fetcho:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.11/HH13

Topic: D.13. Motor Neurons and Muscle

Title: Copper nanoparticles and or ionic copper (II) causes neurotoxicity and cardiotoxicity in Zebrafish embryos

Authors: *E. CUEVAS[1], W. TRICKLER[2], M. G. PAULE[2], S. ALI[2], J. KANUNGO[2]
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Abstract: Copper oxide nanoparticles (Cu-NPs) are frequently used in medical devices, paints, fabrics or as antimicrobials. Their industrial applications may lead to the contamination of aquatic ecosystems. The toxicological and human health risks of NPs in the environment are hard to evaluate due to a lack of knowledge about the mechanisms by which NPs interact with biological systems. In this study, we investigated the toxicity of Cu-NPs (60nm) and ionic copper (II) in wild-type (WT) zebrafish embryos and *hb9-GFP* transgenic zebrafish (each *Danio rerio*, AB-strain) embryos. Here, the effects of bare Cu-NPs were compared to those seen after exposure to the mass equivalent ionic form of copper (II) ($CuCl_2$) at various concentrations (1.25-to-20 ug/ml). Toxicity was evidenced by phenotypic changes in zebrafish embryos including survival, heart rate, motor neuron development and absorptive permeability. Both Cu-NPs and $CuCl_2$ were lethal to zebrafish embryos at 20 μg/ml (within 24-hrs) and 10 μg/ml (within 48-hrs), with $CuCl_2$ being more toxic at equivalent mass concentrations. Similarly, the heart rate was significantly reduced following exposure to either Cu-NPs or $CuCl_2$ in a concentration-time dependent manner. Additionally, the embryo permeability studies showed that exposure to either Cu-NPs or $CuCl_2$ (5 ug/ml) for 24-hrs significantly increased the topical absorption of the fluorescent tracer 6-coumarin (6CM). Furthermore, embryos treated with either Cu-NPs or $CuCl_2$ (2.5 μg/ml for 48-hrs) showed a significant reduction (nearly 2-fold) in spinal motor neurons. These results indicate that both $CuCl_2$ and Cu-NPs can be toxic to zebrafish embryos causing significant neurotoxicity and cardiotoxicity at exposure levels that do not cause lethality.

Disclosures: E. Cuevas: None. **W. Trickler:** None. **M.G. Paule:** None. **S. Ali:** None. **J. Kanungo:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Topic: D.13. Motor Neurons and Muscle

Support: Secyt-UNC/FONCyT PICT-PRH-86

Title: Myelin-Associated Glycoprotein modulates programmed cell death of motoneurons during early postnatal development via NgR/p75NTR receptor-mediated activation of RhoA signaling pathway

Authors: *A. PALANDRI[1], M. V. ROZES SALVADOR[1], J. WOJNAKI[1], A. L. VIVINETTO[1], R. L. SCHNAAR[2], P. H. H. LOPEZ[1]
[1]Inst. De Investigacion Mercedes Y Martin Ferre, Cordoba, Argentina; [2]Pharmacol. and Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Myelin-Associated Glycoprotein (MAG) is a minor constituent of the nervous system selectively expressed at the periaxonal layer of myelinated axons. By engaging multiple axonal receptors, including Nogo-receptors (NgRs), MAG exerts a nurture effect on axons it ensheaths. Pharmacological activation of NgRs has a modulatory role on p75NTR-dependent postnatal apoptosis of motoneurons (MNs). However it is not clear whether this observation reflects a physiological role of NgRs in MNs development. NgRs are part of a multimeric receptor complex which includes p75NTR, Lingo-1 and gangliosides. Upon ligand binding, this multimeric complex activates RhoA/ROCK signalling in a p75NTR-dependent manner. The aim of this study was to analyze a possible modulatory role of MAG on MNs apoptosis during postnatal development. A time course study showed that Mag-null mice suffer a loss of MNs during the first postnatal week. Also these mice exhibited increased susceptibility in an animal model of p75NTR-dependent MNs apoptosis induced by nerve-crush injury, which was prevented by treatment with a soluble form of MAG (MAG-Fc). The protective role of MAG was further confirmed in *in vitro* models of p75NTR-dependent MN apoptosis including the MN cell line MN1. Lentiviral expression of shRNA sequences targeting NgRs on these cells abolished protection by MAG-Fc. Analysis of RhoA activity using a FRET-based RhoA biosensor showed that MAG-Fc activates RhoA. Pharmacological inhibition of p75NTR/RhoA/ROCK pathway or overexpression of a p75NTR mutant lacking binding to RhoA completely blocked MAG-Fc protection against apoptosis. The role of RhoA/ROCK signaling was further confirmed in the nerve-crush model, where pre-treatment with Y27632 (ROCK inhibitor) blocked pro-survival effect of MAG-Fc. Overall these findings identify a new protective role of MAG as a modulator of apoptosis of MNs during postnatal development by a mechanism involving p75NTR/RhoA/ROCK signaling pathway. In addition our results highlight the relevance of the nurture/protective effect of myelin on neurons.

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Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Topic: D.13. Motor Neurons and Muscle

Support: NHMRC APP1065884

Title: Developmental changes in dendritic morphology and distribution of excitatory and inhibitory synaptic inputs of mouse hypoglossal motor neurons

Authors: *M. C. BELLINGHAM, R. KANJHAN, M. J. FOGARTY, P. G. NOAKES
Univ. of Queensland, Brisbane, Australia

Abstract: Hypoglossal motor neurons (XII MNs) play a vital role in suckling, eating, vocalization and breathing. XII MNs (n=103) in brainstem slices from C57Bl6 mice (n=36) aged embryonic day 17 (E17) to postnatal day 28 (P28) were filled with Neurobiotin, labeled with Cy3-avidin, and then immunohistochemically labeled with antibodies against pre- and post-synaptic marker proteins at glutamatergic (VGLUT2 and PSD95) and GABAergic (VGAT and GABAα1) neurochemical synapses. The morphology of single XII MNs was digitally imaged and synaptic marker immunofluorescence was processed to visualize postsynaptic puncta within the XII MN, and presynaptic puncta within 1 μm of these postsynaptic puncta. The dendrites of XII MNs from areas thought to innervate different tongue muscles showed similar morphology in most, but not all, features. Morphological properties of XII MNs were established prior to birth, not differing between E17-18 and P0-1. MN somatic volume gradually increased for the first 2 weeks post-birth. The complexity of dendritic branching and dendrite length of XII MNs increased throughout development. MNs in the ventromedial XII motor nucleus, likely to innervate the genioglossus, frequently (42%) had dendrites crossing to the contralateral side at all ages, but their number declined with postnatal development. Unexpectedly, dendritic spines were found in all XII MNs at all ages, and were primarily localized to XII MN somata and primary dendrites at E18-P4, increased in distal dendrites by P5-P8, and were later predominantly found in distal dendrites. Dye-coupling between XII MNs was common from E18 to P7, but declined strongly with maturation after P7. Axon collaterals were found in 20% (6 of 28) of XII MNs with filled axons; collaterals terminated widely outside and, in one case, within the XII motor nucleus. Glutamatergic neurochemical synapses increased in density in distal dendrites with age, while GABAergic neurochemical synapses were common on soma and proximal dendrites at all ages. These results reveal new morphological features of mouse XII MNs, and suggest that dendritic projection patterns, spine density and distribution, dye-coupling patterns, and excitatory and inhibitory synaptic inputs show specific developmental changes in mice. Funded by Australian NHMRC grant APP1065884.

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Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Support: Italian Ministry of Instruction, University and Research, PRIN HandBot project (CUP: B81J12002680008)

Italian Ministry of Health, NEMESIS project

Neurohand project (DTB2 Filas)

Title: Analysis of efferent microneurography recordings for ENG-driven hand neuroprosthesis

Authors: *F. M. PETRINI[1,2,3], S. RASPOPOVIC[1,5], J. RIGOSA[1,5], M. CAPOGROSSO[1,5], F. GIAMBATTISTELLI[4], L. ZOLLO[3], E. GUGLIELMELLI[3], S. MICERA[1,5]
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Abstract: Few attempts are in the literature to control a hand prosthesis by means of electroneurographic signals (ENG). However, currently there are still no reliable ENG-driven prosthesis. For this reason, it is important to access the peripheral nerves of humans without surgery in order to investigate the ability to decode features of neural control of the hand motion. Microneurography (MNG) is an established minimally invasive technique for the study of human peripheral nervous system (PNS). However the MNG studies are mainly focused on the afferent PNS, while investigations on the efferent side are lacking. MNG studies on the efferent PNS could potentially allow to identify the best techniques for processing and decoding the ENG signals. We conducted MNG sessions on two healthy volunteers and acquired efferent signals from their median nerve along with the electrical activity of the hand muscles (EMG) innervated by it. In particular, in order to seek for efferent signals, a neurologist placed the electrode in the fascicles that presented ENG correlated with the hand EMGs but not with muscles stretch or skin touch. This expert overview was necessary to minimize and eventually avoid the acquisition of tactile or proprioceptive signals. During the trials the subjects were asked to execute several grasps/movement with hand and wrist, at different forces and velocities while MNG was acquired. The recorded signal was wavelet denoised to find the occurrences of the spikes, which were subsequently projected in the principal component space and clustered with a superparamagnetic classifier. As a result of the sorting, we found a population of 10 neurons

with a firing rate in the range 0-10 Hz. We discovered that from the ENG signal it is possible to detect the type of grasp/movement performed by the subjects (e.g., tridigital, cylindric grasp or rest): indeed, different neuron groups show distinct firing activity during particular grasps. We found, also, that the neurons firing rate have a strong relation with the force exerted during the grasps. This relation has been modeled mathematically. Finally, we observed that when identical tasks are executed at increasing velocities, the average (positive) derivative of the neurons firing rate has a significant increase. These results represent an important neurophysiological insight into the way the PNS decodes the control of different movements, their velocity and force, supporting the idea that MNG could represent a method to deeply investigate the way the motoneurons control the volitional limbs motion.

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Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

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Topic: D.13. Motor Neurons and Muscle

Support: Fondecyt grant N° 1110433

Anillo grant N° ACT1109

Title: Stereological estimation of the number of cells and VGluT1, VGluT2 and VIAAT immuno-positive varicosities in the trigeminal, facial and ambiguus motor nuclei of the mouse brain

Authors: *P. HENNY, M. FAUNES, S. FERNANDEZ, A. ONATE
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Abstract: Mammalian branchiomeric motor nuclei are involved in vital non-locomotive behaviours such as suckling, mastication, swallowing, whisking, facial expression and the production of vocalizations. Trigeminal (Vm), facial (VIIm) and ambiguus (Amb) brainstem motor neurons innervate the jaw, facial, and pharynx/larynx/oesophagus muscles, respectively. The activity displayed by these neurons is controlled by primary excitatory afferents and various second order sensory brainstem nuclei, as well as premotor neurons from the medullary, pontine

and midbrain reticular formation, most of which are glutamatergic GABAergic or glicinergic. In order to clarify the influence that these neurotransmitters have on controlling the activity of neurons of Vm, VIIm and Amb we estimated the number of varicosities immunopositive for the vesicular glutamate transporter subtypes 1 (VGluT1) and 2 (VGluT2) and for the vesicular transporter for GABA and glycine (vesicular inhibitory amino acid transporter, VIAAT, also known as VGAT), using an unbiased stereological technique, the optical fractionator. To further examine differences in density, we also estimated the volume and cell number for these nuclei and their subdivisions. According to our results, Vm, VIIm and Amb contain ~1,200, ~3,400 and ~800 cells, respectively. Cells in Amb are much more densely packed than in Vm and in VIIm. These nuclei exhibit different patterns of glutamatergic and GABA/glycinergic innervation. Vm receives the highest proportion of VGluT1+ varicosities among the other cranial nuclei, most likely originating from trigeminal mecencephalic nucleus afferents carrying propioceptive activity from jaw closing muscles, associated with the role of Vm in mastication. VIIm shows a surprisingly higher density by VGluT2+ and VIAAT+ varicosities than Vm and Amb. This high degree of innervation likely reflects the numerous and various inputs needed for the VIIm to take part in the wide range of complex behaviours in which it is involved. The high cell density in the Amb, along with a relatively low density of terminals may reflect its more crucial involvement in stereotyped and reflexive type of movements.

Disclosures: **P. Henny:** None. **M. Faunes:** None. **S. Fernandez:** None. **A. Onate:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.16/HH18

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant NS062200

NIH Grant NS069616

Title: Role of subthreshold Ca-dependent potassium currents in motoneuron firing properties

Authors: *R. H. LEE[1,2], C. S. MITCHELL[1]
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Abstract: While there is extensive observation and theory regarding the role of Ca-dependent potassium (i.e. SK channel) currents during motoneuron firing, little has been done on the sub-threshold aspect of this current. We have recently hypothesized that this current plays a small but critical role in regulating firing rate stability and input-firing rate (F-I) gain by interacting directly with spike initiating persistent sodium currents. In the work presented here, we examine this hypothesis both experimentally and theoretically. Experimentally, we observe subthreshold outward currents consistent with SK activation and that these currents vary in a manner consistent with decreasing the F-I gain as predicted. On the theoretical side, we demonstrate how this current stabilizes F-I gain to prevent excitotoxic excursions in firing rate as the motoneuron and its innervated muscle fibers fatigue.

Disclosures: R.H. Lee: None. **C.S. Mitchell:** None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.17/HH19

Topic: D.13. Motor Neurons and Muscle

Support: Edmonton Civic Employees.

University of Saskatchewan

Title: Spinal nerve root and rootlet stimulation

Authors: *J. A. NORTON
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Abstract: The spinal nerve roots remain functional after a spinal cord injury, and respond to electrical stimulation as well as conducting reflexes. In a previous study we implanted electrodes on the anterior nerve roots between L2 and S2 in individuals with chronic, complete spinal cord injuries. The large number of muscles innervated from each nerve root, often with non-synergistic actions meant that we were unable to generate functional standing or stepping, although good exercise through cycling was achieved. In this study we investigated the innervation pattern of nerve rootlets in a rodent model. The rootlets are the bundles of fibres that exit the spinal cord and coalesce to form the nerve roots. Electrical stimulation, under inhalational anesthesia was performed with multi-channel EMG recordings in the hind limbs.

Using a constant current stimulator (and 200μs pulse widths) we evaluated the thresholds for eliciting the first and second muscle responses. We also looked at whether these muscles were synergistic, and where they were recorded the threshold for non-synergistic muscles. Our previous studies looked at contralateral thresholds and defined these as crosstalk and identified k as the ratio between the 2 thresholds. We use the same parameter for both contralateral thresholds and ipsilateral ratios between 2 muscle responses. Universally we found across all rootlets (221) that there was a k value than we saw on the roots (62) for both ipislateral thresholds, non-synergistic thresholds and contralateral thresholds. Electrical stimulation of nerve rootlets appears to be more selective, and offer more degrees of control than nerve root stimulation. This offers the potential for this type of stimulation to be a part of future multi-modal neural prosthesis.

Disclosures: J.A. Norton: None.

Poster

247. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 247.18/HH20

Topic: D.13. Motor Neurons and Muscle

Support: NIH R24 HD50821-07

Title: Estimating the time course of excitatory postsynaptic potential in human motoneuron

Authors: *X. HU[1,2], N. L. SURESH[2], B. JEON[2], W. Z. RYMER[2]
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Abstract: The ability to estimate the time course of excitatory postsynaptic potential (EPSP) permits a systematic characterization of human motoneuron properties. The rising edge of the EPSP is typically estimated from the discharge probability of motoneurons. However, the estimation of the falling phase of the EPSP is insecure because of the absence of discharges due to afterhyperpolarization. In this study, we proposed a more rigorous approach estimating the falling edge of the EPSP using sub-threshold paired electrical stimulations. Specifically, we applied a conditioning electrical stimulation of the median nerve innervating the flexor carpi radialis muscle. The conditioning stimuli were set at a sub-threshold level (i.e., no H-reflex was recorded) to avoid afterhyperpolarization. Test stimuli with the same amplitude were applied with a random latency ranging from 4 to 60 ms with respect to the conditioning stimuli. The

probability of triggering H-reflexes from the test stimuli reflects the time course of the EPSP from the conditioning stimuli. The impulse function was then used to quantify the falling phase of the EPSP. Using this approach, we were able to capture the time course of EPSP in motoneurons non-invasively, which can be used as a potential tool to evaluate the properties of human motoneurons in healthy and disease states.

Disclosures: X. Hu: None. **W.Z. Rymer:** None. **N.L. Suresh:** None. **B. Jeon:** None.

Poster

248. Small Networks and Plasticity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 248.01/HH21

Topic: D.15. Basal Ganglia

Support: NIH Grant K99 NS0806524

Title: Synaptic plasticity of two genetically distinct populations of GPe neurons after dopamine depletion

Authors: *K. J. MASTRO[1,2], H. A. HOLT[3], A. H. GITTIS[3,2]
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Abstract: The basal ganglia (BG) are set of subcortical structures critical for movement and cognition. The external segment of the globus pallidus (GPe) is a central nucleus in the BG and contains a heterogeneous population of neurons. Previously, we identified two genetically distinct populations that express Parvalbumin (PV) or Lim homeobox 6 (Lhx6) protein in healthy transgenic mice. The populations differ in their topographic organization, intrinsic physiology and axonal projections. These results provide tools to study cell-type specific roles of the GPe in BG function during health and disease. Here, we utilized transgenic mouse lines to assess changes in axonal projections and synaptic physiology under control (saline) and dopamine depleted (6-hydroxydopamine) conditions. First, we identified anatomical targets and projections densities of each GPe cell-type by expressing EYFP under viral transfection in Lhx6-cre or PV-cre mice treated with saline or 6-OHDA. Under conditions of low dopamine, downstream BG targets were differentially altered in Lhx6 and PV populations. Specifically, there was a significant decrease in projection densities of the PV-GPe population to the subthalamic nucleus (STN) and parafascicular nucleus but no change in Lhx6-GPe projections to these areas. To

identify alterations in synaptic physiology, we recorded mini inhibitory spontaneous currents (mIPSCs) in acute brain slices from Lhx6 or PV reporter mice. Under control conditions, mIPSC frequency was greater onto Lhx6-GPe than PV-GPe neurons. After dopamine depletion, mIPSC frequency decreased onto Lhx6-GPe neurons and increased onto PV-GPe neurons. To investigate excitatory synaptic inputs, we focused on the major source of excitation from the STN which has been heavily implicated in disease. Here, we recorded the evoked synaptic response of the STN inputs onto the two GPe populations to measure the amplitude and paired pulse ratio. The amount of facilitation in the two populations was altered in the disease condition. Specifically, Lhx6-GPe neurons became more depressive in conditions of low dopamine. The anatomic and synaptic changes that occur after dopamine depletion suggests a reorganization of the circuit that may contribute to the complex symptomology associated with basal ganglia dysfunction.

Disclosures: K.J. Mastro: None. **H.A. Holt:** None. **A.H. Gittis:** None.

Poster

248. Small Networks and Plasticity

Location: Halls A-C

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Topic: D.15. Basal Ganglia

Support: IMPULSA03

DGAPA-UNAM IN202814, IN202914

CONACYT 154131

Title: Differential gabaergic contribution during corticostriatal activation

Authors: *A. B. GARCÍA[1], R. HENÁNDEZ-MARTÍNEZ[1], J. E. PÉREZ-ORTEGA[1], V. G. LÓPEZ-HUERTA[2], J. BARGAS[1], E. GALARRAGA[1]
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Abstract: Feed-back inhibition or that arising among striatal projection neurons (SPNs) axon collaterals has been found to be profoundly disturbed during dopamine (DA) depletion in a rodent model of Parkinsonism (Lopez-Huerta et al., 2013). We intend to initiate a similar study with feed-forward inhibition and compare the two. We recorded SPNs in an *in vitro* corticostriatal slice preparation while sensorimotor cortex was stimulated with a concentric

bipolar electrode (50 μm in diameter). Orthodromic corticostriatal synaptic responses were evoked after a train of four pulses of 0.1 ms duration and of increasing intensity. We performed both electrophysiological (whole cell recordings) and calcium imaging recordings (fluo-8) to follow the activity of one to dozens of cells simultaneously with single cell resolution. Corticostriatal axons make convergent mono- and polysynaptic contacts with both SPNs and interneurons (INs). The GABAA receptor antagonist, bicuculline (10 μM) increased orthodromic response in direct SPNs (dSPNs) and decreased the same response in indirect SPNs (iSPNs) along a whole series of stimulus intensities. The reason for this discrepancy is explained in an accompanying poster. Calcium imaging allowed the acquisition of neuronal recruitment curves during increasing stimulus intensities before and after bicuculline application. Blockade of GABAA receptors tripled the number of activated neurons for the same stimulus indicating that cortical transmission reaches SPNs in parallel to interneurons so that SPNs integrate a convergence of cortical excitation and INs inhibition thus allowing quantify the amount of feed-forward inhibition as compared to feed-back inhibition. The goal is to compare these two classes of inhibition in control and DA depleted circuits since it is known that feed-back inhibition is lost during dopamine deprivation while feed-forward inhibition is enhanced. The question is: to what extent a disturbance in the circuit due to DA depletion arises from these unbalanced inhibitory entries?

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Poster

248. Small Networks and Plasticity

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Topic: D.15. Basal Ganglia

Support: IMPULSA03

DGAPA-UNAM IN202814, IN202914

CONACYT 154131

Title: Striatal microcircuit plasticity

Authors: *E. LARA-GONZALEZ, M. DUHNE, J. BARGAS
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Abstract: Learning and memory theories propose: 1) that different types of long term synaptic plasticity such as LTP and LTD are capable to shape the synaptic weights of neuronal microcircuits, and 2) That these changes in synaptic weights induce observable changes in functional connectivity and therefore in circuit trajectories for the preferential flow of activity. In addition, for this to happen, neuronal elements conforming the ensembles or neuron pools conforming the network have to recycle. In order to observe whether those phenomena can be revealed in the striatal microcircuit we used dynamic calcium imaging techniques to record the activity of dozens of neurons simultaneously, with single cell resolution, while applying plasticity protocols. Striatal microcircuits consist in the presence of spontaneous peaks of neuronal co-activation (network states) which exhibit recurrence, alternation, and reverberant behavior (Carrillo-Reid et al. 2008). After a plasticity protocol (given in the cortex), we observed that control network states changed their composition, functional connectivity and number of neuronal elements. Previously silent neurons became active, previously active neurons became silent and neurons from one network state migrated to other network state giving as a result a reconfiguration of the microcircuit. We infer that these are the circuit level analogues of the change in synaptic weights and correspond to the circuit correlates of a forming memory trace. Clearly, training stimulus generates a new microcircuit using previous and new neurons and connections while discarding others, suggesting that on coming memory traces are a combination of new connections with previously existing ones.

Disclosures: E. Lara-Gonzalez: None. **M. Duhne:** None. **J. Bargas:** None.

Poster

248. Small Networks and Plasticity

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Topic: D.15. Basal Ganglia

Support: IMPULSA03

DGAPA-UNAM IN202814, IN202914

CONACYT 154131

Title: Network theory applied to living neural circuits distinguish between normal and pathological conditions

Authors: *J. E. PÉREZ-ORTEGA, M. DUHNE, E. LARA, V. PLATA, D. GASCA, A. HERNÁNDEZ-CRUZ, E. GALARRAGA, J. BARGAS
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Abstract: By using whole-cell recordings, dynamic calcium imaging to visualize the activity of dozens of neurons simultaneously with single cell resolution, and network theory algorithms, the functional configuration of the striatal microcircuit was revealed. The network was visualized as a graph in which the nodes are individual neurons and correlated firing between them represents the links. Differences in activity were used to distinguish hierarchical architectures. It is shown that network topology satisfies a "small world" configuration (Watts and Strogatz, 1998) in that the circuit is highly clustered —it has small communities—, like a regular lattice, and still have a small characteristic path length —or high efficiency— as a random graph. Degree distribution (links per node) could be fitted by a "power law" denoting the hierarchical configuration, where "hub-nodes" improve efficiency by interconnecting different communities conformed by neuron sets. Whole-cell recordings disclosed interneurons as "hub-nodes". This topology depended on corticostriatal feed-forward activation of "hub-nodes" because the decorticated striatal microcircuit failed to keep its hierarchical structure, decreased its resilience, and increased its characteristic path length, diminishing its efficiency. Interestingly, a similar change in circuit topology was found when the striatal network was deprived of dopamine using the 6-OHDA rodent model of Parkinson´s disease. In addition, inverse functional configurations were found in a rodent model of L-DOPA induced dyskinesia. Nevertheless, network measurements suggested that both the Parkinsonian and the dyskinetic microcircuits significantly decreased their efficiency and the slope parameter of their degree distributions. In conclusion, change in hierarchical architecture that causes failures in "hub-nodes" connectivity is one main characteristic of pathological circuits.

Disclosures: J.E. Pérez-Ortega: None. **M. Duhne:** None. **E. Lara:** None. **V. Plata:** None. **D. Gasca:** None. **A. Hernández-Cruz:** None. **E. Galarraga:** None. **J. Bargas:** None.

Poster

248. Small Networks and Plasticity

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NIGMS/NIH PRAT Training Program GM000002 07

Title: Motor deficits induced by a selective loss of dopamine D2 receptors in striatal indirect pathway neurons

Authors: *J. C. LEMOS[1], A. R. KAPLAN[1], D. M. FRIEND[2], J. H. SHIN[1], M. RUBINSTEIN[3], A. V. KRAVITZ[2], V. A. ALVAREZ[1]
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Abstract: The direct and indirect pathways are the two main outputs from the striatum that control motor output in complementary and sometimes opposite ways. GABAergic medium spiny neurons that express D1 receptors form the direct pathway (dMSNs) and those expressing D2 receptors (D2Rs) form the indirect pathway (iMSNs). D2Rs on iMSNs may have a critical role in regulating basal striatal circuit function and motor behavior, yet testing these hypotheses have been difficult using conventional pharmacological techniques because D2Rs are present on several different cell types in the striatum. We generated a cell-specific D2R knockout mice that lacks D2R selectively in iMSNs, referred to here as iMSN-D2 KO mice (Drd2loxP/loxP;A2a-cre+/-). Characterization of the cre expression pattern in these mice shows 80% colocalization with a specific marker for iMSNs (met-enkephalin). iMSN-D2 KO mice display a significant suppression in locomotor activity in the homecage as well in an open field. Moreover, iMSN-D2 KOs showed impaired performance on a motor skill task as assayed by the rotarod test. However, this motor impairment was not apparent in animals place in a forced swim test suggesting that these mice are capable of movement in certain contexts. Surprisingly, despite this reduced locomotor activity, they concurrently show enhanced responsivity to novelty. These behavioral phenotypes were not due to decreased evoked dopamine release in the striatum. The observed motor deficits were rescued by selective activation of Gi coupled DREADD receptor (hM4Di) expressed in iMSNs demonstrating that activation of the Gi signaling pathway in iSPNs in critical for facilitating sustained locomotion. *In vivo* recordings made in the dorsal striatum of awake behaving iMSN-D2 KO mice revealed a decrease in firing rate of MSNs compared to controls. In an *ex vivo* slice preparation we observed increased frequency and amplitude of GABA-A mediated mIPSC in both the dorsal and ventral striatum MSNs. There are several sources of GABAergic innervation onto MSNs, one of which is GABAergic axon collaterals emanating from D2-containing iMSNs that synapse onto to neighboring dMSNs and iMSNs. Optogenetic activation of these collateral inputs is sufficient to decrease the excitability of neighboring dMSN. D2-like agonist inhibited the collateral inputs via activation of presynaptic

D2R in iMSNs of WT mice, but not iMSN-D2 Kos mice. Thus, loss of D2Rs from iMSNs abolish the inhibition of GABA release from these collateral inputs and contributes to the increase GABA tone and the reduction in locomotor activity observed in iMSN-D2 KO mice.

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Poster

248. Small Networks and Plasticity

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Topic: D.15. Basal Ganglia

Support: NSERC PDF-438487-13

Title: Presynaptic active zone proteins RIM1αβ are required for normal corticostriatal transmission and striatal-based behaviors

Authors: *D. A. KUPFERSCHMIDT, D. M. LOVINGER
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Abstract: The presynaptic scaffolding proteins RIM1αβ (RIM1) coordinate key active zone processes involved in fast neurotransmitter release and mediate various forms of presynaptic plasticity. Genetic deletion of RIM1 results in several behavioral abnormalities and learning deficits. Given the near ubiquitous neuronal expression of RIM1, the specific cell types and circuits contributing to the altered transmission and behavior seen following RIM1 deletion are unknown. Using Cre recombinase-conditional RIM1 knockout mice crossed with Emx1-Cre mice that express Cre in the vast majority of excitatory neurons in neocortex and hippocampus (Emx1:RIM1 KO), we find that loss of RIM1 in these excitatory neurons, including corticostriatal projection neurons, alters corticostriatal transmission and striatal-based behaviors. Whole-cell recordings of excitatory transmission in medium spiny neurons of the dorsolateral striatum reveal that Emx1:RIM1 KO mice show enhanced paired-pulse facilitation and reduced synaptic depression during 10-Hz trains, suggestive of impaired release probability at these synapses. Phenocopying global RIM1α KO mice, Emx1:RIM1 KO mice show elevated novelty-induced locomotion but normal motor learning on the accelerating rotarod. In contrast to global RIM1α knockout mice, Emx1:RIM1 KO mice show normal prepulse inhibition of acoustic startle. Preliminary results suggest that Emx1:RIM1 KO mice may also show enhanced

responding for food on random interval and progressive ratio schedules. Our findings implicate cortical pyramidal cells as important mediators of select behavioral impairments seen in global RIM1α KO mice, and reveal novel roles for RIM1-dependent processes in corticostriatal transmission and striatal-based behaviors.

Disclosures: D.A. Kupferschmidt: None. **D.M. Lovinger:** None.

Poster

248. Small Networks and Plasticity

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Program#/Poster#: 248.07/HH27

Topic: D.15. Basal Ganglia

Support: NIH K08

Title: Striatal indirect pathway activity is reduced in a mouse model of dyskinesia

Authors: *A. B. NELSON[1,2], D. NATHANIEL[1], A. KREITZER[1]
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Abstract: Dystonia is characterized by abnormal activation of normally opposing muscle groups. In most cases here is no neuropathological correlate, suggesting that the symptoms may be caused by abnormal circuit function, rather than frank cell loss. Some models of basal ganglia function suggest dystonia may be associated with aberrant activity in the input nucleus of the basal ganglia, the striatum. One hypothesis is that an imbalance in the striatal direct- and indirect-pathways triggers dystonia. To investigate the circuit basis of dystonia, we have employed a combination of electrophysiology and optogenetics to probe the activity of the striatum in a mouse model of dystonia. Paroxysmal nonkinesigenic dyskinesia (PNKD) transgenic mice carry the human gene mutation associated with human PNKD, and recapitulate the human phenotype, which consists of attacks of dyskinesias, including dystonia, triggered by caffeine, alcohol, or stress. Using optogenetic labeling of striatal medium spiny neurons (MSNs) forming the direct pathway (dMSNs) and indirect pathway (iMSNs) during *in vivo* single-unit recordings in freely moving mice, we have found that dyskinetic attacks are associated with profound decreases in iMSN activity, with more modest changes in direc pathway activity. Dyskinesia can be recapitulated in wild-type mice by optogenetically boosting synchronous activity in dMSNs, suggesting that an imbalance in the activity of the two pathways is sufficient

to cause dyskinesia. In addition, we find that PNKD mice show evidence of aberrant endocannabinoid-dependent long-term depression in the indirect pathway, which may be a cellular substrate for the firing changes seen *in vivo*. In support of these synaptic changes being relevant for the generation of involuntary movements, we find that blocking endocannabinoid receptors *in vivo* can prevent dyskinetic attacks. These findings suggest that an imbalance in striatal direct and indirect pathways is both necessary and sufficient to produce dyskinesias in mice.

Disclosures: A.B. Nelson: None. **D. Nathaniel:** None. **A. Kreitzer:** None.

Poster

248. Small Networks and Plasticity

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Topic: D.15. Basal Ganglia

Support: F32NS080589

P50NS071669

Title: Activity of basal ganglia output neurons in Parkinsonian mice

Authors: *C. J. LOBB, D. JAEGER
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Abstract: Changes in the firing rate and pattern of basal ganglia output neurons are hypothesized to underlie motor deficits in Parkinson's disease. To test this hypothesis, we performed behavioral and electrophysiological experiments on two groups of mice: saline-treated (control) and unilaterally treated 6-OHDA (Parkinsonian) mice. Both injections were done in the medial forebrain bundle. Injection of 6-OHDA in mice caused a profound depletion of striatal dopamine along with a loss of nigrostriatal dopaminergic neurons. 6-OHDA-treated mice had robust behavioral deficits showing reduced locomotion and increased rotations in the cylinder and arena tests. In one set of electrophysiological experiments, we acutely recorded the activity of substantia nigra pars reticulata (SNpr), the major output nucleus of the basal ganglia, from the above control and Parkinsonian mice under urethane anesthesia. SNpr neurons recorded from anesthetized 6-OHDA-treated mice displayed a strong ~1 Hz oscillatory firing pattern in contrast to control mice which fired spikes in a regular firing pattern. The mean firing rate was also

significantly reduced in 6-OHDA-treated mice (control inter-spike interval (ISI): 41±6 ms, n = 3; 6-OHDA ISI: 288±56 ms, n = 15). Analysis of spiking of SNpr neurons from 6-OHDA-treated mice with local field potentials recorded in cortex revealed that SNpr neurons fired in phase with cortical slow-wave activity. Experiments were also performed in chronic experiments in the awake state. To do this, a headplate was attached to the skull with dental acrylic and a craniotomy made leaving the dura intact. Head-fixed mice were trained on a simple behavioral paradigm (a 10% sucrose reward was given at a fixed interval of 60 s with each reward preceded by a cue light). SNpr recordings were made from these awake, head-fixed mice during the holding period between rewards. Results from these experiments are currently under investigation and will be included in the final poster.

Disclosures: C.J. Lobb: None. **D. Jaeger:** None.

Poster

248. Small Networks and Plasticity

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Program#/Poster#: 248.09/HH29

Topic: D.15. Basal Ganglia

Support: Royal Society of NZ Marsden Fund

Title: Dopamine reinforcement signals modify the direction of spike timing dependent synaptic plasticity in the striatum

Authors: *S. D. FISHER[1], Y. F. ZHANG[1], M. J. BLACK[1], W. C. ABRAHAM[2], J. N. J. REYNOLDS[1]
[1]Anat., [2]Psychology, Univ. of Otago, Dunedin, New Zealand

Abstract: Plasticity of cortical synapses onto spiny projection neurons in the striatum is thought to critically underlie the formation of sensorimotor associations between actions and outcomes. Previously we have shown that potentiation of cortical motor signals is most effective when they are followed by a temporal conjunction of depolarization and dopamine. A potential source of this conjunction is the superior colliculus (SC), due to its pathways to the striatum via thalamic nuclei and substantia nigra pars compacta (SNc). Moreover, these pathways can be engaged by light stimuli that have been potentiated in the SC through pairings with a primary reinforcer. In recent years the precise timing between pre and postsynaptic activity, termed spike timing-dependent plasticity (STDP), has been proposed as a generic mechanism to modify synaptic

weights. However, during reinforcement learning, STDP is unlikely to be the sole determinant of the direction of plasticity. Reinforcement of an action can occur seconds later, much longer than the millisecond timing relevant to STDP. We therefore hypothesized that, *in vivo*, corticostriatal plasticity is determined not only by STDP but also by behaviorally relevant reinforcement signals. To investigate this hypothesis, we made intracellular recordings from spiny projection neurons in urethane-anesthetized rats. In STDP experiments, presynaptic cortical activity both preceded ('pre-post') and followed ('post-pre') postsynaptic spiny neuron activity by 5-10 ms, during 60 pairings at 0.1 Hz. The STDP condition was paired with a sensory input (light flash) one second later, and a dopamine signal (SNc electrical stimulation) a second after that, to secondarily reinforce the light flash. Under these conditions, pre-post STDP pairings potentiated cortically-induced post-synaptic potentials (+13% EPSP slope at 20 min, $p < 0.05$) whereas post-pre STDP pairings resulted in robust depression (-28% EPSP slope at 20 min, $p < 0.05$). This is in sharp contrast with the findings from *in vitro* experiments where post-pre potentiation is often found. Furthermore, pre-post STDP without sensory and dopamine signals resulted in depression (-7% EPSP slope at 20 min, $p < 0.05$), suggesting that the presence of salient sensory reinforcement signals modifies the direction of STDP. The finding of pre-post potentiation in the striatum aligns with traditional Hebbian views of plasticity, and emphasizes the need to investigate this phenomenon *in vivo* where neuromodulator levels are physiological. These results demonstrate that the synaptic weighting of cortical inputs to spiny neurons can be enhanced through pairing with a salient sensory stimulus.

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Poster

248. Small Networks and Plasticity

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Title: A putative vglut1-positive cortico-pallidal glutamatergic projection: Differential organization between the internal and external globus pallidus

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Yerkes Res. Ctr., Udall Ctr. Excel. For Parkinson's Dis. and Dept. of Neurol., Atlanta, GA

Abstract: It has long been known that both the internal and external pallidal segments (ie GPi and GPe) receive massive glutamatergic innervation from vesicular glutamate transporter 2 (vGluT2)-positive neurons in the subthalamic nucleus (STN). In this study, we present further evidence for the existence of a vesicular glutamate transporter 1 (vGluT1)-positive input to the monkey and human globus pallidus. Because the main source of vGluT1 terminals in the forebrain is the cerebral cortex, our data suggest the existence of a putative “cortico-pallidal” glutamatergic system in primates. Although both GPe and GPi contain vGluT1-positive terminals, their overall pattern of distribution is strikingly different between the two pallidal segments. While they are evenly distributed throughout the whole extent of GPe, they are confined to the peripallidal and medialmost “limbic” regions of GPi, a pattern of distribution similar to that of pallidohabenular neurons in monkeys. In contrast, the core of GPi is almost entirely devoid of vGluT1 terminals in normal monkeys and humans. At the electron microscopic level, vGluT1-positive terminals in both GPe and GPi vary in size (~0.5-1.5 μm in diameter), are densely packed with round synaptic vesicles, contain occasional mitochondria and form asymmetric synapses. However, their pattern of synaptic connectivity dramatically differs between the two pallidal segtments. While almost 90% vGluT1-positive terminals in GPi target dendritic shafts of various sizes, vGluT1 terminals form synapses almost exclusively with “spine-like appendages” or small-diameter dendrites in GPe. In comparison, vGluT2-positive terminals display a very similar pattern of synaptic connectivity between GPe and GPi, i.e. in contact with dendritic shafts of various sizes in both nuclei. In conclusion, this study suggests the existence of a direct vGluT1-containing cortical projection that displays a differential degree of regional and synaptic specificity in GPe and GPi of monkeys and humans. In GPi, cortico-pallidal terminals are distributed to subserve specific relationships with limbic-related pallidohabenular neurons. In GPe, these terminals establish highly specific synaptic connections with spine-like structures and distal tips of pallidal dendrites. A cortico-pallidal connection could possibly allow for a direct route through which cortical influences could bypass the striatum and the subthalamic nucleus, to reach GPe and GPi neurons. The characterization of the exact cortical origin, and physiological significance of such a cortico-pallidal system in normal and parkinsonian states is under study.

Disclosures: **Y. Smith:** A. Employment/Salary (full or part-time):; Emory University, Atlanta, GA. **A. Mathai:** None. **J. Pare:** A. Employment/Salary (full or part-time):; Emory University, Atlanta, GA. **R.C. Moot:** None.

Poster

248. Small Networks and Plasticity

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Topic: D.15. Basal Ganglia

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NIH/NCRR Grant OD P51 OD11132

Title: Loss of glutamatergic thalamic input (vGluT2-positive) in deep layers (layer VI) of primary motor cortex (M1) of MPTP-treated parkinsonian monkeys

Authors: *R. M. VILLALBA[1], J.-F. PARE[1], T. WICHMANN[2], Y. SMITH[2]
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Abstract: The primate primary motor cortex (M1) receives a substantial innervation from the mesocortical dopamine system. Previous findings from our laboratory have shown that there is a significant loss of tyrosine hydroxylase (TH)-positive innervation of M1 (Weinkle et al., 2011, Soc. Neurosci. Abstr. 883.2) and a reduction in the spine density on cortical layer V pyramidal neurons (Smith et al., 2013, Soc. Neurosci. Abstr. 240.01) in M1 of MPTP-treated parkinsonian monkeys. Functional studies indicate that M1 corticospinal neurons decrease their firing rate and display abnormal discharge patterns in parkinsonian monkeys (Pasquereau and Turner, 2011, Cerebral Cortex 21:1362). These pathophysiologic changes in M1 pyramidal neurons are likely to be associated with remodeling of the glutamatergic innervation of M1 in the parkinsonian state. The goal of the present study was to examine whether the glutamatergic input from the thalamus, identifiable by its expression of the vesicular glutamate transporter type 2 (vGluT2), also undergoes changes in its density and ultrastructural features when monkeys are rendered parkinsonian by treatment with MPTP. Our preliminary results comparing the intensity of vGluT2 immunostaining at the light microscopy level (measured with ImageJ) in M1 in control and MPTP-treated parkinsonian monkeys showed an 80-85% decrease in the intensity of vGluT2 immunoreactivity in deep cortical layers (lower layer V/layer VI), while the intensity vGluT2 immunostaining increased by 20-25% in layers II-III. Preliminary electron microscopy results of

vGluT2-positive axo-spinous synapses in control and MPTP-treated animals in deep cortical layers in M1 have shown also different morphologies. While in control animals the postsynaptic densities (PSDs) of axo-spinous vGluT2 synapses were highly variable in shape ranging from macular to perforated with multiple active zones, the PSDs of axo-spinous synapses formed by vGluT2 terminals in parkinsonian monkeys were largely macular. Quantitative studies are in progress to characterize in more detail the ultrastructural features and pattern of synaptic connectivity of these thalamocortical synapses in control and parkinsonian monkeys. A deeper understanding of changes in the synaptology of the thalamocortical system that is associated with the development of parkinsonism will help us to better understand how cortical pathology contributes to aspects of the pathophysiology in Parkinson's disease.

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Poster

248. Small Networks and Plasticity

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Topic: D.15. Basal Ganglia

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NIH/ORIP Grant P51 OD11132 (infrastructure grant supporting the Yerkes Center)

Title: Effects of stimulation of the internal globus pallidus on thalamic activity patterns in parkinsonian monkeys

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Abstract: Stimulation of the internal globus pallidus (GPi) is one of the major functional surgical treatments used for advanced Parkinson's disease (PD). The effects of this intervention on electrical activity patterns in downstream targets of GPi output, specifically the thalamus, are not known. This series of experiments examined these effects in two Rhesus monkeys that were rendered moderately parkinsonian by treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), using standard electrophysiologic recordings before and after

treatment with MPTP. In the post-MPTP state, we recorded the spiking activity of neurons in the ventral thalamus before and during electrical stimulation of the ventrolateral motor territory of GPi with macroelectrodes (bipolar 120 Hz stimulation, 0.5 mm inter-contact distance, biphasic stimuli, 100μs/phase, 200 μA). The stimulation had modest effects on the animals' parkinsonism. Our analysis of the spiking activity of neurons showed a slight reduction of firing and increased bursting (as compared to the normal state) in the basal ganglia- and cerebellar-receiving areas of the ventral thalamus. Spectral analyses revealed an increase in the 3-13 Hz range of frequencies and a reduction in the gamma-range of frequencies in the basal ganglia-receiving area of the thalamus, as well as an increase in the 1-3 Hz range in the cerebellar-receiving territory. GPi stimulation had no effects on the distribution of spikes within inter-stimulus intervals and only minimally increased firing. However, the stimulation markedly reduced oscillations in the 13-30 Hz ranges in both territories. The results confirm that oscillatory and non-oscillatory characteristics of spontaneous thalamic activity are altered in the parkinsonian state. Electrical stimulation of GPi did not entrain thalamic activity patterns, but changed oscillatory activity in the ventral thalamus towards more normal levels.

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Poster

248. Small Networks and Plasticity

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Topic: D.15. Basal Ganglia

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Title: Localization of Cav3.1 T-Type calcium channels in the monkey thalamus: light and electron microscopic immunocytochemistry using subtype-specific antibodies

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Abstract: The motor dysfunction of Parkinson's disease (PD) results from degeneration of the nigrostriatal dopaminergic system, and the consequent functional changes of basal ganglia-thalamocortical circuits. Changes in thalamic activity, including an increase in burst discharges of thalamic neurons, are associated with the development of parkinsonism. In part, the abnormal bursting activity in PD may involve de-inactivation of T-type calcium channels (Cav3) following neuronal hyperpolarization. It is unclear whether this abnormal thalamic burst activity is the result of altered hyperpolarization from basal ganglia inputs, or changes in the localization and function of T-type calcium channels in the thalamus. As part of an effort to address the involvement of T-type calcium channels in abnormal thalamic activity, we studied the cellular, subcellular and subsynaptic localization of the Cav3.1 channel in the ventrolateral (VL) and centromedian/parafascicular (CM/Pf) thalamic nuclei, the main thalamic targets of basal ganglia outflow, in normal monkeys and in monkeys rendered moderately parkinsonian following chronic exposure to MPTP. At the light microscopic level, strong Cav3.1 neuropil immunoreactivity was found throughout the monkey thalamus, with the exception of the reticular nucleus. The intensity of immunolabeling in CM/Pf was lower than in VL. There was no obvious difference in the overall pattern and intensity of immunostaining between normal and parkinsonian monkeys. At the electron microscopic level, most Cav3.1 immunoreactivity was found in dendritic shafts of various sizes. In VL, 40-50% dendritic profiles displayed Cav3.1 immunoreactivity. At the subcellular level, aggregates of Cav3.1 immunoperoxidase and immunogold labeling were commonly found in the post-synaptic densities of putative asymmetric glutamatergic synapses, suggesting a role in excitatory transmission. Occasional pre-synaptic labeling was also encountered. There were no significant differences in the pattern of subcellular and subsynaptic localization of Cav3.1 between normal and parkinsonian monkeys. Thus, Cav3.1 is widely expressed in the basal ganglia-receiving nuclei of the thalamus in normal and MPTP-treated monkeys. Any involvement of these channels in increased thalamic bursting activities in the parkinsonian state is likely mediated by altered hyperpolarization of thalamic neurons rather than changes in the expression of Cav3.1 channels in the thalamus.

Disclosures: E. Chen: None. **J. Paré:** None. **S. Jenkins:** None. **Y. Smith:** None. **T. Wichmann:** None.

Poster

248. Small Networks and Plasticity

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Title: Electrophysiological effects of local administration of a selective T-type calcium channel blocker on thalamic neurons

Authors: *A. DEVERGNAS[1,2], Y. MA[1,2], I. HAMADA[1,2], C. K. JONES[3,4], C. W. LINDSLEY[3,4], Y. SMITH[1,2,5], T. WICHMANN[1,2,5]
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Abstract: Traditional antiparkinsonian dopamine replacement therapy is effective, but often leads to significant side effects that limit the usable dose range in many patients. There is, therefore, a need to develop non-dopaminergic approaches for the treatment of parkinsonian motor signs. One such approach is to use medications that normalize firing pattern abnormalities, specifically the known increases in burst firing, that result from nigrostriatal dopamine loss in the basal ganglia-thalamocortical circuits. In this study, we used the highly selective T-type calcium channel blocker, ML218, to test the hypothesis that some of the increased bursting in the thalamus is dependent on the activation of T-type calcium channels, we examined the effects of T-type calcium channel blockade on thalamic firing in parkinsonian monkeys. To do so, we examined the electrophysiological effects of local microinjections of ML218 on the activity of neurons in the ventral anterior and ventrolateral nucleus of the thalamus in 3 MPTP treated parkinsonian monkeys. Given the large size of thalamocortical neuronal cell bodies compared with interneurons, most of the recorded cells in our sample are likely thalamic projection neurons. The intra-thalamic injections of ML218 were done with a custom-built injectrode, consisting of a standard tungsten recording microelectrode glued to a thin silica injection tube. ML218 administration (2.5mM solution in artificial cerebrospinal fluid, 0.5µl, 0.1- 0.2µl/min) did not selectively change parameters descriptive of bursting in the thalamus (such as the proportion of spikes within bursts, the frequency of burst, or the maximal rate of firing within bursts). However, we observed prominent changes in other parameters. Thus, of 43 recorded thalamic neurons 18 (42%) showed a decrease, and 13 (30%) displayed an increase in their firing

rate. Both changes were seen within and between bursts. We also found that ML218 exposure reversed some of the MPTP treatment-induced changes in oscillatory power of thalamic firing, by decreasing oscillations in the 3-30 Hz range, and increasing the frequency above 30 Hz. These unexpectedly heterogenous effects of ML218 may be due to the fact that T type calcium channels in the monkey thalamus are widely expressed in thalamic interneurons and at pre-synaptic locations in addition to projection neurons.

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Poster

248. Small Networks and Plasticity

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Topic: D.15. Basal Ganglia

Support: NIH/ORIP grant P51-OD011132 to the Yerkes National Primate Research Center

NIH/NINDS grant P50-NS071669 (Udall Center grant)

Title: Optogenetic modulation of cortico-thalamic interactions in monkeys

Authors: *A. GALVAN[1], X. HU[2], Y. SMITH[1], T. WICHMANN[1]
[1]Yerkes Res. Ctr. and Dept. Neurol., [2]Yerkes Res. Ctr., Emory Univ., ATLANTA, GA

Abstract: The cerebral cortex and relay thalamic nuclei are reciprocally connected. Information transfer from cortical inputs to thalamic neurons cannot be studied with conventional electrical stimulation methods because the stimulation affects indiscriminately cortical projection neurons, interneurons, cortico-cortical connections, and (antidromically) thalamocortical fibers. In this study, we used optogenetic tools to selectively activate the terminals of projections from the primary and supplementary motor cortex (M1, SMA) to the ventrolateral (VL) motor thalamus in rhesus macaques. We compared the efficacy of two excitatory opsins, ChR2 and the red-shifted C1V1(TT), and studied the electrophysiological responses of thalamic cells elicited by optical activation of their cortical afferents. Two monkeys received recording chambers for access to motor cortices and thalamus. We injected the M1 and SMA with 30 µl of AAV5-CamKII-C1V1 (E122T/E162T)-EYFP in one hemisphere and AAV5-CamKII-hChR2 (H134R)-EYFP in the other hemisphere. After at least 6 weeks, optrodes (standard tungsten electrodes glued to 0.2 mm

OD optical fibers) were introduced into M1, SMA or the VL to light-activate opsin-expressing neurons (in cortex) or their terminals (in thalamus), while simultaneously recording the extracellular activity of neurons in the vicinity of the stimulation site. We found that 64/104 (61%) of cortical neurons and 65/165 (39%) of thalamic neurons responded to nearby optical stimulation in the C1V1 transfected hemispheres (in two monkeys). In one monkey (so far) we have recorded 64 cortical and 32 thalamic neurons in the ChR2 transfected hemisphere, and found that 20 cortical neurons (32%) and 8 thalamic neurons (25%) responded to light stimulation. For both opsins, the responses of cortical neurons to 20 ms light pulses were, in most cases, short-latency increases in firing rates. However, the responses of thalamic neurons to light stimulation of C1V1 or ChR2-expressing cortical terminals were far more variable, with many neurons showing decreases, or combinations of increases and decreases of firing. In most cases, thalamic responses were only elicited with long (500 ms) light pulses. The prolonged time to respond, and the fact that stimulation of the (excitatory) opsins lead to inhibitory effects in some thalamic neurons, suggest that at least some of the physiological effects of cortical terminals stimulation could be mediated through activation of polysynaptic circuits.

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Poster

249. Grasping Dexterity

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: EU IP grant no. 248587

EU IP grant no. 601165

Title: Multi-digit position and force coordination during unconstrained grasping

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Abstract: Small children learn to grasp objects before learning to walk or to talk. Grasping and handling objects found in daily life (e.g., cell phones, books, door handles, etc.) is one of the basic ways in which humans interact with their environment. Yet, grasping is a complicated task

when considered at the cognitive level. Grasping and manipulation tasks are highly unconstrained as an infinite number of combinations of digit positions and forces distributions can lead to stable grasps. This is due to redundancies at different levels of the sensorimotor system. In this work, we investigate this redundancy problem by examining how humans control grasping of a hand-held object using three, four and five digits in response to external perturbations. Our results revealed a similar variability in digits' initial placement when grasping with different number of digits. Moreover, the distribution of digit normal forces were modulated depending on the number of digits used, their locations, and the type of the external perturbations. Principal component analysis revealed that more than 95% of the digit force variance was accounted by the first two components. Finally, participants learned to compensate the external torque within the first perturbations within each trial during the holding phase. We propose that that the redundancy problem was addressed by the central nervous system in a similar fashion on a trial-to-trial basis in terms of digits' initial placements, but differently in terms of digits normal force distribution that was controlled online depending on the number of digits actively involved in the grasp.

Disclosures: **A. Naceri:** None. **M.O. Ernst:** None. **M. Santello:** None.

Poster

249. Grasping Dexterity

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Topic: D.05. Visual Sensory-motor Processing

Support: Canadian Institutes of Health Research

Natural Sciences and Engineering Research Council of Canada

Alberta Innovates - Health Solutions

Title: Haptic grasping configurations in early infancy reveal different developmental profiles for visual guidance of the reach vs. the grasp

Authors: *J. M. KARL, I. Q. WHISHAW
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Abstract: The Dual Visuomotor Channel theory proposes that reaching consists of two movements mediated by separate but interacting visuomotor pathways that project from occipital

to parietofrontal cortex. The Reach transports and orients the hand to the target while the Grasp opens and closes the hand for target purchase. Adults use vision to integrate the Reach and the Grasp into a single prehensile act. Young infants produce discrete preReach and preGrasp movements, but it is unknown how these movements become integrated under visual control throughout development. Highspeed 3D video analysis and linear kinematics were used to examine 4 to 24 month old infants, as well as blindfolded and sighted adults, as they reached to grasp a vertical rod. Hand orientation and aperture were measured when the hand first made contact with the target to determine the extent to which vision was used to orient the Reach vs. close the Grasp prior to contact. The youngest infants resembled blindfolded adults in that they failed to orient and close the hand prior to target contact. Between 9 and 24 months, infants were able to orient the Reach prior to target contact as accurately as sighted adults, but Grasp closure took longer to develop and even 24 month old infants failed to substantially close the hand prior to target contact. Infants of all ages differed from sighted adults, who oriented, opened, and closed the hand prior to target contact. The results suggest that the Reach and the Grasp are adaptively uncoupled in early infancy to capitalize on different sensory cues - vision for the Reach and haptics for the Grasp. Due to relatively delayed development of visual guidance for the Grasp, complete integration of the two movements into a single visually guided action occurs over a prolonged time period lasting into early childhood. The results support the proposition of the Dual Visuomotor Channel theory that the Reach and the Grasp are separate movements derived from different neural origins. They extend the original theory by indicating that haptic inputs also access the Reach and Grasp pathways and that it is likely these, rather than visual inputs, that shape the initial development of separate Reach and Grasp pathways in parietofrontal cortex.

Disclosures: **J.M. Karl:** None. **I.Q. Whishaw:** None.

Poster

249. Grasping Dexterity

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Program#/Poster#: 249.03/II6

Topic: D.17. Voluntary Movements

Support: DFG (SCHE 1575/1-1)

Title: Grasp force coding in F5 and AIP in a delayed grasping task

Authors: *R. W. INTVELD, H. SCHERBERGER
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Abstract: Studies focusing on the neural representation of hand forces have traditionally targeted the primary motor cortex (M1) due to its direct connections to the corticospinal tract. Few studies have also looked at premotor areas, where a stronger representation of movement planning is found. In this study we focused on the macaque ventral premotor cortex, also known as area F5, because of its strong relation to grasping movements, and on the anterior intraparietal area (AIP) that is directly connected to F5. AIP is highly active during the planning and execution of grasping movements, but its role in the control of grasp force is virtually unknown. We trained a macaque monkey on a delayed grasping task, in which a manipulandum (handle) was grasped with the right hand with one of two grip types, either a power grip or a precision grip. Every grip had to be held for 1 second at one out of three force levels. Both the particular grip type and the required amount of force were cued to the monkey in the beginning of each trial. We then recorded neural activity from F5 and AIP in the left hemisphere, contralateral to the moving arm, with chronically implanted floating microelectrode arrays (FMAs; MicroProbes for Life Sciences). Two 32-channel FMAs were implanted in each area (total of 128 electrodes). We found that single unit activity in F5 and AIP was strongly modulated by both grip type and grasping force. Response to grip type was similar in both areas. However, grasping force was more strongly coded in F5 than in AIP during most epochs of the task. Only during the cue presentation and the holding phase, when the monkey was actively maintaining the force level, similar proportions of AIP neurons showed force modulation as in F5. Neural modulation was also different in both areas, with F5 neurons showing more often an increase in activity with increased hand force, whereas AIP neurons were modulated to similar degree toward an increasing and decreasing force level. These preliminary results demonstrate a clear, but potentially different involvement of AIP and F5 in the planning and execution of grasp forces.

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Poster

249. Grasping Dexterity

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Topic: D.05. Visual Sensory-motor Processing

Support: ERC-2010-StG-260607

FWO-G082711N

Title: The effect of electrical microstimulation of the macaque anterior intraparietal area during grasping

Authors: *R. VAN EUPEN[1], P. JANSSEN[2]
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Abstract: The macaque anterior intraparietal area (AIP) is known to be crucial for object grasping. In humans, disruption of neural activity by transcranial magnetic stimulation (TMS) over parietal cortex during grip adjustment delays the grasp time, suggesting that the human homologue for AIP may be crucial for online control of grasping movements. In an effort to better localize the disruption of neural activity, we used single-cell recordings and electrical microstimulation in AIP of two macaque monkeys while the animals performed a reach-to-grasp task, in which a sudden change in the orientation of the object required an adjustment of the grip. Monkeys had to maintain stable fixation on the object for 450 ms before a light illuminated the object. After a delay period, the light switched off and an auditory go-cue instructed the animals to reach, grasp and pull the object to obtain a liquid reward. In the non-perturbation (NP) condition, the object orientation did not change during the trial. In the perturbation (P) condition, the object was rotated quickly by 90 degrees, 360 ms before the go-cue. Prior to the microstimulation experiment, we recorded single-unit activity. Almost all neurons (16/19, 84%) showed a modulation of their firing rate when object perturbation occurred. This modulation was in accordance with the neuron's orientation selectivity in 53% (7/14) of the orientation-selective neurons. In order to interfere with normal AIP activity, we electrically stimulated in 50% of both the P and NP trials (biphasic, 300 Hz, 300-500mA) in the 400 ms epoch before the go-cue, i.e. the epoch in which visual updating with the new object orientation in the object perturbation condition was possible. In both monkeys, AIP microstimulation shortened reaction times in the NP condition ($p < 0.001$). However, in the P condition, AIP microstimulation also significantly shortened reaction times ($p < 0.001$; main effect perturbation: $p = 0.67$; interaction: $p < 0.001$). In contrast, no significant effect of AIP microstimulation on the reach-to-grasp time was observed. No effect of microstimulation on reaction time or reach-to-grasp time was observed when stimulating in neighboring area LIP or in area 5 in the medial bank of the intraparietal sulcus. Thus, electrical microstimulation of AIP did not disturb reach-to-grasp movements and even shortened reaction times, irrespective of object perturbation. These results suggest that AIP microstimulation may enhance neural sensitivity in the motor system during object grasping.
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Disclosures: R. Van Eupen: None. **P. Janssen:** None.

Poster

249. Grasping Dexterity

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Topic: D.17. Voluntary Movements

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SNF CRSII3-147660/1

Dr. Wilhem Hurka Foundation

Title: Calcium imaging in motor cortex of mice grasping regular or irregular ladder rungs reveals cortical control of forelimb movement parameters according to relevance

Authors: W. OMLOR[1], A.-S. WAHL[2], H. LÜTCKE[1], C. VON ACHENBACH[2], M. VAN 'T HOFF[1], H. KASPER[1], M. WIECKHORST[1], M. E. SCHWAB[2], *F. HELMCHEN[1]
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Abstract: The horizontal ladder paradigm is frequently used to quantify deficits of cortically controlled skilled motor acts in rodents modeling Parkinson's disease or stroke. However, the role of the motor cortex during skilled locomotion on ladder rungs remains poorly understood at the level of neuronal networks. In transgenic mice expressing channelrhodopsin-2 in neocortical layer 5 neurons, we optogenetically mapped the motor cortex and identified a region, in which equivalent forelimb movements - involving all proximo-distal joints - were generated across animals. In the respective forelimb area of each mouse we applied calcium imaging using yellow cameleon Nano140 to record the activity of layer 2/3 neuronal networks while the head-fixed animal was moving across regularly or irregularly spaced rungs on a ladder wheel. Forelimb kinematics such as changes in shoulder, elbow, wrist and digit joints were simultaneously quantified using high-speed videography. For the regular rung pattern, the activity of neuronal subsets was correlated mainly with digit joint angles, which were encoded most precisely in the cell population. For the irregular pattern, neuronal subsets showed high correlations with digit and additionally shoulder joint angles, providing enough information to predict these kinematic parameters based on the population activity. We propose that skilled grasping under the two conditions demands cortical fine-control of digit joints while the unpredictable reaching distance on the irregular pattern requires in addition increased cortical shoulder control. Our findings demonstrate how motor cortex networks dynamically orchestrate selective movement parameters according to their relevance in the specific task and movement sequence.

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Poster

249. Grasping Dexterity

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Topic: D.17. Voluntary Movements

Support: advanced ERC Parietalaction

Title: Grasp-specific modulation of corticospinal excitability during observation of person vs. hand actions

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Abstract: It is well established that observing actions performed by others can modulate corticospinal output. Studies have shown that this so-called 'motor resonance' can be modified by a variety of different contexts. Recently, brain imaging studies in monkeys (Nelissen et al 2005) have revealed differential activations within the ventral premotor cortex (PMv) when an individual versus only a hand is observed grasping an object. However, whether this differential processing in PMv has a distinct effect on corticospinal output remains unknown. To address this issue, we asked subjects to observe a series of videos or static images in which a whole person or merely the hand was seen reaching and grasping a peanut (precision grip) or an apple (whole hand grasp). Thus, subjects (n=18, right-handed) observed 8 conditions pseudorandomly presented in 4 blocks (20 trials/condition) in a 2x2x2 factorial design (video vs. static image; whole person vs. hand; precision grip vs. whole hand grasp). Using transcranial magnetic stimulation (TMS), we examined motor evoked potentials (MEPs) in the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) at the point that the hand contacted the object. TMS intensity was adjusted to produce an MEP size of approximately 1 mV (~115% rMT) in the FDI. Within each block baseline MEPs were also collected at random during the inter-trial-interval and used to normalise each muscle. Subjects were instructed to fixate a central dot on the screen and remain with their hands relaxed throughout each block. To make sure subjects attended the stimuli, they had to report when the fixation dot was dimmed (1/8 trials). We found that

observing a video of a whole person or hand performing a precision grip significantly increased MEPs in the FDI compared to ADM. Conversely, ADM MEPs were increased compared to FDI during observation of a person or hand performing a whole hand grasps. In contrast, observing a static image of a person or hand interacting with the objects revealed no significant differences in FDI and ADM MEPs across grasp. Further analysis suggests a specific effect of observing a whole person on FDI MEPs only during precision grip. Our results indicate that observing actions performed by a whole person leads to modulation of corticospinal excitability in a grasp-specific fashion, similar to observation of actions merely performed by a hand. They are consistent with the view that multiple premotor sectors projecting to the primary motor cortex respond to action observation, one of them being a human homologue of F5c.

Disclosures: **K.L. Bunday:** None. **J.M. Kilner:** None. **R.N. Lemon:** None. **M. Davare:** None. **G.A. Orban:** None.

Poster

249. Grasping Dexterity

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NIH Grant NS050256

Title: Object-specific single neuron and population activity in rhesus macaque primary motor cortex during a reach-to-grasp task

Authors: *R. N. TIEN[1,2], S. PEREL[3,2], A. B. SCHWARTZ[1,2]
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Abstract: Neurons in primate primary motor cortex (M1) display patterns of activity related to finger and hand movement during object grasping and manipulation. Previous studies have correlated M1 single unit and population activity to wrist and hand kinematics, represented by joint angles (JAs). In this study, we ask if, and how, higher-order task features, specifically information about the object being grasped, are represented in M1 neural activity. To this end, we recorded wrist and finger JAs concurrently with well-isolated single M1 neuron firing rates

(FRs) from two rhesus macaques performing a reach-to-grasp task. The subjects grasped a diverse set of objects presented at various spatial locations and orientations. We found that JA profiles were consistent over repeated reaches to the same object regardless of spatial location. This allowed us to construct a preferred joint angle (PJA) vector for each object in JA space, defined as the mean of each JA feature over all reaches to one object. These PJAs were used to construct a linear classifier that could identify the grasped object from JA values during single trials with high (>90%) accuracy. Multiple linear regression was then used to construct a PJA vector for each neuron. We found that the mean FR of each neuron was proportional to the angular distance between the neuron's PJA and the PJA of the object being grasped, suggesting that individual M1 neurons systematically prefer movements toward certain objects over others, analogous to the classical finding that M1 neurons systematically prefer movements in specific directions in three-dimensional space. We then examined the neural data as a pseudo-population by constructing object-specific preferred firing rates (PFRs) in neural space, defined as the mean FR for all neurons across all reaches to one object. Multi-dimensional scaling and cluster analysis revealed that the object-specific PJAs were clustered in a similar way to the object-specific neural PFRs, indicating that movements toward objects that were similar in terms of JAs were represented similarly in M1. Finally, we asked if M1 activity carried object-related information beyond that found in the JAs alone. First, for each neuron, object-specific PJAs were constructed using FR and JA data from reaches to only one object at a time. We found that a neuron's PJA could change significantly depending on which object was grasped. We also found that objects could still be classified using only the residual FRs after the variance due to changes in JAs was subtracted away. These findings suggest that M1 population activity could carry object-specific information beyond simply representing kinematics.

Disclosures: **R.N. Tien:** None. **S. Perel:** None. **A.B. Schwartz:** None.

Poster

249. Grasping Dexterity

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Topic: D.17. Voluntary Movements

Support: BBSRC David Phillips fellowship (UK)

Title: The role of anterior intraparietal area in updating weight to size mapping for skilled grasp

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Abstract: Grasping and manipulating objects require the brain to extract useful information from multiple sensory sources, in particular vision and haptics (touch). When lifting objects, fingertip forces rely on the integration of a sensorimotor memory acquired from previous visuo-haptic experience and online visual cues. However, how the cortical grasping circuit combines vision to haptics with a differential gain during planning and execution of grip-lift movements is still unknown. Since the anterior intraparietal area (AIP) is part of the cortical grasping circuit and involved in multisensory integration of visual and haptic cues, we hypothesized it is a good candidate for controlling the planning of fingertip forces by encoding a specific weighting between sensorimotor memory and online visual cues. Here we used conflicts between vision and haptics to test their relative gain in biasing force planning for the next lift. Subjects (n=12) interacted with a virtual reality environment to grasp haptic objects simulated by two Phantom robots while they received online visual feedback via a 3D screen. Object size (2 or 7 cm height) and weight (1 or 3.5 N) were varied pseudoramdomly. In 20% of trials, size and weight were incongruent (i.e. small-heavy or large-light objects). We quantified grip force rate peak (GFR) as a behavioural read-out of force planning. We also applied theta burst transcranial magnetic stimulation (cTBS) over AIP to test its causal role in combining vision to haptics for controlling force scaling. As expected, we first found that GFR was significantly higher (23% increase) for large objects compared to small ones, irrespective of the size or weight of previous objects. Interestingly, a visuo-haptic conflict in the previous trial biased the sensorimotor memory effect. GFR was significantly lower (11% decrease) when the previous object was large-light compared to small-light. Conversely, there was a significant increase in GFR (16%) when the previous object was small-heavy compared to large-heavy. Applying cTBS over AIP changed these effects of visuo-haptic conflicts on GFR scaling by altering the relative weighting between sensorimotor memory and visual cues. These results show that the cortical grasping circuit can rapidly adapt to a new mapping between weight and size and that AIP is causally involved in combining haptics with visual cues for the online control of force during skilled grasp.

Disclosures: **M. Davare:** None. **A. Nuruki:** None.

Poster

249. Grasping Dexterity

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Program#/Poster#: 249.09/II12

Topic: D.17. Voluntary Movements

Title: Laterality of grasp-related activity in macaque areas AIP and F5

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Abstract: In primates, the anterior intra-parietal area (AIP) and area F5 of the ventral premotor cortex (PMv) play key roles for the transformation of visual target information into appropriate motor plans for object manipulation. While much research has focused on how these areas represent grasp planning and execution signals for contra-lateral movements, very little is known about grasp planning for the ipsi-lateral hand. To study this, we trained a female macaque monkey to perform a delayed grasping task, in which a handle was grasped in one of 5 distinct orientations with the contra- or ipsi-lateral hand with a power or precision grip. On any given trial, cue lights and an auditory cue indicated the appropriate grip type and the hand to be used, respectively. During the task we recorded single- and multi-unit activity simultaneously from AIP (n = 201) and F5 (n = 173) in the right hemisphere. In order to quantify the laterality of neural tuning, we compared the activity of each unit during contra- and ipsi-lateral grasps. During the instruction and movement epochs, firing rates were on average 25% higher during contra-lateral trials in both areas. However, most units were modulated by grasps of either hand, suggesting task dependency regardless of the hand used. Interestingly, grip type tuning was 1.5 times more prevalent during contra-lateral trials, suggesting a more functionally significant role. The hand used for grasping was significantly encoded during the instruction epoch in 38% of units in AIP and 56% of units in F5 (3-Way ANOVA). During movement execution this tuning increased in AIP (50%), but decreased in F5 (36%). Grip type and handle orientation tuning was present in 16% of units in AIP during instruction, while grip type tuning was present in 25% of F5 units. Grip tuning was always maximal during grasp execution. Interestingly, many units switched their preferred grip or hand during memory or movement, suggesting a flexible encoding of task parameters during grasp preparation and execution. To further elucidate the functional role of contra- and ipsi-lateral activity during grasp preparation, we calculated the functional correlation between AIP and F5 during the instruction epoch (i.e., how similarly task conditions were encoded in the neural state spaces of AIP and F5). We found that task conditions were encoded similarly in AIP and F5 during contra-lateral grasps (r-value: 0.74), but not during ipsi-lateral grasps (r-value: 0.07). Taken together, our results suggest that AIP and F5 are very active during grasp movements of either hand, but functional communication between AIP and F5 might take place primarily during contra-lateral grasping.

Disclosures: J.A. Michaels: None. **H. Scherberger:** None.

Poster

249. Grasping Dexterity

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Topic: D.18. Brain-Machine Interface

Title: An EMG-based state machine decoder for the detection of hand posture and grip force for reach-to-grasp tasks

Authors: *A. GAILEY, M. SANTELLO, P. ARTEMIADIS, M. ISON
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Abstract: The loss of a hand can dramatically affect the ability to perform activities of daily living, hence quality of life. To date, EMG-controlled prosthetic hand systems are based on decoding electromyographic (EMG) signals from the arm to discriminate among hand postures and specific types of grasps, i.e., two-digit precision grasp, three-digit precision grasp, and the full-hand grasp. However, these systems do not discriminate hand pre-shaping prior to object contact from hand posture at contact with the object. To address this gap, we asked able-bodied subjects (n = 4) to perform a reach-to-grasp task while recording surface EMG from five electrodes placed around the circumference of the upper forearm. Subjects were instructed to pre-shape their hand, contact the object, apply a specific amount of force (which varied across trials) on a force-sensing device, lift the object, hold it for a few seconds, replace the object on the table, and release it. The state machine decoder contains three basic states: (1) resting state, (2) preparatory grasping state, and (3) object contact state. States 2 and 3 consisted of four sub-states, each denoting a different type of grasp. A multiclass machine learning classifier classified among the four different grasp types in state 2 and the resting state. Next, for a given identified grasp type, we used another machine learning classifier to discriminate the preparatory grasping state from the contact state. When contact with the object was detected, a sigmoidal fit to the sum of multiple EMG amplitudes was used offline to relate the co-contraction levels of the muscles to the total grip force on the object. We were able to distinguish among different grasp types within the preparatory grasp state, with an accuracy ranging from 92% to 97%. However, the decoder predicted the contact event to occur earlier or later than the actual contact (average: -0.64 and +0.56 s, respectively, across subjects) A similar error magnitude was found for the detection of object release (range: -0.61 to +0.22 s). For prediction of grip force across subjects, the average normalized root mean squared error was relatively small (20% for the two-digit grasp, three-digit grasp and five-digit precision grasp; 18% for the full-hand grasp). These preliminary results show that the preparatory grasping pose alone is sufficient to discriminate

among four different grasp types, and that pattern recognition techniques can reasonably detect changes in EMG signal patterns when transitioning from a preparatory grasping pose to contact with the object. Future work will explore transitions between grasp types for in-hand object manipulation.

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Poster

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Topic: D.17. Voluntary Movements

Support: CIHR Grant MOP126158

Banting Postdoctoral Fellowship

Title: Object-directed action sequences decoded from human frontoparietal and occipitotemporal networks

Authors: ***J. P. GALLIVAN**[1], I. S. JOHNSRUDE[2], R. FLANAGAN[2]
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Abstract: Object manipulation tasks (e.g., drinking from a cup) typically involve sequencing together a series of distinct motor acts (e.g., reaching towards, grasping, lifting and transporting the cup) in order to accomplish some overarching action goal (e.g., quenching thirst). Although several studies in humans have investigated the neural mechanisms supporting the planning of visually guided movements directed towards objects (such as reaching or pointing), only a handful have examined how manipulatory actions--those that occur after an object has been grasped--are planned and represented in the brain. Here, using event-related functional MRI and pattern decoding methods, we investigated the neural basis of real-object manipulation using a delayed movement task in which participants first prepared and then executed different object-directed action sequences that varied either in their complexity or final action goals. Consistent with previous reports of preparatory brain activity in non-human primates, we found that activity patterns in several frontoparietal areas reliably predicted entire action sequences in advance of movement. Notably, we found that similar sequence-related information could also be decoded from pre-movement signals in object- and body-selective occipitotemporal cortex (OTC). These

findings suggest that both frontoparietal and occipitotemporal circuits are engaged in transforming object-related information into complex, goal-directed movements.

Disclosures: J.P. Gallivan: None. **I.S. Johnsrude:** None. **R. Flanagan:** None.

Poster

249. Grasping Dexterity

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Topic: D.05. Visual Sensory-motor Processing

Support: European Commission Grant Cogsystems FP7-250013

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Title: Connectional heterogeneity of the macaque dorsal prefrontal area 46 (46d)

Authors: E. BORRA, M. GERBELLA, V. GIORGETTI, S. ROZZI, *G. LUPPINO
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Abstract: The prefrontal cortex is composed of distinct sectors differentially involved in executive functions. Recent connectional evidence showed that ventral area 46 (46v) could contribute to motor behavior through topographically organized connections with inferior parietal and ventral premotor areas involved in hand-, arm-, and eye-related sensorimotor circuits (Gerbella et al. 2013. Cereb Cortex 23:967-87). In the present study, we placed injections of neural tracers (11 injections, 4 macaques) in different parts of dorsal area 46 (46d) to examine whether and how this area could contribute to different aspects of motor control. The results showed rostrocaudal connectional gradients within area 46d. The caudalmost part of area 46d displayed connections with prefrontal (8B, 8A, 8r, 8/FEF), premotor (F7/SEF), and inferior parietal (LIP) oculomotor areas. Other connections involved the fundus of the principal sulcus, the posterior cingulate cortex, and the ventral part of medial (PGm) and superior (V6A) parietal areas. In contrast, a relatively more rostral sector of area 46d, at about 3-6 mm from the caudal tip of the principal sulcus, showed robust connections with several arm-related areas. Specifically, this prefrontal sector was densely connected to both the premotor areas F7 and F2 and the dorsal part of parietal area V6A. Weaker connections involved other arm-related premotor (F6 and F5) and parietal (PGm and PG/Opt) areas. In the prefrontal cortex, the

connections were dense with area 46v and weaker with areas 9, 8B, 8r, and 12l. In the cingulate cortex, dense connections involved area 24c and weaker areas 24a, 24b, 23a, and 23b and the posterior cingulate cortex. Finally, tracer injections placed further rostrally in area 46d showed a different connectivity pattern characterized by connections mainly with the prefrontal areas 10, 9, rostral 46v, 45A, and 12l, the orbitofrontal area 11, and the premotor area F7. Other connections involved the posterior cingulate cortex, the superior temporal area STP, and the insula. Altogether, the present data show that area 46d displays general rostrocaudal connectional gradients similar to those observed in area 46v. Specifically, the caudal part of area 46d hosts two different sectors: a caudal one connected to inferior parietal and frontal areas involved in controlling oculomotor behavior and a more rostral one connected to superior parietal and dorsal premotor areas involved in visually guided arm reaching movements. The present data suggest that area 46d contribute to the control of oculomotor behavior and of reaching-grasping movements based on visuo-spatial information and behavioral guiding rules.

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Poster

249. Grasping Dexterity

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Topic: D.05. Visual Sensory-motor Processing

Support: CIHR Grant

NSERC

Title: Similarity of representations in human dorsal- and ventral-stream brain regions during object viewing and grasping

Authors: *S. FABBRI[1,2], K. STUBBS[2], R. CUSACK[2], J. C. CULHAM[2]
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Abstract: One influential model of the visual system has emphasized the segregation between vision-for-action in the dorsal stream (occipital to parieto-frontal cortex) vs. vision-for-perception in the ventral stream (occipital to temporal cortex). Functional magnetic resonance

imaging (fMRI) studies comparing levels of activation have shown some support for the functional segregation of the two visual streams. For example, areas in the dorsal stream (such as the anterior intraparietal sulcus, aIPS) have shown higher activation during grasping vs. reaching while areas in the ventral stream (such as the lateral occipital cortex, LOC) have not. However, newer multivoxel pattern analysis (MVPA) approaches have the potential to reveal effects beyond those manifested by activation levels alone. Here we used representational similarity analysis to explore how 6 geometrical shapes (sphere, cylinder, plate, cube, bar, and disk) presented in 3 sizes (small, medium, and large) are decoded in the human brain during different tasks. By correlating the patterns of brain activity measured with functional magnetic resonance imaging (fMRI) while 12 participants passively viewed or grasped each of the 18 objects, using a precision grip with 2 or 5 digits, or a whole hand grasp, we were able to measure the extent to which visual, motor, or both dimensions, are represented in different regions in the human brain. Cluster analysis revealed that pattern of activity in LOC shared similarities with ventral and dorsal premotor cortices (PMv, PMd), aIPS and primary motor cortex (M1). This similarity was mainly driven by sensitivity in these regions to differences between tasks. Within these regions, object properties, like shape and size, were processed during grasps to various extents. Moreover, object features were also represented in LOC and aIPS during passive viewing. These results suggest stronger similarity in visual processing between the two visual streams than previously indicated by brain activation levels alone, in line with evidence in monkeys of direct connections between the two streams.

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Poster

249. Grasping Dexterity

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Topic: D.17. Voluntary Movements

Support: National Science Foundation Grant EFRI-1137229

Title: Are there interlimb differences in lower extremity stability and dexterity?

Authors: *L. LEVEY[1], A. B. SAWERS[1], M. LYLE[2], L. H. TING[1]
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Abstract: Interlimb differences in upper extremity motor performance have been proposed to arise from the lateralization of motor function whereby one cortical hemisphere specializes in trajectory and direction control for dexterous arm functions (Sainburg, 2002), while the other hemisphere specializes in maintaining stable postures (Bagesteiro, 2003). Here, we sought to test whether similar interlimb differences in stability and dexterity exist in the lower extremities, and whether such interlimb differences in motor function correspond to foot preference. We tested the ability of the lower limb to maintain stability by measuring the magnitude and variation of the center of pressure (CoP) velocity during single limb standing (SLS) with eyes closed. We tested lower limb dexterity using the lower extremity dexterity (LED) test (Lyle, 2013), which examines how well the leg can dynamically interact with an unstable surface. During the LED test, subjects are positioned in an upright partially supported posture and use one leg to compress a spring prone to buckling using the highest force that can be sustained for 16 seconds. Higher force production on this task has been correlated to motor agility (Lyle, 2013). Ten unimpaired adults (25±4.0 years) performed both tests. Each leg was tested in a randomized order. Interlimb differences were identified for each test suggesting limb specialization. The magnitude ($p < 0.001$) and variation ($p < 0.05$) of the CoP velocity during SLS was found to be significantly different between legs, suggesting there is a leg specialized for maintaining stability. The magnitude ($p < 0.01$) but not the variation ($p = 0.57$) of the maximal vertical GRF during the LED test was also found to differ significantly between legs, suggesting there is a leg specialized for dexterity. However, we found no consistent pattern of specialization across individuals. Six participants performed both tests better on the same leg suggesting that one leg was specialized for both stability and dexterity, while four participants performed each test better on different legs suggesting that one leg was specialized for stability and the other was specialized for dexterity. Additionally, no relationship was identified between performance of either of the tests and Schneider's foot preference assessment (Schneider, 2010). Our results suggest that interlimb differences in the lower limb may not be governed by similar hemispheric specialization as the upper limbs. Nevertheless, tests of dexterity and stability may be effective in explaining differences in lower limb motor coordination observed in gait and balance.

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Poster

249. Grasping Dexterity

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Topic: D.17. Voluntary Movements

Title: Correlates between motor cortical output and control of the digits during common-practiced hand dexterity tasks

Authors: *Z. A. RILEY[1], A. W. MEEK[2], J. WILLIAMS[2], N. R. ECKERT[2]
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Abstract: Few situations in ordinary living require use of the digits to such a degree that cortical outputs are significantly modulated. Examples of this are the use of digits for braille reading in blind individuals, or playing an instrument as a professional musician. However, both of these are extreme examples where there was either damage elsewhere in the nervous system or the task required significant skill training. For unimpaired individuals the only tasks that could reach comparable daily use would be cellular phone text messaging or typing on a computer keyboard. The purpose of the present study was to examine motor cortical outputs in muscles of the hand and forearm (abductor pollicis brevis [APB], abductor digiti minimi [ADM], first dorsal interosseus [FDI], and flexor carpi radialis [FCR]) relative to text messaging and keyboard typing ability. Transcranial magnetic stimulation was used to examine cortical representations and stimulus recruitment curves for the aforementioned muscles in 10 healthy subjects (range: 20-34 yrs). This was compared with their performance of a functional texting task (FTT) and functional keyboard task (FKT). Both tasks required completing as many 45-character long phrases as possible in 2-minutes, each on a cell phone keyboard and on a standard computer keyboard. The number of correct characters yielded a performance score, and the number of correct characters / characters possible determined an accuracy score, for each task. After the texting and keyboard tasks the individuals cortical representations and recruitment curves were examined. Subjects had mean scores of 338±65, and 559±103 characters on the FTT and FKT, respectively. The scores were significantly higher in the FKT ($p \leq 0.001$). The accuracy scores were higher for the FKT as well (98.3±2.8% vs 97.5±1.3%, $p = 0.02$) A stepwise regression model including the slope of the recruitment curve, and volume, center-of-gravity (CoG), and area of the cortical representation of APB was used to determine if the individual FTT scores and accuracy could be predicted. Only the volume and CoG terms were able to predict 79.3% of the variance in the FTT score ($p = 0.004$). There were no predictors of the FTT accuracy. The same variables were included for all muscles in a model to examine predictors of FKT scores and accuracy, though none were significant predictors. The results of this study suggest that cortical outputs, specifically APB representation volume and CoG, can help predict cellular phone texting ability. The differences observed between the FTT and FKT could be due to the focused nature of texting (using only thumbs) versus the distribution of activity across all digits in keyboard typing.

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Poster

249. Grasping Dexterity

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Title: Facilitating supplementary motor area using near-infrared spectroscopy mediated neurofeedback improves postural stability but not hand dexterity

Authors: *H. FUJIMOTO[1,2], M. MIHARA[2], N. HATTORI[1], M. HATAKENAKA[1], H. YAGURA[1], T. KAWANO[1], H. OTOMUNE[2], I. MIYAI[1], H. MOCHIZUKI[2]
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Abstract: Background: There is accumulating evidence that the supplementary motor area (SMA) is involved in various aspects of motor control including postural control, inter-limb coordination, and coordinating temporal sequences of actions. However, its potential as a candidate for therapeutic target promoting functional recovery after stroke remains controversial. Objective: To investigate whether the SMA facilitation affects the hand dexterity and/or postural control in cause-and-effect manner, we used near-infrared spectroscopy (NIRS) mediated neurofeedback (NF) The system aims to induce neuromodulation via voluntary control of cortical activities by real-time presentation (Mihara M et al. Stroke 2013;44:1091-8). Method: Twenty healthy right handed subjects participated in this study (M: F = 7: 13, 28.1 ± 4.6 years old). As a marker for cortical activation, we used oxygenated hemoglobin derived signal measured by using a 50-ch continuous wave NIRS system with 4 short distance channels for correcting the extra-brain contamination. NF session consisted of 16 repetitions of 5second - task and 8-16 second rest periods. Subjects were asked to control their SMA activation according to the feedback signals without specific strategic instruction. Each subject received the REAL session in which their own SMA activation was fed back and the SHAM session in which the SMA activation of other subjects was fed back, with an interval of more than one week. Before and after each NF session, postural stability and hand dexterity were assessed using cumulative

length of center of pressure (COP) displacement during standing and 9-hole PEG test score (9HPT) with non-dominant hand respectively. NF effect on the SMA activation was assessed using comparison between first 6 blocks and last 10 blocks in each NF sessions. Behavioral changes were analyzed using repeated measures ANOVA with $p<0.05$ as significant. Results: Group analysis of cortical activation revealed that the SMA activation was enhanced only in REAL condition. COP length was maintained in REAL condition whereas it slightly increased after NF in SHAM condition. On the other hand, 9HPT scores did not change after NF in both REAL and SHAM condition. There was significant interaction between group (REAL vs. SHAM) and time ($F1,38=6.2$; $P<0.05$) on COP length, but there was no significant interaction on 9HPT, suggesting that the SMA facilitation improved postural stability but not hand dexterity. Conclusion: Our findings suggest a cause-and-effect relationship between the SMA and postural control, and provided the rationale for SMA modulation as therapeutic target for balance disorder after stroke.

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Poster

249. Grasping Dexterity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 249.17/II20

Topic: D.17. Voluntary Movements

Title: Visual and haptic guidance to facilitate inter-manual transfer of handwriting skills

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Abstract: Survivors of cerebrovascular accidents or amputation involving the dominant hand are forced to transfer their handwriting skills to their non-dominant hand. This process typically takes several months of training by occupational therapists and the overall success is highly subject-dependent. The goal of this study is to explore the mechanisms underlying the inter-manual transfer of handwriting skills and to investigate whether technological solutions can facilitate this process. In a previous study we demonstrated that robot-assisted training may accelerate inter-manual transfer. Specifically, we found that in order to facilitate learning, haptic guidance must account for the temporal aspects of the movement. Here we go one step further,

by asking whether the benefit for learning comes from haptic guidance or from temporal information, irrespective of the modality (either visual or haptic). The experimental apparatus consisted of a manipulandum with three degrees of freedom (Novint Falcon), with a customized pen-grip connected to the robot endpoint. A computer screen placed horizontally below the pen tip displayed a top view of a notepad. The robot recorded the pen tip movements that, when in contact with the screen, were continuously displayed as a red trace. We compared three different assistance modalities. In the static visual (SV) modality, subjects could see a static trace of the reference template. In the dynamic visual (DV) modality, subjects were presented with an animated version of the reference template (i.e. a moving target). In the dynamic visual + haptic (DVH) modality, subjects were provided visual guidance (as in DV) but in addition the robot generated forces toward this moving target, with a magnitude proportional to the target-pen distance. At the beginning of each trial, a letter was displayed and subjects had to reproduce it in writing. Subjects were divided into three groups (SV, DV, DVH). Training took place in one-hour sessions during three consecutive days. Before and after each training phase, subjects were tested through free-hand drawing of the same letters used in training, but without assistance. Across days, the magnitude of assistive forces was gradually reduced. An assessment procedure, inclusive of the trained letters and non-trained letters was repeated at the beginning of day 1, at the end of the third day of training and on the day after (day 4) to assess retention. Early results suggest that the final performance - learned handwriting movements - exhibits between-groups differences both spatial and temporal aspects. Specifically, temporal information seems crucial for learning.

Disclosures: I. Tamagnone: None. **V. Sanguineti:** None.

Poster

249. Grasping Dexterity

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 249.18/II21

Topic: D.17. Voluntary Movements

Title: Time-of-day effects on brain activity during the imagination and execution of finger opposition movements

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Abstract: During motor imagery a person internally simulates a movement without actually performing it. It has been shown that similar neural processes and brain areas are activated during mental and actual actions. Imagined actions are based on internal predictive models. Interestingly, motor prediction is influenced by the time of the day, and consequently by the amount of daily physical activity. In particular, the accuracy of motor prediction, assessed by calculating the temporal equivalence (isochrony) between actual and imagined actions, significantly varies according to the time of the day. Better performance (i.e., isochrony between actual and mental movements) is achieved in the afternoon than in the morning. This phenomenon can be explained by a continuous update of internal predictive models, by means of self-supervised learning, throughout the day. Here, we performed an fMRI study (n=15) at 7 a.m. and at 2 p.m. During each session, subjects had to actually and mentally perform a simple sequence of finger opposition movements (thumb-to-index, medium, ring and little) as fast as possible. Motor performance was recorded by using a magnetic-resonance-compatible engineered glove. The sequence was repeated five times and the duration of the whole task was calculated; during motor imagery, the subjects were instructed to tap the index with the thumb at the beginning and the end of the task. Actual and imagined movements were faster at 2 p.m. than 7 a.m. Motor prediction, assessed by the absolute difference (a.d.) between actual and mental movements, was better at 2 p.m. (a.d. = 1.7±1.3 s) than 7 a.m. (a.d. = 2.9±1.7 s). For actual movements, the fMRI analysis revealed that brain activity in the morning was more distributed and involved the primary motor cortex, the supplementary motor area (SMA), and the cerebellum. In the afternoon, the areas involved were similar but more localized and lateralized to the left brain and right cerebellar hemisphere, as a result of a better reorganization and optimization of circuits involved in the control of fine finger movements. For mental movements, at 7:00 a.m. we found very extensive activation of the SMA and the involvement of the left premotor areas, but the right cerebellum showed very low activity. At 2:00 p.m., the areas involved were similar to those activated in the morning but with reduced activations. Interestingly, the left parietal lobe was also recruited. Our results indicate that motor performance level and brain areas activation varied during the day. This should be taken into account when planning a rehabilitation program based on motor imagery.

Disclosures: L. Bonzano: None. **L. Roccatagliata:** None. **G.L. Mancardi:** None. **C. Papaxanthis:** None. **M. Bove:** None.

Poster

249. Grasping Dexterity

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 249.19/II22

Topic: D.17. Voluntary Movements

Title: Role of contralateral anterior intraparietal sulcus in coordinating digit force to position for dexterous manipulation

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Abstract: During dexterous manipulation, humans have an exquisite ability to coordinate fingertip forces as a function of digit placement. However, the underlying neural mechanisms are not well understood. The anterior part of intraparietal sulcus (aIPS) is known to be involved in the control of force and hand shaping during grasping movements. Thus, aIPS is a potential candidate for controlling digit force modulation to position. We addressed this issue by inducing 'virtual lesions' of aIPS contralateral to the hand interacting with the object. Eight subjects generated torque (70 Nmm) on a virtual object rendered by two haptic devices attached to the fingertips to control the lateral motion of a cursor to intercept a falling ball. For Exp#1, visual feedback of the object was not allowed throughout the trial. Object width (large, L; small, S) was changed unexpectedly across trials, thus requiring subjects to accurately modulate digit forces based on somatosensory feedback of digit positions at contact. We hypothesized that digit force-to-position modulation without visual feedback of digit placement would require aIPS. aIPS was disrupted using single-pulse transcranial magnetic stimulation (spTMS) at (Early) or 100 ms (Late) after object contact. For Exp#2, subjects performed the same task with visual feedback of the object, but its width was changed unexpectedly at grasp onset. This required subjects to use visual feedback of the new object width to adjust grip aperture, and change the digit forces they had originally planned in order to perform the manipulation task. We hypothesized that aIPS is necessary for enabling force-to-position coordination based on visual feedback of object width. aIPS was disrupted using spTMS at grasp onset. Conditions were separated according to when the object width remained the same (LL, SS) and was different (LS, SL) across and within trials. Subjects were able to generate adequate torque on the object to achieve the task goal. For Exp#1, early vs. no TMS increased peak force rate (FR) by 2.01±1.4 N/s for the LL condition. For SS and LS conditions, early vs. no TMS delayed accurate scaling of forces by >100 ms. Late TMS did not interfere with the magnitude of peak FR nor timing of force profiles. For Exp#2, TMS at grasp onset delayed the time to achieve 10% of peak grasp velocity by 150 and 70 ms for SL and LS conditions, resp. The perturbing effects of TMS over aIPS on hand shaping also impaired FR scaling, but in a condition-dependent manner. Our findings suggest the role of aIPS in integrating somatosensory feedback of digit position for control of forces, and visual feedback information of object width for predictive control of force-to-position control.

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Poster

249. Grasping Dexterity

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Topic: D.18. Brain-Machine Interface

Support: Tateishi Foundation 2031013

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JSPS Grant-in-Aid for Scientific Research on Innovative Areas 26119533

Title: Classification of hand shapes for dexterous control of a five-fingered robot hand using neural signals in the macaque inferior temporal cortex

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Abstract: Introduction: Previous researches on brain-machine interface (BMI) have demonstrated dexterous hand prosthetic control by decoding motor cortex signals associated with finger movements. These approaches are, however, not applicable for patients with paralysis resulting from the damaged motor cortex. On the other hand, considering our ability to visually imagine intended hand movements and the fact that neurons in the inferior temporal (IT) cortex are involved in the visual representation of body parts including hand, it is conceivable that neural signals in the IT cortex are also available for dexterous control of prosthetic hands. To assess the feasibility of this alternative approach, we demonstrated a prosthetic hand emulator control based on the classified hand shapes using IT neural activity. **Method:** Multi-unit activity was recorded from 190 electrodes chronically implanted in the IT cortex of a macaque monkey while the animal viewed images of three different hand-signs (rock, scissors, and paper). The recorded data was converted to the spike count with the time window of 100 ms and processed to classify which image was viewed at a time (3 hand sign images or blank screen) with linear discriminant analysis. Offline processing was used to evaluate the classification performance. The decoded output was then used to control a five-finger moveable robotic hand (Handroid,

ITK co. ltd.) with a cosmetic cover made of RTV silicone rubber fabricated by Satoh Giken co., which imitates a real human hand. **Results: C**lassification accuracies were very high as 82.0% on average (chance level performance is 25%) when all data acquired from 190 ch during one recording session was included in the analysis. Hand shape was decoded with highest accuracy (88.8%) at the latencies of 400 ms after visual stimulus onset, followed by gradual decrease in accuracy down to 64.0 % until the offset of stimulus. Data from 2 trials per image is enough to train a classifier that achieves 72.6 % correct classification on average. Analysis on data randomly sampled across different sessions shows that neural signals from more than 350 ch provide higher classification accuracy than 90 % on average. These results suggest that the dexterous control of a prosthetic hand will also be feasible if decoding the ensembles of several hundreds of neurons in the visual cortex.

Disclosures: R. Hayashi: None. **S. Saga:** None.

Poster

249. Grasping Dexterity

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Topic: D.17. Voluntary Movements

Support: NIH Grant NS079471

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Title: Localization of grasp domains in frontal and parietal cortex with intrinsic optical imaging in behaving monkeys

Authors: *O. A. GHARBAWIE[1], R. M. FRIEDMAN[2], A. W. ROE[2]
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Abstract: A parietal-frontal network in the primate brain is central to the sensorimotor integration for grasping. Nodes of the network include zones in anterior and posterior parietal cortex that are connected through parallel pathways to nodes in motor and premotor cortex. Our understanding of the functional dynamics of the network nodes has emerged primarily from single unit recordings, which provided invaluable insight about how neurons are tuned to stages of prehension. Nevertheless, the spatial clustering of grasp-related neurons within each network node remains unknown. We have adopted a multi-pronged strategy that involves optical imaging,

intracortical microstimulation, and single unit recording in behaving monkeys, to investigate the functional organization of the grasp network nodes. We trained a macaque monkey to reach, grasp, and lift a knob with its preferred hand. A chronic chamber implanted in the contralateral hemisphere provided access to the hand and forelimb representations in primary motor (M1), dorsal premotor (PMd), ventral premotor (PMv), and areas 1 and 2. Optical imaging maps were collected under 630 nm wavelength illumination during all stages of the grasp task and in various control conditions. A prominent activation domain (~ 2 mm^2) characterized the response pattern in M1. Intracortical microstimulation in and near the M1 domain evoked hand movements including flexion or extension of the digits, or wrist dorsiflexion. Similarly, single unit recordings from the same domain showed that neurons modulated their firing rates as the hand approached and grasped the target. Smaller domains were identified in PMd and PMv and their activation generally led the temporal pattern of the M1 domain. Three activation domains in parietal cortex were sequentially driven in a mediolateral pattern. Receptive field mapping with multi-unit recordings indicated that those domains were in rostral aspects of area 2. Moreover, the medial domain overlapped the forearm representation, whereas the lateral domains overlapped representations of the palm and digits. Our multi-pronged approach allows us to gain novel insight about the spatial organization of the grasp domains in frontal and parietal cortex and to resolve their functional dynamics.

Disclosures: O.A. Gharbawie: None. **R.M. Friedman:** None. **A.W. Roe:** None.

Poster

250. Reaching Control: Action and Sensation

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01HD040289

NIH Grant F31NS086399

Title: Do spatial and temporal cues interact during proprioceptive estimates of arm motion?

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Abstract: Humans can estimate the visual consequences of movement using proprioception (i.e. the sense of limb position and motion). Here we asked how well people could judge the timing of passive arm movements that occurred at different speeds, and thus moved different spatial extents. Healthy young controls performed a spatial-temporal psychophysical task in a KINARM robot where the arm was moved in the horizontal plane. They were first shown a dot that represented the position of the fingertip of their unseen arm. The dot disappeared and the robot passively moved the subject's hand directly to the right of its initial position at a constant velocity. During the movement, a visual dot appeared briefly, moved at the same velocity of the fingertip, and then disappeared. The subjects' task was to compare the onset of the dot to their felt fingertip and they were told that the dot might appear before their fingertip reached the dot onset location (early) or after their fingertip passed that location (late). Subjects then were asked to verbally indicate whether the dot appeared too early or too late relative to their ongoing movement. The paradigm was a two alternative forced choice task method of constant stimuli using temporal shifts of the dot including 0, ±200, ±400, ±600, and ±800 msec. We tested each subject on the task three different times with three different velocities (7, 9, and 11 cm/sec). For each velocity the temporal shifts were the same, but faster velocities produced larger spatial shifts. This allowed us to determine whether subjects could utilize additional information from different sized spatial shifts, or were solely relying on the temporal information. To analyze proprioceptive acuity, we fit a logistic function to each subject's data in the temporal shift domain. We then calculated the slope of each fit. We hypothesized that people would have better acuity (i.e. steeper slopes) when the spatial shifts were larger for the faster speeds. However, this was not the case. Acuity was no different for the temporal shifts across the different speeds, indicating that subjects did not benefit from traveling farther for a given time shift. This occurred despite the fact that the spatial extent traveled changed by a minimum of 22% for the same temporal shift. We conclude that the nervous system does not appear to benefit from clear spatial cues in augmenting this type of temporal proprioceptive task.

Disclosures: H.M. Weeks: None. **A.J. Bastian:** None.

Poster

250. Reaching Control: Action and Sensation

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Topic: D.17. Voluntary Movements

Support: STW grant 12160

Title: The effect of force level on the force reproduction error changes with force direction and arm posture

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Abstract: Golgi Tendon Organs and tactile sensors provide the central nervous system with sensory information about forces. The CNS integrates these sensory signals and the resulting force estimate can comprise systematic and random errors due to sensorimotor uncertainties. Previous studies show a force reproduction error, being the systematic error, where subjects generate too high forces when reproducing externally applied target forces [1] or self-generated target forces up to 40N [2,3] and generate too low forces when reproducing self-generated forces of 130N and up [3]. So far force perception has only been looked at in one degree of freedom and one direction, not taking into account that force perception might not behave isotropically. The goal of this study was to assess the effect of force level on the force reproduction error in different force directions and arm postures (shoulder and elbow angles) in the horizontal plane. We hypothesize that the force reproduction error will decrease with increasing force level as shown previously [3], but will differ in size for each force direction. Subjects (n=36, all right handed, 24 men) were divided into three groups and were instructed to match an onscreen target force (10N, 40N or 70N) in magnitude and direction (8 directions, 45 degree increments, in random order) with visual feedback and subsequently reproduce the same force vector without visual feedback in two arm postures. Per subject and arm orientation, ellipses were used to describe the force estimates of both reference and reproduction trials. The orientation, the direction of the principal axis, of the reproduction ellipses did not change with force level, but changed with arm posture and always pointed towards the shoulder. In the direction perpendicular to the principal axis, subjects generated too high forces for 10N and too low forces for 70N which is in accordance to results in one degree of freedom [3]. However, in the direction of the principal axis the force reproduction error increased with force level. These results show that the effect of force level on the force reproduction error depends on force direction and on arm posture. References: 1 Shergill SS; Bays PM; Frith CD; Wolpert DM. Two eyes for an eye: the neuroscience of force escalation. Science, 301(5630):187, 2003 2 Walsh LD; Taylor JL; Gandevia SC. Overestimation of force during matching of externally generated forces. Journal of Physiology, 3:547-557, 2011 3 Onneweer B; Mugge W; Schouten AC. Human force reproduction error depend upon force level. Wold Haptics Conference 2013

Disclosures: B. Onneweer: None. **A.C. Schouten:** None. **W. Mugge:** None.

Poster

250. Reaching Control: Action and Sensation

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Topic: D.17. Voluntary Movements

Support: DARPA award number: N66001-10-C-4056

NSF GRFP Grant No DGE-1247842

Title: Simultaneous multichannel microelectrode recording from macaque primary motor cortex and primary somatosensory cortex during an intuitive reach to grasp task

Authors: *S. N. FLESHER[1,2], A. B. SCHWARTZ[1,3], R. A. GAUNT[1,4,2]
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Abstract: The role of somatosensory feedback in the control of movement is a fundamental question in motor control. The goal of this current study was to identify and characterize functional relationships and connections between primary somatosensory cortex (S1) and primary motor cortex (M1) by simultaneously recording from areas M1 and S1 in a behaving rhesus macaque performing an out-center reach to grasp task. Two sets of intracortical microelectrode arrays, each containing an array of 88 recording electrodes placed in area M1, and an array of 32 recording electrodes placed in area 1 of S1. The M1 arrays were placed, based on cortical landmarks, in the upper arm representation of M1 and the S1 arrays were placed in the hand and finger representation of area 1. Placement of the S1 arrays was verified by receptive field mappings. The subject then performed reaches to a target located in one of four positions in three-dimensional space, grasped the target object, maintaining contact as it was translated to a central location. The target object was attached to a presentation robot through a compliant link to enable manipulation and positioning of the object. The object was instrumented with a force sensor to measure the pressure with which it was being grasped as well as a three-axis accelerometer to provide details about how the object was being manipulated during the transport phases. The object itself incorporated the reward delivery system, which was positioned at the subject's mouth at the end of a successful trial. During all phases of the movements, neural activity was recorded from the electrode arrays in M1 and S1. All channels were spike sorted online. We sought to identify changes in S1 firing based on the state of the reach, and correlate peak firing rates to object contact and other movement characteristics of the reach and grasp. We classified the states of the reach as reach, grasp and carry, and release and return, and identified

changes in the neural activity among states. The majority of units (87.3%) from the S1 array strongly preferred the grasp and carry phase to the release and return phase. A slight preference (67.67%) was also shown for the reach phase over the release phase. The results provide insight into the role of sensory feedback during a motor task. The relationships between these cortical populations during the different phases of the task may indicate a shift in information being relayed by the sensory feedback during a motor task. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No DGE-1247842 and DARPA award number: N66001-10-C-4056.

Disclosures: **S.N. Flesher:** None. **A.B. Schwartz:** None. **R.A. Gaunt:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

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Topic: D.17. Voluntary Movements

Support: Helen "Bessie" Pliner Endowed Professorship

Title: Precision of fine and gross pointing movements differs across visual conditions

Authors: *J. M. HONDZINSKI, S. A. WINGES
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Abstract: People often produce reaching and pointing movements which undershoot remembered target locations in darkness relative to an illuminated environment. This “dark phenomenon” of undershooting has been observed for straight arm and multi-joint arm movements. Because fine motor skills normally require greater precision than gross motor skills, this study was designed to determine whether the dark phenomenon also exists for a fine motor skill pointing task. Specifically, we questioned whether similar outcomes would exist for pointing movements to remembered target locations using the finger or whole upper limb. Since we recently showed that the gravitational pull cannot explain endpoint precision differences between visual conditions, we reasoned that mass differences between the finger and arm body segments would be negligible. If true, a pointing motor program would dominate the task control resulting in similarities in fine and gross precision control for each visual condition. Young adults with no reported neurological impairments produced pointing movements to real targets and remembered target locations in complete darkness (DARK) or normal room lighting

(LIGHT) from standing and side lying body orientations. Gaze was anchored on target or remembered target locations during the pointing movement. Three targets were located at a 1.5 m distance directly in front of subjects. Target levels included the shoulder, eye, and mid trunk. Subjects began with either the arm flexed up by the ear before producing ARM movements or the horizontal supported arm with finger extended at about a 45 degree angle, pointing above/past the eye level target, before producing FINGER movements. Six pointing movements were performed to each target in each in each visual condition when pointing with the ARM or FINGER in standing and side lying orientations. Movements of the shoulders, elbow, metacarpophalangeal (MCP) joint of the second digit, and the fingertip of the dominant pointing limb were recorded. Subjects often ended pointing movements in the DARK short of those in the LIGHT regardless of body part used when standing. This undershooting was observed, but less common in the side lying condition. The greater movement at the MCP joint produced greater variability, likely accounting for greater similarities between visual conditions in this condition. Conclusion: The phenomenon of undershooting remembered target locations in the dark exists for different body segments. The similar endpoint precision which exists for FINGER and ARM pointing movements suggests similar control strategies for endpoint accuracy regardless of the gross or fine motor control needed.

Disclosures: J.M. Hondzinski: None. **S.A. Winges:** None.

Poster

250. Reaching Control: Action and Sensation

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Topic: D.17. Voluntary Movements

Support: JST, CREST

Title: Fine-tuned force control under consistent visual feedback of object motion

Authors: *S. TAKAMUKU, H. GOMI
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Abstract: The ability to precisely estimate the forces involved in our action is essential for dexterous motor control. Visual feedbacks during dynamic object manipulation can contribute to this estimation in two ways. First, feedback of hand position can improve our estimation of endpoint forces from muscle tensions by providing information on arm posture. Secondly,

feedback of object motion can also contribute to our estimation of endpoint forces based on inverse dynamics, the estimation of force from motion. Here, to elucidate the potential contributions of the two computational processes, we examined grip forces during moving a simulated spring-mass-damper system in a cyclic manner while providing visual feedback(s) of the position of the hand and/or the position of the mass. In addition to the visual feedback, the damping factor of the system was also varied so that the phases of the load force, relative to the hand motion, differ between conditions (i.e., two-way factorial design of visual feedback and damping factor). The interest was on how each visual feedback contributes to adjusting the grip force to the timings of the load forces. If our visual feedback is to contribute by providing information on arm posture, the feedback of the hand position would improve our grip force control. Alternatively, if the feedback is to contribute based on inverse dynamics calculation, the feedback of the object motion would contribute. Analysis of cross-correlation between the grip force and the load force revealed that visibility of object motion tends to improve the synchrony of the two forces. Meanwhile, we found no evidence to support the idea that visibility of the hand improves our grip force control in this situation. Our result was consistent with the idea that the visual feedback directly contribute to our force control based on inverse dynamics, rather than indirectly contributing based on improving our estimations of arm posture. In terms of dynamic force control, seeing the objects we control rather than our hand is likely to lead to a better performance.

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Poster

250. Reaching Control: Action and Sensation

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Topic: D.17. Voluntary Movements

Title: Predictive coding and sensory gating in autism spectrum disorders

Authors: *J. FINNEMANN, P. FLETCHER, C. TEUFEL, D. WOLPERT, J. INGRAM, K. PLAISTED-GRANT
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Abstract: Background: Motor deficits are reported for as much as 80% of ASD cases (Green, 2009) and yet their aetiology and relationship with the core symptoms of autism remains unexplored. Since motor difficulties correlate with scales of autism severity (Hilton et al., 2011)

and predict daily life skills (Kopp et al., 2010), a better understanding of the deviant mechanisms in individuals with ASD bears direct relevance to developing intervention strategies and support. Based on the 'connectivity theory of autism' (Belmonte et al, 2004; Cherkassky et al., 2006) which states that autism is characterised by an increase in short-range connections and reduced long-range connectivity, one might speculate that differences in motor cognition will be found in those tasks that depend on the long-range connections between M1 and the premotor and parietal areas whereas functionality of short-range connections between M1 and somatosensory areas is intact. Whereas sensorimotor areas are involved in the post-predictive feedback loop, predictive processes are thought to be driven by premotor areas (Schubotz & von Cramon, 2002). Thus one might expect differences in predictive processes in individuals with ASD, which has indeed been suggested in a recent Bayesian model of autistic perception (Pellicano & Burr, 2012).
Objectives: To review and refine the ideas about predictive mechanisms of sensorimotor processing in ASD vis-à-vis the emerging models about active inference (Friston 2012, 2013) and predictive coding (Friston & Kiebel, 2009, Friston, 2009). It has been suggested that atypical perception in ASD might be the result of aberrant neuromodulatory control of the synaptic gain in specific parts of the cortical hierarchy (Friston, Lawon & Frith, 2012; Lawson et al., 2014)
Experiment: A well-established way to investigate prediction in motor processes at the behavioural level is to measure the sensory attenuation of self-generated versus externally-generated tactile input. In the experiment a lever - via a torque motor - exerts a mild pressure to the participants' index finger. The volunteers' response then varies depending on the condition: participants are asked to 'match' the pressure either by pressing on the lever with their other index finger or by adjusting a slider which controls the torque motor. The slider is a potentiometer which transduces a force gain at the ratio of 0.5N/cm. Where possible results of this experiment will be linked to measurements of somatosensory gating measured before and during movements of the individual to differentiate between central and peripheral mechanisms of SEP gating.

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Poster

250. Reaching Control: Action and Sensation

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Topic: D.17. Voluntary Movements

Support: Natural Sciences & Engineering Research Council of Canada

Canada Foundation for Innovation

Title: Pantomime Grasping attenuates tactile sensitivity of the moving limb

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Abstract: A multitude of events bombard our sensory systems at every moment of our lives. Thus, it is important for the sensory cortex to gate unimportant events. Tactile suppression is a well-known phenomenon defined as a reduced ability to detect tactile events on the skin before and during movement. Previous experiments (e.g., Chapman et al., 1987; Milne et al., 1988; Williams et al., 1998) found detection rates decrease prior to and during finger movement but most experiments examined tactile gating in simple motor tasks, such as index finger abduction. But, there is no reasonable expectation to utilize sensory feedback in finger abduction. Hence, many studies examined tactile gating in various visuo-motor tasks such as pointing (Buckingham et al., 2010), juggling (Juravle & Spence, 2011), grasping (Colino et al., 2014; Juravle et al., 2011), and during normal gait (Duysens et al., 1995; Morita et al., 1998; Staines et al., 1998). The present study examined how tactile detection changes in response to task demands. Participants performed two tasks in two separate experimental sessions: a "reach-to-grasp" session and a "pantomime reach" session. Nine human participants used their right hand to reach and grasp a cylinder in both sessions. Session order was counterbalanced across participants. Tactors were attached to the index finger and the forearm of both arms and vibrated at various epochs relative to a "go" tone. Vibrations could occur at 0 ms relative to imperative cue up to 360 ms post-cue in 60-ms intervals. Only one tactor was randomly activated in any given trial and at one interval relative to the imperative cue. Vibration time was renormalized trial-by-trial relative to reaction time. When participants performed reach-to-grasp targets, tactile acuity decreased at the right forearm before movement onset (Colino et al., 2014) but no suppression was observed at the right index finger or at the left limb. However, when participants performed pantomimed grasping, similar pattern was observed except at the right index finger. Sensitivity decreased at the right index finger suggesting that task demands modify sensory-motor circuitry (see Fink et al., 2014). These results indicate that the task affects gating dynamics in a temporally- and contextually-dependent manner and implies that feedforward motor planning processes can modify sensory signals.

Disclosures: F.L. Colino: None. **G. Binsted:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.08/JJ2

Topic: D.17. Voluntary Movements

Support: DFG-Grant Fi1567/4-1

Title: The effect of effector movement on body- and gaze-centered spatial coding of proprioceptive-tactile reaching

Authors: *S. MUELLER, K. FIEHLER
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Abstract: There is converging evidence that the reach target and the hand are represented in multiple spatial reference frames at the same time, and that the influence of each reference frame on reaching depends on the current sensory context (Pouget et al., 2002). In a previous study we showed that effector movement between target presentation and response led to a shift from gaze-independent to gaze-dependent spatial coding of somatosensory targets (Mueller & Fiehler, 2013, 2014). However, which reference frame was used when it was not a gaze-dependent one remained unspecified. In the present experiment we investigated whether proprioceptive-tactile reach targets are represented in a body- and/or a gaze-centered reference frame, and whether the contribution of body- and gaze-centered reference frames varies with the presence of an effector movement before the reach. Participants were asked to reach to an unseen finger of their left hand which was indicated by a mechanical touch (the proprioceptive-tactile target). Reaches were performed with the right hand in total darkness and with the head fixed. In order to test for body- and gaze-dependent coding of reach targets, we varied (i) the starting location of the reaching hand, (ii) the target location, and (iii) gaze direction. Effector movement was manipulated in 2 separate blocks that differed in whether the left hand was kept stationary at the target location throughout the trial (stationary condition) or actively moved to the target location, received a touch and was moved back before the reach (moved condition). We compared horizontal reach errors of trials that were similar in gaze coordinates but different in body coordinates and vice versa using a correlation approach. Under the assumption that two movements that are represented in the same reference frame also produce similar errors, significant correlations indicate the use of the respective reference frame (body- or gaze-centered). With regard to gaze-dependent coding we only obtained significant correlations in the moved condition but not in the stationary condition, which is in line with our previous results. Interestingly, we found significant correlations in body coordinates in both conditions (stationary

and moved), indicating a predominant use of a body-centered reference frame in the stationary condition and the use of both body- and gaze-centered reference frames when the target hand was moved before the reach. Our results suggest that gaze-centered coding of somatosensory reach targets depends on the presence of an effector movement, while body-centered coding is immanent.

Disclosures: S. Mueller: None. **K. Fiehler:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.09/JJ3

Topic: D.17. Voluntary Movements

Support: STW 12160

Title: Haptic guidance needs to be intuitive not just informative to improve human motor accuracy

Authors: *W. MUGGE, I. A. KULING, E. BRENNER, J. B. J. SMEETS
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Abstract: Humans make both systematic and random errors when reproducing learned movements. Haptic guidance can improve human motor performance through assistive forces that provide additional information about the desired movement. Forces that guide the operator to the target feel intuitive and provide information about the direction and distance to the target (through force scaling). Our study examined whether haptic guidance needs to be intuitive to be useful, or whether it is the additional information about the target's position with respect to the hand that improves the operator's performance in a position reproduction task. We applied force fields that scaled the force exerted to the subject's arm linearly with the distance to the target, exerting no force at the target position. Non-guiding force fields do not affect position accuracy[1], so we hypothesized that without time constraints subjects would reproduce a prior position equally accurately with 90 degrees (Perpendicular) or 180 degrees (Opposing) rotated guidance as with guidance directed towards the target (Assisting), because the guidance forces provide the same information to the subjects. Ten subjects (30.8 (SD 9.3) years, 6 male and 4 female, all right handed) made a series of reaches to visible targets with a haptic device (PHANToM Premium 3.0/6DoF). The reaches were performed in pairs of trials towards the same target, first

with and then without visual feedback about their hand position. The end-point accuracy (systematic error) and precision (random error) in the blind trials were assessed in four conditions (blocked design): no, assisting, opposing and perpendicular guidance. Both the accuracy and precision were significantly better with assistive guidance than in all other conditions. With opposing and perpendicular guidance, subjects took significantly longer to complete a reach and their performance did not differ from performance without haptic guidance. This study shows that haptic guidance only improved position reproduction when using it was intuitive. Subjects ignored the same information when presented non-intuitively, even in this static case without time constraints. References 1. Kuling IA; Brenner E; Smeets JBJ. Proprioception Is Robust under External Forces. PLoS One 2013 8(9)

Disclosures: W. Mugge: None. **I.A. Kuling:** None. **E. Brenner:** None. **J.B.J. Smeets:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.10/JJ4

Topic: D.17. Voluntary Movements

Title: The effects of aging on proprioceptively guided reaching movements in three dimensional space

Authors: *T. I. GONZALES[1], T. S. SCHAAP[2], T. J. W. JANSSEN[3], S. H. BROWN[1]
[1]Univ. of Michigan, Ann Arbor, MI; [2]VU Univ., Amsterdam, Netherlands; [3]Amsterdam Rehabil. Res. Inst., Reade, Netherlands

Abstract: Aging is associated with impaired upper limb proprioceptive acuity, as reflected by decreased position matching accuracy with increasing task complexity and movement extent. Studies have shown that older adults have greater difficulty reproducing limb position when interhemispheric transfer of proprioceptive feedback is required (Adamo et al., 2007,2009; Herter et al., 2014) although limb asymmetries, thought to reflect non-dominant specialization for position sense (Sainburg 2002; Goble et al. 2006), persist in older populations (Adamo et al. 2007). Most studies have primarily used single joint or planar paradigms to examine age-related changes in proprioception. It is unclear whether these changes can be generalized to more complex multi-joint movements, where additional sensory feedback may affect performance. For example, King & Karduna (2013) found no differences in matching accuracy between the preferred and non-preferred arms when young adults performed a proprioceptive reaching task in

the vertical plane. Since age-related declines in cognitive function may impair the ability to integrate multiple sources of sensory feedback (Goble et al. 2009), deficits in position matching ability in older adults may persist when tasks are performed in three dimensional space. The accuracy with which young and older participants reproduced remembered reference hand positions was assessed under different experimental conditions. Participants matched reference positions located directly to the front or 45° to the side relative to the midline using the preferred and non-preferred arms. Either the same (i.e. ipsilateral matching) or the opposite (i.e. contralateral matching) arm was used to reproduced the reference position. No significant differences in matching accuracy were found between young and older participants when matching ipsilaterally. When matching contralaterally, accuracy was significantly worse in older participants for reference positions located to the side, which may reflect age-related changes in the perception of peripersonal space (Ghafouri & Lestienne 2000). In contrast to previous studies, accuracy did not differ between the preferred and non-preferred arms in either group. These results extend previous findings demonstrating age-related impairments in proprioceptively guided arm movements when interhemispheric transfer is required. Additional sensory feedback when reaching in three dimensional space may attenuate differences in limb asymmetries found in previous position matching studies.

Disclosures: **T.I. Gonzales:** None. **T.S. Schaap:** None. **T.J.W. Janssen:** None. **S.H. Brown:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.11/JJ5

Topic: D.17. Voluntary Movements

Support: NSF Grand EFRI-1137172

Title: Precision of arm position sense strongly depends on arm configuration

Authors: *K. OH[1,2], B. I. PRILUTSKY[1,2]
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Abstract: Arm position sense is not uniform across the arm workspace. Several studies have shown that precision (random error) of reproducing a target hand position is significantly lower when the hand is away from the body than when it is closer to the body; similarly, precision of

reproducing target hand positions in the left-right direction is lower than that in the forward-backward direction (van Beers et al 1998; Wilson et al. 2010). The non-uniformity of hand position sense precision is poorly understood. It is generally agreed that the muscle spindles are a major contributor to arm position sense, and their signals encode joint angles. We hypothesized, based on known geometric properties of a two-segment kinematic chain, that less precise hand position sense observed in locations away from the body may be caused by relatively smaller changes in the joint angles for the same hand displacement. To test this hypothesis, we conducted a geometric analysis of a two-segment kinematic chain representing the human arm for the hand horizontal workspace. The analysis involved derivation of the Jacobian for the arm model, and the associated norm, condition number, eigenvalues and eigenvectors to investigate the error amplification of hand position due to small errors in joint angles. The analysis revealed the theoretical distribution of hand position error amplification in the horizontal workspace. To test the theoretical predictions for the hand position sense precision, 7 healthy subjects performed two arm position matching tasks without visual feedback using a bimanual robot Kinarm (BKIN, Canada). In the first task, Kinarm repeatedly moved the dominant right hand in 4 positions in the horizontal workspace in random order, and the subject was instructed to match joint angles of the left arm to the joint angles of the right arm. In the second task, the subjects were instructed to match the left hand movement distance and direction from the initial hand position to those of the right hand. The experimental precision distribution at each target location was described by a best-fit ellipse and compared with the theoretical predictions. The results demonstrated close similarity in terms of the area and orientation of the precision distribution between the experiments and theoretical prediction. The theoretical and experimental precision level was lower in the left-right direction than in the forward-backward direction, in agreement with previous studies (van Beers et al 1998; Wilson et al. 2010). We concluded that the arm configuration contributes substantially to the precision of arm position sense across the arm horizontal workspace.

Disclosures: **K. Oh:** None. **B.I. Prilutsky:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.12/JJ6

Topic: D.17. Voluntary Movements

Support: NIH/NIGM T32 GM081741-06

Title: Identification of functional muscle synergies for the control of gravitational loads during reaching movements

Authors: *E. V. OLESH[1], W. J. TALKINGTON[2], V. GRITSENKO[2]
[1]Erienne V Olesh, Morgantown, WV; [2]Human Performance, Physical Therapy Div., West Virginia Unversity, Morgantown, WV

Abstract: Forces produced during multi-segmental limb motion constitute a challenging set of parameters that must be controlled by the central nervous system. However, the modalities of neural control signals that reflect the controlled parameters and produce muscle contractions are a matter of active debate. We have addressed this issue by investigating the role that gravitational and motion-related joint torques may play in the formation of muscle synergies for movements of the arm. We recruited healthy subjects to perform reaching tasks in a center-out paradigm. These reaching tasks originated from either a medial (center of the trunk) or lateral (side of the body) starting position. The hand movements were either in a vertical or horizontal planes. Both limbs were tested. Using a virtual reality system (Oculus Rift and Vizard, WorldViz Systems), a total of fourteen targets were arranged around each starting location in a circular pattern. Subjects were instructed to start at one of the four initial positions (two for each limb) and move as quickly and as accurately as possible to the target of interest, which was indicated by a color change. Each trial was completed when the subject returned to the initial starting position. Fifteen movements to each target were completed in a randomized order. Electromyography data was recorded from twelve muscles of each arm using Motion Lab systems. In addition, kinematic data was collected using the Impulse motion capture system (Phasespace). Data were then processed using custom scripts written in Matlab (MathWorks). Motion capture data were used to calculate joint angles for shoulder, elbow, and wrist. These angular kinematics were then used to calculate joint torques, which were subdivided into gravitational and motion-related components. We then applied a decomposition analysis to examine the relationship between joint torque components and muscle activity profiles. We have shown that joint torque components can be used to decompose muscle activity profiles for individual movements. This suggests that separate functional muscle synergies may exist for the production of voluntary motion and for the compensation for loads due to gravity.

Disclosures: E.V. Olesh: None. **V. Gritsenko:** None. **W.J. Talkington:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.13/JJ7

Topic: D.17. Voluntary Movements

Support: BioX Neuroventures Fabrication Grant, Stanford University

Title: Haptic fMRI: Mapping neural activation during planning and reaching for unconstrained three degree-of-freedom tasks

Authors: *S. MENON, M. YU, H. GANTI, K. BOAHEN, O. KHATIB
Stanford Univ., Stanford, CA

Abstract: Recent advances in combining haptic interfaces, robots that are designed to monitor and perturb human motion, with high resolution functional magnetic resonance imaging (Haptic fMRI) have enabled non-invasive experiments that study spatial reaches, visually guided trajectory tracking, and object manipulation in virtual worlds [1]. Here, we use Haptic fMRI to demonstrate that unconstrained three degree-of-freedom motions can elicit heterogeneous neural activation at the millimeter spatial scale. The activation is task specific and spans motor, somatosensory, and pre-motor cortex; the former two exhibit motion-related activation and the latter also exhibits planning related activation (Fig. 1). In contrast with classical limb-mapping experiments [2,3], where different joints elicit correlated neural activation in individual voxels, our fMRI time-series measurements differ dramatically across brain regions and tasks. Such activation time-series are not well explained by generalized linear models with canonical haemodynamic response functions. To compensate, we used an impulse response model to factorize the time-series for each voxel into task-specific components, which helped capture neural activation heterogeneity. Plotting the impulse response model predictions on an inflated brain helped localize motor planning to a sub-section of pre-motor cortex (see Fig. 1, inflated brains; c.s, central sulcus). The primary motor and somatosensory cortices, in contrast, exhibit a reduction in signal during planning and, with some parts of pre-motor cortex, a wide-spread increase during reaching. The lack of time-series correlations at a millimeter spatial scale indicates that our experiment is unaffected by common fMRI artifacts like head motion or magnetic field drift, which induce low spatial-frequency correlations across brain regions. 1. Menon, S., e.a. Proc. IEEE EMBC, 4137-42, July (2013). 2. Meier, J. D. and Graziano, M. S. A. e.a. J Neurophysiology 100, 1800-1812 (2008) 3. Strother, L., e.a. Eur J Nsci 36(9), 3291-3298 (2012).

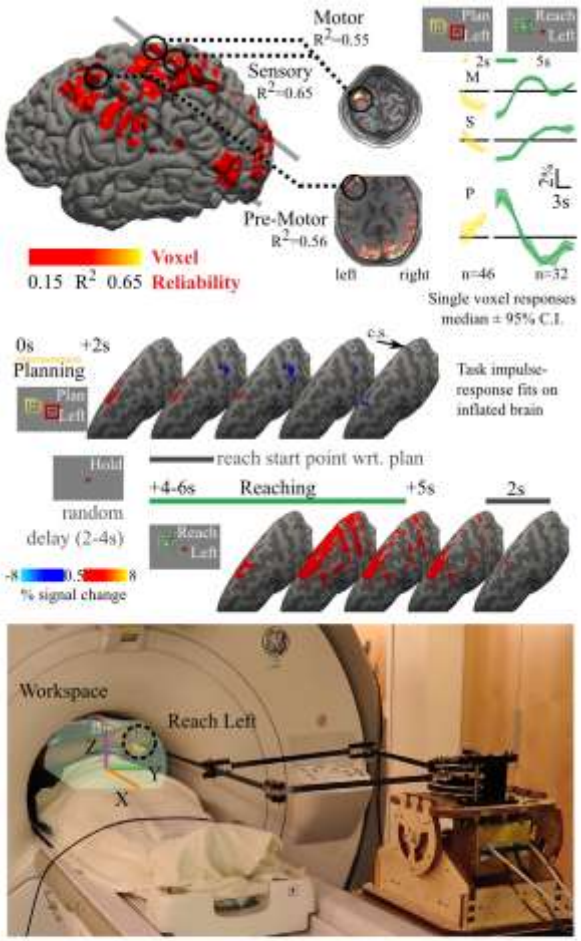

Figure 1. *Top*. Neural activation patterns across motor regions measured with Haptic fMRI. *Mid*. Planning (above) and motion (below) related activation. *Bottom*. Subjects control a virtual ball on a screen by moving the haptic interface. Visual cues specify planning (yellow; see top right) and reaching (green).

Disclosures: S. Menon: None. **M. Yu:** None. **H. Ganti:** None. **K. Boahen:** None. **O. Khatib:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.14/JJ8

Topic: D.17. Voluntary Movements

Support: Biotechnology and Biological Sciences Research Council

Medical Research Council

Title: Using single pulse microstimulation to assess excitability of cortical microcircuits

Authors: *D. S. SOTEROPOULOS
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Abstract: Intracortical microstimulation has been (and still is) one of the mainstay techniques used in movement neuroscience for assessing motor cortical function and connectivity in animal models. Single pulse intracortical microstimulation (sICMS) is well suited to assess cortical connectivity as it often elicits responses in other nearby cortical neurones and the short (<2ms typically) artefact duration means that short latency responses can be reliably measured (using a Peri-Stimulus Time Histogram - PSTH). Whilst these responses are used to characterise how cortical sites interconnect, this technique has not been used to assess connectivity changes during behaviour. Changes in response probability during behaviour may provide a measure of cortical excitability but there are a number of factors that need to be accounted for. An important factor is the non-stationary spiking rate during behaviour - changes in background activity are likely to affect the impact of the cortical stimulus on spiking probability even if there is no underlying change. The feasibility of this approach was tested on motor cortical data collected from an awake behaving monkey during microstimulation. We first examined whether we could model the early excitatory response of cortical neurones often seen in response to sICMS. A non-leaky "integrate and fire" neurone model of the cortical cell was created, that matched the interval spike statistics and time varying spiking rate of the real neurone. A simulated excitatory post-synaptic (EPSP) response was then added to the input of the simulated neurone at regular intervals - this corresponded to the "sICMS". The size of this EPSP was adjusted so that the response in the simulated PSTH matched that in the real PSTH. This allowed us to test what the expected changes in response size would be at different background rates, assuming a constant EPSP amplitude. This was applied to cortical neurones responding at short latency to callosal stimulation or to cortical neurones responding to microstimulation of an adjacent microelectrode. For the callosally responding cells (n=3), the changes in response probability with background firing rate of the real cells was well matched by our simulations, as expected given that axonal tract stimulation should produce an invariant post-synaptic response. For cortical neurones responding to sICMS (n=3) this was not the case, particularly for higher firing rates (which typically corresponded to movement epochs). This discrepancy may correspond to changes in cortical excitability during movement.

Disclosures: D.S. Soteropoulos: None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.15/JJ9

Topic: D.05. Visual Sensory-motor Processing

Support: Kessler Foundation

The Healthcare Foundation of NJ

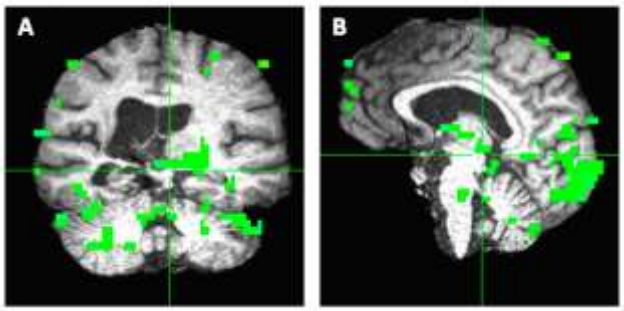
NIH/NICHD/NCMRR Grant K24HD062647-01

Title: Spatial bias, the superior colliculus and prism adaptation

Authors: *A. CHAUDHARI[1,2], A. BARRETT[1,2]
[1]New Jersey Med. Sch., Newark, NJ; [2]Kessler Fndn., West Orange, NJ

Abstract: Objective: To demonstrate that externally modifying superior colliculus activity may lead to observable changes in spatial bias. Background: Sprague 1966 first described an experiment where ablation of the contralesional superior colliculus (SC) led to improvements on spatial tasks in cats with induced parietal neglect. Since then, studies have shown that: (1) isolated deactivation of the SC may result in asymmetric spatial behavior, and (2) right visuoparietal damage may reduce input and activity in the ipsilesional SC. We hypothesized that stimulating the right SC may alter spatial performance bias. We also compared this effect with prism adaptation. Methods: A.A. is a 29-year-old woman who presented with spatial bias and extinction following a right midbrain/collicular hemorrhage. On Visit 1, A.A. underwent a computerized line-bisection task with and without SC stimulation with dual rotating checkerboards and right hemi-patching. For comparison, on Visit 2, A.A. underwent the task before and after one prism adaptation training session (PAT; Goedert et al., 2013). Results: A significant change in line bisection error was observed with both SC stimulation [baseline mean=-2.1±6.53mm, stimulation mean=+2.2±5.36mm; $p<0.01$] and PAT [pre-prism mean=-3.0±5.07mm, post-prism mean=-0.7±5.42mm; $p<0.05$]. However, both SC stimulation and PAT shifted Aiming, motor-intentional errors rightward. This rightward shift tended to be higher in far space (SC $p<0.05$; PAT $p=0.07$). Discussion: Superior colliculus activation and PAT may alter spatial bias via related mechanisms. In our participant, who had right SC damage, left SC activation may have resulted from visual stimulation. We observed reduction in leftward Aiming bias, which may have especially affected far space. Further research investigating SC stimulation and its long-term effects may results in novel or more effective treatments for disorders such as spatial neglect.

Figure 1: fMRI scan of the participant in coronal (A) and sagittal (B) sections. Voxels in green exhibited significant differences in activity with and without SC stimulation. Cross hairs are placed at the approximate location of the superior colliculus, though the structure is difficult to distinguish because of the hemorrhage.



Disclosures: **A. Chaudhari:** None. **A. Barrett:** None.

Poster

250. Reaching Control: Action and Sensation

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 250.16/JJ10

Topic: D.05. Visual Sensory-motor Processing

Support: NIH Grant NS011862

Title: Object grasp by the ipsilateral and contralateral hands evokes similar responses from posterior parietal cortex (PPC) neurons

Authors: *E. P. GARDNER[1], J. CHEN[1], J. L. BAKER[2]
[1]Dept Physiology/Neuroscience, New York Univ. Sch. Med., NEW YORK, NY; [2]Brain Mind Inst., Weill Cornell Med. Col., NEW YORK, NY

Abstract: The contralateral hemisphere has traditionally been implicated in sensorimotor control of each hand. However, we previously observed PPC responses to actions of either hand during natural grasping. To compare cortical representation of the hands during skilled prehension, we trained macaques to perform a reach-to-grasp instructed delay task, and recorded neural responses bilaterally in PPC. The protocols assess responses evoked by the left and right hands performing the same grasp task, and quantify firing rates when both hands act together. Five objects (represented as white icons on a monitor screen) were tested per session: two near each hand, and one at the midline. Icons of the rewarded object(s) were colored red after both hands were placed on the start keys; the animal maintained this posture during an 800-ms delay until the cue color changed to green. The animal was trained to reach, grasp and pull the object(s),

hold it for >800 ms, and then relax the grasp. The hand closest to the object was typically used to perform the task. Neural activity was recorded simultaneously from arrays of 32 implanted microelectrodes placed at symmetric locations in the PPC hand area of both hemispheres. Firing rates were compared during 8 planning and execution stages. Common patterns of activity occurred when the animal used the left, right, or both hands. Responses in the superior parietal lobule (SPL) to reach, grasp and pull by either hand were similar in time course, but were generally higher in rate to the contralateral hand. IPL neurons encoded the time course of hand actions, regardless of which hand was used to accomplish task goals. Firing rates during the delay period, when hand use was presumably planned, were lower than during action stages (reaching, hand shaping, grasping and pulling). Bimanual trials, when both hands performed the same actions in tandem, evoked prolonged spike trains that form a composite of responses to each hand alone. Bimanual spike trains reflected the timing of hand actions in single knob trials, and the trial duration was lengthened when both hands acted together. Hand preferences--percentage of trials in which each hand was used to grasp objects at the midline--are reflected in the left-right grasp sequence of bimanual trials, with the preferred hand leading the less preferred one. Bimanual coding may thereby play an instructive role in motor equivalence and skill learning when each hand performs the same movements.

Disclosures: E.P. Gardner: None. **J. Chen:** None. **J.L. Baker:** None.

Poster

251. Reaching Control: Movement Selection and Strategy

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 251.01/JJ11

Topic: D.17. Voluntary Movements

Support: NRC Grant 13-B-01

MOTIE,Korea Grant 10046150

Title: Cognitive motor interference regarding upper extremity function among stroke patients

Authors: *J.-H. SHIN[1], S.-D. EUN[1], S. JEONG[1], S. LEE[1], J. LEE[1], S. KIM[2], J. LEE[3], H. CHOI[1]
[1]Rehabil., Natl. Rehabil. Ctr., Seoul, Korea, Republic of; [2]Hanyang Univ., Seoul, Korea, Republic of; [3]Seoul Natl. Univ. Bundang Hosp., Seongnam, Korea, Republic of

Abstract: Background: Cognitive deficit after stroke is such a common symptoms that that cognitive aspects should be regarded as an important issue in the stroke patients with motor impairments. The concept of "cognitive motor interference" has been arisen to explain the situation, where cognition and motor interferes each other. We sought to characterize the cognitive motor interference during reaching in stroke patients and normal controls and to identify the relationship between motor and cognitive performance. Methods: A total of 16 stroke patients and 12 normal control subjects were enrolled. They completed midline reaching with five different kinds of cognitive tasks (COWAT categorized test, serial 7 subtractions, auditory clock, and judgment of line orientation test), or without task. The reaching performance was measured with the help of motion analysis system (VICON, Oxford, UK) using plug-in upper limb model market set. Generalized estimation equations was applied for statistical analysis using SPSS 18.0 Results: 1) Stroke versus control During reaching using affected or non-dominant hand, forward peak velocity showed significant difference between cognitive tasks and groups (stroke, normal control), and backward peak velocity showed interaction (cognitive task x group) and significant difference between groups. During reaching using unaffected or dominant hand, forward peak velocity showed significant difference between groups, and backward peak velocity showed significant between cognitive tasks. 2) Affected versus unaffected hemisphere During reaching using affected hand, there was no difference between affected hemispheres and cognitive tasks in terms of forward and backward peak velocity. During reaching using unaffected hand, forward peak velocity differs according to the cognitive task, but not affected hemisphere. 3) Mild versus moderate motor impairment During reaching using affected hand, forward peak velocity was different according to the cognitive tasks and backward peak velocity was different according to the motor impairment severity. During reaching using unaffected hand, there was no difference between different cognitive tasks and motor impairment severity. Conclusion: These findings demonstrate the cognitive motor interference in upper extremity performance among stroke and normal control, which is more evident with stroke patients. The interference depends on the cognitive task, and it was definite according to the motor impairment level. Thus we hypothesize the cognitive motor interference is derived from combination of motor and cognition capacity.

Disclosures: J. Shin: None. **S. Eun:** None. **S. Jeong:** None. **S. Lee:** None. **J. Lee:** None. **S. Kim:** None. **J. Lee:** None. **H. Choi:** None.

Poster

251. Reaching Control: Movement Selection and Strategy

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 251.02/JJ12

Topic: D.17. Voluntary Movements

Support: NIH NS064046

James S. McDonnell Foundation

Title: Abnormal muscle patterns underlie poor upper limb control during reaching in children with dystonia

Authors: *M. BERTUCCO[1], C. NGUYEN[1], T. D. SANGER[2]
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Abstract: Childhood dystonia has been defined as a movement disorder in which involuntary muscle contractions cause twisting and repetitive movements, abnormal postures, or both. Abnormal upper extremity movements are a disabling and poorly understood symptom of this disorder. Kinematic studies have shown increased random movement variability of multiple joints during reaching that may be the external consequence of the inability to suppress unwanted components of movement due to basal ganglia dysfunction. Little is known about the pattern of muscle activity that leads to abnormal postures in children with dystonia during multijoint goal-directed movements. Therefore, the aim of this study was to describe the spatio-temporal characteristics of muscle activity that underlie impaired movement execution during multjoint reaching in childhood dystonia. Twenty-one children with primary and secondary dystonia (D) and age-matched healthy control children (C) were recruited in the study. The subjects were seated and used the index finger to reach a target (width 3 cm) distanced approximately 90% of the arm's reach length from the body as fast and accurately as possible. Kinematic data were recorded using a magnetic tracking device with eight sensors attached at the bony landmarks of the principal upper limb joints. The electrical activity of eight upper limb muscles was recorded using surface electromyography (EMG). We found significantly longer movement times (on average C = 0.680 and D=1.056 s; $p< 0.01$) and less accurate performance, computed as the endpoint variability at the target, (on average C = 0.46 and D=2.11 cm2; $p< 0.01$) in children with dystonia. Additionally, children with dystonia showed a larger variability (measured as standard deviation) both at distal (hand, C = 4.1 and D=6.1 cm; $p< 0.01$) and proximal joints (shoulder, C = 1.3 and D=1.9, $p< 0.01$). Principal Component Analysis on the kinematic data, computed on the three-dimensional joint trajectory, showed a lower signal-to-noise ratio in group D with respect to group C. The EMG analysis showed an abnormal tri-phasic muscle pattern throughout the movement in group D. Although both groups showed similar magnitudes of co-contraction either at the shoulder and elbow joints, group D displayed a different time-varying co-contraction with a distinctive ramp pattern while approaching the target. The results suggest that abnormal spatio-temporal activation of agonist-antagonist muscles contribute to the

abnormal postures in childhood dystonia, and lead to jerky and imprecise trajectories during reaching.

Disclosures: **M. Bertucco:** None. **C. Nguyen:** None. **T.D. Sanger:** None.

Poster

251. Reaching Control: Movement Selection and Strategy

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 251.03/JJ13

Topic: D.17. Voluntary Movements

Support: ONR Grant N000141210588

Title: Advanced techniques for the computational modeling of human movement

Authors: ***G. G. GAMBLE**, M. YAZDANI, R. HECHT-NIELSEN
UCSD, San Diego, CA

Abstract: Computational modeling has been a successful tool in describing many aspects and characteristics of human movements. While such models typically have a sound theoretical and mathematical foundation, their interpretations and neuronal implementations in the human motor system are not clear. We propose several techniques that enable an analysis of computational models that elucidate the neural implementation and behavior of the underlying motor system. We offer methods which can transform existing, purely mathematical models into models that represent anatomy and physiology more directly, thus rendering these models easier to interpret in terms of the motor system. For example, we show how to convert most optimal control models of movement from utilizing continuous control signals to instead utilizing sparse control signals. Even though sparse signals are discontinuous, the movements that result are continuous and smooth. Such sparse control signals have a clear neuronal interpretation as a sequence of spikes. These control signals are qualitatively simpler than their non-sparse counterparts, yet yield comparable if not better results when applied towards modeling human movements. We demonstrate this by quantitatively comparing this technique to human reaching movements. In addition, we discuss new ways to visually analyze data of human movement as compared to computational model output. Other related techniques will also be discussed.

Disclosures: **G.G. Gamble:** None. **M. Yazdani:** None. **R. Hecht-Nielsen:** None.

Poster

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Topic: D.17. Voluntary Movements

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Title: Bayesian filtering of surface electromyography as a human-computer interface

Authors: *A. FEINMAN, M. BERTUCCO, N. H. BHANPURI, T. D. SANGER
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Abstract: Surface electromyography (sEMG) has shown tremendous promise as a non-invasive human-computer interface (HCI) for patients with motor system impairments, such as amputation or dystonia. In previous work, our group has shown that controlling the sEMG signal (i.e., myocontrol) through the implementation of non-linear Bayesian filtering algorithms for sEMG. opens the possibility of using sEMG to control devices without complex post-processing algorithms. In this study, we used sEMG with Bayesian filtering for real-time control of a robotic arm. Four subjects were given control of two degrees of freedom (vertical and lateral endpoint position). Control of the robot was examined using a paradigm similar to the original Fitts' Law experiment (Fitts 1954). In each trial, a pair of bars was presented to the subject, and they were asked to tap the robot endpoint back and forth between the two bars as quickly as possible without missing. Subjects generated isometric contractions of the FCU and biceps by grasping a rod fixed to their chair, with FCU controlling lateral position and biceps controlling vertical position. When the subject was at rest, the robot endpoint rested in one of the bars. Subjects had to control their contractions and relaxations to tap between the bars. The two bars were identical in shape in each trial, but the width of the bars and/or the distance between the bars varied across trials to generate six indices of difficulty in the range of 1-4 bits. The forward movement of successful forward-and-back movements was analyzed. Movement times of the forward movement for successful trials ranged from 0.6-0.9 seconds across the set of IDs. The index of performance across these 4 subjects with little practice was 9.1 bits/s, as compared to 10.3 bits/s as reported during use of a computer mouse (Card, English, and Burr 1978). This data shows that Bayesian filtering of sEMG has the capacity to serve directly as an HCI for simple movement tasks. Control of more complex systems, such as myoelectric prosthetics, may be improved through use of this method. Furthermore, examining how healthy and impaired individuals learn to use myoelectric devices may provide an insightful comparison to how both healthy and diseased sensorimotor systems control bodily limbs.

Disclosures: **A. Feinman:** None. **M. Bertucco:** None. **N.H. Bhanpuri:** None. **T.D. Sanger:** None.

Poster

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Topic: D.17. Voluntary Movements

Support: NIH R01NS052236

Title: Using non-negative matrix factorization as a filter to improve usability of myocontrol

Authors: ***C. NGUYEN**[1], M. BERTUCCO[1], D. J. BERGER[2], A. D'AVELLA[2], T. D. SANGER[1]
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Abstract: For children with dystonia, prosthetic devices can provide mobility, manipulation, and functional communication. However, the goals of prosthetic control in children with impairments need to be different from those in adults. Children need flexible interfaces whose motions are not described in advance, so they can develop their own movements and explore varying and unpredictable goals. We use electromyographic control ("myocontrol") to accomplish this. Surface EMG is a non-invasive way to capture EMG, but the high level of noise makes it difficult to use in real-world applications. Furthermore, it has proven difficult for even healthy adults to learn myocontrol with multiple degrees of freedom. These issues may be addressed with Bayesian non-linear filtering of EMG and dimensionality reduction from the high-dimensional muscle space. We have developed a Fitts' Law paradigm to assess the information transmission during myocontrol. Previously, we showed that myocontrol in healthy young adults using single muscle control with Bayesian-filtered EMG has a performance level comparable to that of force control. In this study, we tested whether muscle synergies affect the feasibility of myocontrol in young healthy adults, children with dystonia and age-matched controls by comparing 3 different myocontrol conditions: (1) single muscle control, (2) all muscle control, (3) synergy control. Subjects moved a cursor in two-dimensions in an isometric point-to-point reaching task using myocontrol. In single muscle control, one muscle corresponded to one dimension of movement. For our task, the flexor carpi ulnaris moved the cursor horizontally and the biceps moved it vertically. For all muscle control, EMG from 8 muscles was mapped to the 2-dimensional plane using multiple regression with the torque direction. For synergy control,

synergies were identified using non-negative matrix factorization. Fitts' Law was used to calculate the average index of performance (IP) for each synergy in the analogous 1-D task. The 3 synergies with the highest IPs were then used to control the cursor in the 2-D task. The speed-accuracy tradeoff across the 3 different myocontrol conditions was similar in controls, however, performance improved for children with dystonia. Results suggest that dimensionality reduction may act as an additional, personalized filter in noisy EMG. This work will also improve our understanding of the existence of synergies in dystonia and direct future research in rehabilitation.

Disclosures: C. Nguyen: None. **M. Bertucco:** None. **T.D. Sanger:** None. **D.J. Berger:** None. **A. D'Avella:** None.

Poster

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Topic: D.17. Voluntary Movements

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Title: An empirical validation of the cost of effort during arm-reaching

Authors: *A. A. AHMED
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Abstract: An optimization approach has proven particularly successful in developing theoretical models of movement control. The underlying theory is that the brain optimizes movement over the following cost function that includes a cost for error and a cost for effort, representing the intuitive idea that one generally seeks to move in the most accurate and most efficient manner possible. Models implementing such cost functions have done an impressive job explaining behavioral observations at multiple levels of analysis and in multiple tasks. However, there is an increasing amount of experimental observations that deviate from optimality. This highlights a fundamental assumption that limits this approach's explanatory power. The difficulty of directly measuring movement effort costs forces one to make a priori assumptions about how the

movement effort costs are represented. Models that penalize effort as a function of squared force, squared torque, square rate of toque development have all been able to predict human motor behavior, without any experimental validation of the actual effort cost. Here we measure effort costs directly in the form of metabolic rate, via expired gas analysis, in a variety of reaching tasks, to determine the function that most appropriately represents effort costs during human reaching movements. Subjects made planar reaching movements while grasping the handle of a robotic arm. They reached at a number of different speeds, to different distances, at prescribed curvatures, and against different resistances. During all reaching movements we measured metabolic rate via expired gas analysis. We developed a two-link arm model and simulated the reaching movements in the experiments. For each movement, we calculated effort costs that have been historically successful in predicting reaching trajectories or metabolic cost in locomotion: mechanical work, torque squared, rate of torque development squared, and more. We also calculated the sum of absolute force, as it has been hypothesized that effort should track linear force. Model-calculated efforts costs were compared to experimentally measured effort costs to determine the best representation of effort cost during reaching movements. Preliminary results show that a linear cost on force and mechanical work did a poor job of predicting movement trajectories, compared to torque-squared. Thus far, these findings provide empirical support for the quadratic effort term commonly implemented in optimal control models of arm-reaching.

Disclosures: A.A. Ahmed: None.

Poster

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Topic: D.17. Voluntary Movements

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Title: The two learning systems in motor control: reward vs. sensory prediction errors

Authors: *P. VASWANI[1], R. SHADMEHR[2]
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Abstract: The difference between the expected and actual consequences of action, called sensory prediction error (SPE), is a critical signal for motor learning. However, many studies

have demonstrated that reward can influence behavior. Typically, these two goals are congruous; actions that minimize SPE also maximize reward. However, the neural bases of learning from these two signals are separate. When faced with conflicting signals, how does the brain select behavior? We asked subjects to make reaching movements and, using two viscous force fields or visuomotor rotations, presented subjects with two sensory consequences (cursors) for each action. If subjects can learn actions that maximize reward, they should learn the mapping of actions to one consequence and ignore the large errors in the other. Instead, subjects chose actions that minimized SPE across trials, forsaking reward. We found that even when subjects were taught a rewarding solution, when presented with both perturbations unpredictably, they chose actions that minimized SPE but did not maximize reward. Next, we presented subjects with both sensory consequences in a single trial. In this case, there was no SPE, but both perturbations were present. Subjects did not adapt to the mean perturbation, and instead selected a rewarding solution. To verify that the reward (points) in our task was affecting behavior, we presented subjects with both perturbations simultaneously, but consistently rewarded only one of the consequences. We observe that subjects always selected the rewarding solution. Therefore, when faced with no SPEs, actions maximized reward. However, when faced with SPEs, actions appeared blind to reward. Finally, we asked if a strong reward signal could overcome the hegemony of learning from SPE. We provided subjects with a mild SPE by coloring one of the two cursors, unpredictably. When both cursors were rewarded, i.e. in the presence of a strong reward cue, subjects' behavior was biased by the SPE, but they were able to maintain behavior that resulted in task success. However, when we reduced the frequency of the reward, in the presence of the mild SPE, subjects no longer maintained a solution that received reward, and instead reduced the SPE. Sensory prediction error provides a strong learning signal to the motor system that dominates behavior, even when it results in a reduction in reward. In the absence of an SPE, the motor system can maximize reward. Only when SPE is mild and a strong reward signal is provided do we observe a balance between the two systems. Otherwise, sensory prediction error dominates motor learning.

Disclosures: P. Vaswani: None. **R. Shadmehr:** None.

Poster

251. Reaching Control: Movement Selection and Strategy

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Topic: D.17. Voluntary Movements

Support: NIDRR Grant H133E080024

Title: Does task engagement level affect how we move?

Authors: *Y.-A. CHEN, Y.-C. CHUNG, B. KIM, C. WINSTEIN
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Abstract: High subjective engagement level is consistently found when motor tasks are performed under virtual or augmented reality conditions. In previous work, attentional demand was greater during a reach task to a virtual target compared to a real target. More importantly, engagement level was generally higher in the more difficult virtual compared to the easier real target condition. This study investigated the impact of engagement level on movement strategy by comparing reach kinematics for those with low- or high-engagement ratings during virtual target (VT) and real target (RT) conditions. Using a within-subject design, 15 participants (59-88 yrs old) performed reach movements to targets arranged in a circular 8-target pattern; there were 48 reaches per condition and each from standing and stepping. A Kinect™ camera positioned in front captured each reach; position data were resampled at 12Hz. Movement onset/offset was defined as the first time at which the tangential velocity of the wrist virtual marker was above/below 1 cm/s. Kinematic variables included movement time (MT), time to peak velocity (TTPV), and time after peak velocity (TAPV). Participants' self-assessed engagement to each condition was acquired using a 5-level Likert scale for a single item administered after reaching. Participants were categorized into low-, neutral- and high- engagement (E) groups based on scale scores. Reaches from standing to VT for participants with high-E showed longer MT than those with low-E; the longer MT was attributed to longer TAPV. Interestingly, MT for reaches from stepping to VT were independent of engagement. However, in spite of similar MT, the high-E group demonstrated shorter TTPV and longer TAPV than the low-E group. For easier reaches to RT, there was no evidence that engagement level modulated any kinematic measure. These findings provide indirect evidence that engagement level may impact movement strategy, but only for relatively difficult tasks. Reaches to RT were relatively easy (from open-ended post-interview); RT reaches exhibited a symmetric velocity profile suggestive of a default strategy regardless of engagement level. In contrast, during more challenging VT reaches, a high-E level was associated with slower movements and a prolonged homing-in phase. We speculate that high-E may harness more attentional resources toward goal achievement. These preliminary group level findings suggest an interaction between engagement level and task difficulty on movement strategy. Further analysis of individual-subject variability within engagement level groups is needed to confirm or refute the group level analysis.

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Poster

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Title: Characterization of visuomotor behavior in patients with schizophrenia under a 3D-depth inversion illusion

Authors: *J. NGUYEN, S. M. SILVERSTEIN, T. V. PAPATHOMAS, E. B. TORRES
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Abstract: Evidence has shown that patients with schizophrenia (SZ) are less susceptible to experiencing depth-inversion illusions (DIIs) (Keane et al., 2013; Dima et al., 2009; Koethe et al., 2006; Schneider et al., 2002). DIIs produce illusory motion and perceived depth reversal of scenes or objects in which physically concave angles are perceived as convex and vice versa (Papathomas, 2007). It has been hypothesized that the observed reduction in sensitivity to DIIs may be due to reduced ability to apply stored knowledge to visual perceptual processes. How this anomaly translates to sensory motor processes and kinesthetic sensing is largely unexplored in SZ. We examine the signatures of motor output variability of continuous reach-to-grasp motions in a number of patients with SZ in relation to typically developing controls. They are asked to reach for an embedded target on a 3D-DII. Specifically, we study motions with different levels of intent: the goal-directed reach deliberately aimed at the target, and the transitional, non-instructed retraction of the arm as subjects bring their hand to rest. The signal-to-noise patterns embedded in the motor output variability of these continuous motions are a form of kinesthetic re-afference. From trial to trial, their modulation and central control depend on the continuous returning afferent stream, which those motions themselves cause. In typical controls, their stochastic signatures discriminate between the different levels of intent, but these patterns are disrupted in clinical populations, including autism and Parkinson's disease (Torres et al., 2013; Yanovich, et al., 2013). Since little is known about sensory-motor integration and kinesthetic re-afference sensing in SZ, especially in the transitional movement domain, we here aim to characterize any perturbations that may arise in addition to visual perceptual impairments. Two 3D stimuli were used: (1) a proper-perspective in which perspective-painted cues are congruent

with the bottom-up signals of binocular disparity and motion parallax, and (2) a reverse-perspective, in which the painted cues competed with bottom-up signals, eliciting bistable percepts of: (a) veridical depth and (b) illusory reverse-depth percept in which concave parts are perceived as convex and vice versa. The control subjects viewed the stimuli and grabbed at planar disk targets while we recorded their movements. Their signatures of motor output variability could automatically discriminate levels of intent and illusory state. We discuss signatures of motor output variability for the SZ patients in relation to the control subjects.

Disclosures: J. Nguyen: None. **T.V. Papathomas:** None. **S.M. Silverstein:** None. **E.B. Torres:** None.

Poster

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Title: Identifying MI/PMv neural ensemble states associated with motor errors using spike train similarity analysis

Authors: *T. C. WHALEN[1], C. E. VARGAS-IRWIN[2,3], J. P. DONOGHUE[5,2,4,3]
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Abstract: Neural encoding of movement-related variables is frequently examined within the context of instructed delay tasks. Analysis typically focuses on trials where the subject correctly interprets the instructions, choosing an action previously associated with a specific cue. Here, we compare neural activity patterns associated with correct and incorrect action selection: in addition to decoding what action was ultimately executed, we use neural activity patterns to identify trials in which the instructional cue was not correctly interpreted. We trained three male rhesus macaques to perform a reach-and-grasp task in which a cue for one of two grips is given followed by a go cue. Data was recorded from motor cortex (M1) and ventral premotor cortex (PMv) using chronically implanted microelectrode arrays. Single unit activity was sorted using an automated density-based algorithm (Vargas-Irwin and Donoghue 2007). We projected spiking activity during the delay between grip and go cues into a spike train similarity space (SSIMS) to evaluate the relationship between spiking patterns. The SSIMS algorithm produces a low-dimensional representation of neural activity where the distance between points quantitatively represents the similarity between recorded ensemble states. We find that correctly executed trials produce a consistent progression of ensemble states that clearly separate clusters associated with specific grip strategies. Error trials diverge from this set of trajectories, traversing ensemble states which are not normally encountered in correctly executed trials, despite identical actions being performed. Error trajectories diverge from normal trajectories at various times, including errors occurring during cue encoding as well as grip selection. Using SSIMS models, we successfully differentiate between correct and error trials for a given action with accuracy significantly higher than chance. We find no significant differences between classification accuracy in M1 and PMv, and find that small ensembles of approximately ten neurons are generally sufficient for classification. These findings suggest the existence of a neural signature of errors present in cortical motor areas. Early detection of these possible error states may contribute to designing more accurate neural decoding algorithms for use in brain computer interfaces.

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Poster

251. Reaching Control: Movement Selection and Strategy

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Topic: D.17. Voluntary Movements

Title: Inverse vs forward dynamics analysis of join control during arm movements

Authors: *N. DOUNSKAIA, W. WANG
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Abstract: During multi-joint movements, the limb segments mechanically affect each other, and therefore, the joints cannot be controlled independently. How the brain organizes control of multi-joint movements has been extensively studied. Inverse dynamics models have been used with this purpose for longer than 40 years. They allow the assessment of the contribution of active and passive torques to net torque (NT) at each joint. More recently, the induced acceleration analysis (IAA) developed with the use of forward dynamics models has started to gain popularity. The IAA allows estimation of contribution of active and passive torques to joint accelerations. However, the IAA is more complex because it requires the usage of both the inverse and forward model. In addition, interpretation of passive factors that contribute to joint accelerations is not straightforward. We compared the inverse dynamics analysis and the IAA by applying them to 3D arm movements performed during a free-stroke drawing task. The task requires the production of series of strokes from the center to the perimeter of a horizontal circle while selecting stroke directions in a random order. In our previous studies, subjects performed the free-stroke drawing task while keeping the arm in the horizontal plane. An anisotropic distribution of strokes revealed directional preferences. The inverse dynamics analyses demonstrated that the preferred directions were characterized by a specific control pattern of the shoulder and elbow during which one joint was rotated actively, by muscle torque (MT) and the other joint was rotated predominantly passively, by interaction torque (IT). In the present study, gravitation torque (GT) affected joint motions in addition to IT and MT. The inverse dynamics model was used to compute two characteristics, a contribution of passive torque (PT=IT+GT) to NT and impulses of MT, IT and GT. The IAA was used to compute the contribution to joint acceleration of MT, GT, and other passive torques that depended on joint velocity as well as on MT and GT applied to the other joints. All three analyses revealed the same pattern of joint control in both the preferred and non-preferred directions. The interpretation of the passive mechanical factors influencing joint motions was more straightforward when the inverse dynamics analyses were used. We conclude that, if a general pattern of joint control needs to be assessed, inverse dynamics analyses are adequate, and may be preferable due to simplicity and straightforward interpretation of inter-segmental dynamics effects. However, the IAA may be advantageous for precise assessment of the contribution of various factors to joint accelerations.

Disclosures: **N. Dounskaia:** None. **W. Wang:** None.

Poster

251. Reaching Control: Movement Selection and Strategy

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Topic: D.17. Voluntary Movements

Support: BCS0744747

Title: Influence of gravity on direction preferences of arm movements

Authors: *W. WANG[1], N. DOUNSKAIA[2]
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Abstract: We previously demonstrated directional preferences during horizontal arm movements with the use of a free-stroke drawing task. Subjects produced strokes with the fingertip from the center to the perimeter of a horizontal circle, selecting movement directions in a random order. An anisotropic distribution of strokes revealed preferred directions. They were characterized with a specific joint coordination pattern that included active control by muscle torque (MT) of either the shoulder or elbow (the leading joint) and passive motion, due to interaction torque (IT), of the other (trailing) joint. The wrist was voluntarily fixed in all directions. Here, we investigated the influence of gravitational torque (GT) on the directional preferences. Again, the free-stroke drawing task was used but the circle was orientated in two vertical planes, frontal and sagittal. Also, the arm's joints were allowed to rotate in 3D space. Frequency of stroke production across directions and torques at the three joints were computed. Preferred directions were apparent in both conditions. In all preferred directions, at least one joint, either the shoulder or elbow, was accelerated largely by passive torque (PT=IT + GT). In each condition, there was one preferred direction in which GT also played a dominant role in accelerating the other joint. The wrist was predominantly fixed in all directions. The results supported the previous finding that there is a preference to passively rotate one of the two joints of the shoulder-elbow linkage (the trailing joint). In contrast to horizontal arm movements during which passive motion of this joint was produced by IT generated by motion of the leading joint, both IT and GT contributed to the trailing joint motion production during the non-horizontal movements. In addition, gravity was often exploited to substitute for MT in the production of motion of the leading joint (the joint that generated IT used to rotate the trailing joint). However, the possibility to rotate the leading joint by GT did not make these directions more preferred than those in which the leading joint rotated actively. This finding does not support the minimization of muscle energy expenditure as a primary factor that determines the anisotropic preferences of arm movements. Rather, the revealed directional preferences point to the use of the internal model of inter-segmental dynamics during movement planning for selection of movement directions in which neural control that provides coordination of joint motions during movement execution is simplified by using predominantly passive motion of at least one joint.

Disclosures: **W. Wang:** None. **N. Dounskaia:** None.

Poster

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Title: Electrocorticographic decoding of high-level action goals following verbal instruction

Authors: *G. HOTSON[1], G. MILSAP[2], D. P. MCMULLEN[3], B. A. WESTER[5], W. S. ANDERSON[3], J. W. KRAKAUER[4], N. E. CRONE[4], N. V. THAKOR[2]
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Abstract: Brain-machine interfaces have recently made great strides in allowing subjects to reach out and grasp objects. However, this is only the first step in enabling users to accomplish more complex action sequences. Brain-machine interfaces under direct neural control currently lack the speed and precision to perform complex action sequences to accomplish meaningful activities of daily living. Decoding high-level action goals would therefore be of great benefit, as this would allow for a semi-autonomous brain-machine interface to assume low-level control of the effector to perform the necessary action sequence. Here we show that a high-level representation of goal intention can be found in the high gamma signal of electrocorticography following verbal instruction cues. Subjects were first instructed with one of two high-level goals ("look" or "drink"), then instructed how to accomplish the goal ("arm" or "waist"), and finally they were given a "go" cue. Upon hearing the "go" cue, the subject inspected the water level of a measuring cup or drank from it with a straw. Either task could be performed by either bending at the waist or picking the cup up with their arm, depending on which cue they received. We found that the high gamma activity preceding the go signal was significantly modulated by the initial high-level goal cue ($p<0.05$, Tukey's test with Bonferroni correction for multiple comparisons

across time points). This activation was located in electrodes over the left posterior middle temporal gyrus, an area which has previously been implicated in decoding word semantics (particularly when manipulable tools are involved). An accuracy of 80% was achieved when classifying between the two high level goals when "waist" was the second cue. This classification was done during the time period immediately preceding the "go" cue through linear discriminant analysis with leave-one-out cross-validation. These results suggest that a user's high-level action goal can be decoded from electrocorticography, and can potentially be used to help operate a semi-autonomous brain-machine interface.

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Poster

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DST-IRPHA

Title: Eye-hand coordination: Evidences for interaction at the level of motor planning

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Abstract: Eye-hand coordination appears to be a ubiquitous element underlying many of our skilled behaviors. It is still unclear how eye and hand systems, which are functionally and anatomically separate, are coupled to produce coordinated eye and hand movements when a task demands it. Though it is generally accepted that coordinated movements are generated by the interaction between these two separate systems, the computational architecture underlying such interaction remains unclear. While McPeek and colleagues have shown that eye and hand systems appear to index a common target selection stage, the possibility of interactions occurring between eye and hand effectors during motor planning, is not known. With this particular aim, we recorded the eye and hand movements of human subjects performing fast and slow hand

movements to a peripherally appearing target after a variable fixation time. No explicit instruction was given concerning the eye movements. The velocity instructions were given 1000 ms before the appearance of the target so that the subjects could prepare for the upcoming movement. This negated the confound that the reaction time of the subjects maybe influenced by the added decision making stage regarding the velocity to be employed for the current trial. Since visual and the positional attributes of the target were identical, differences across velocity conditions could be attributed to interactions during motor planning independent of target selection. The comparison between the trials of fast and slow conditions revealed that eye movements, though not explicitly instructed, were affected by the conditions. Eye movements had higher peak velocities when accompanied by a fast hand movement. The correlation between the eye and hand peak velocity, though low, was significant, and was sensitive to the hand velocity. We also observed a trial to trial variation in the amplitude of the eye and hand, which we attributed to inaccuracy at the level of target selection. However, even after the effect of amplitudes was regressed out, we observed a low but significant correlation between peak velocities of the eye and hand, which we attribute to interactions occurring during movement planning.

Disclosures: **A. Gopal P A:** None. **S. Jana:** None. **A. Murthy:** None.

Poster

251. Reaching Control: Movement Selection and Strategy

Location: Halls A-C

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Topic: D.17. Voluntary Movements

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Title: Impedance-based communication for human-robot interaction

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Abstract: The improvement of human-robot interaction is an active area of investigation. Enabling humans and robots to cooperate in attaining common goals has important implications

for a wide variety of activities, including recreation, military applications, and approaches aimed at rehabilitation of sensorimotor function using exoskeletons. Physical interaction between humans and robots has been mainly characterized by the notion of mechanical impedance. However, the extent to which limb impedance can be used to physically convey information about high-level goals, e.g. the preferred direction of motion, remains to be investigated. The present study was designed to quantify human-human physical interactions where one agent ("follower") was instructed to infer the intended direction of motion of another agent ("leader"). The strategy used by human agents, consisting of the "follower" probing the upper limb impedance of the "leader", was then studied during interactions between a human and a robot agent. We found that human subjects could infer the intended direction of the other human agent with a level of accuracy (48.8% on average for all directions) above chance level (16.6%, 1 out of 6 possible directions). We interpreted these findings as evidence for arm impedance being used as a means of communicating preferred directions among cooperating agents. To test this hypothesis, we conducted experiments involving a human subject and a robot arm using the same human-human collaboration. Both roles (leader, follower) were given to both agents (human, robot) in two separate experiments. The results showed that control of the leader's impedance was a critical factor in conveying the preferred direction to the follower. Specifically, when subjects were asked to infer the intended movement direction of a robot, or vice versa (68% and 51% of level of accuracy on average for all directions, respectively), an impedance-based strategy similar to that used during human-human interactions could account for the high percentage of accurate inferences of direction of the planned arm movement. The present findings support the notion of impedance as a viable means of communication between biological and non-biological agents and its future potential for being utilized to communicate high-level goals during cooperative actions.

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Poster

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Topic: D.17. Voluntary Movements

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Title: Independent control of stabilizing reflexes and triggered reactions in the human arm

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Abstract: The long-latency stretch reflex is a motor response to external perturbations of posture within a time window slower than short-latency reflex but prior to the onset of voluntary activity, and plays important roles at the interface of reflexive (rapid and automatic) and volitional (slow and adaptable) control. Previous studies demonstrated two distinct components of the long-latency stretch reflex: stabilizing reflexes opposing the perturbations and triggered reactions releasing pre-planned motor actions. Each of these two distinct functions has been extensively investigated separately, and there is growing consensus that multiple convergent neural pathways contribute to them. However, it is not known how these neural pathways are integrated when both postural stability and prepared motor action are relevant. Our hypothesis was that the stabilizing and triggered components of the long-latency stretch reflex can be modulated independently during arm reaching movements. This hypothesis was evaluated by assessing reflex modulation in mechanical environments that destabilize arm posture in a controlled fashion, independently from the planned movements. Our results demonstrate significant environmental specific modulation (stabilizing reflex) and target dependent modulation (triggered reaction) of the long-latency stretch reflex, and no interaction between them within individual muscles during the transition from posture to movement. These results support independent control of two different components of the long-latency stretch reflex and strengthen the growing consensus of multiple convergent pathways contributing to it: corticospinal pathways for stabilizing reflexes and brainstem pathways for triggered reactions.

Disclosures: H. Lee: None. **E. Perreault:** None.

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Topic: D.17. Voluntary Movements

Support: Falk Medical Research Trust

Title: The role of the cortex in the control of arm stability

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Abstract: Arm control has been studied behaviorally for many years. While there is a good understanding of the systems level processes that mediate arm control and stabilization, the neural mechanisms, particularly those involved in arm stability remain unclear. In the work presented here, we use electroencephalography (EEG) and electromyography (EMG) together with goal directed stabilization tasks to examine what extent co-contraction and intermittent voluntary corrections contribute to stabilization and to understand how brain areas interact over time to stabilize the arm. Ten college aged subjects performed a series of 2D reach and hold tasks using a passive arm support system (recording limb position) to control a cursor projected onto a horizontal display. Each task condition consisted of a point to point movement to a target followed by a 4 second stabilization period. In the Stability condition, the subjects' arm was perturbed during the stabilization period. In the Voluntary condition, subjects were instructed to mimic the perturbation paths from the Stability condition upon reaching the target. In the Co-contraction condition, subjects were asked to co-contract (10 - 20 %) during the stabilization period. In the Point to Point condition, subjects were asked to stabilize at the target with no perturbations applied. EEG data was recorded using a 64 channel electrode system, filtered from 0.1 to 100 Hz, amplified and processed using independent component analysis to remove artifacts. EMG data was recorded from the flexor carpi radialis, extensor carpi ulnaris, biceps, lateral head of the triceps, and the anterior and posterior deltoid, filtered from 10 to 350 Hz, amplified by 1000, root mean squared (100 ms window) and normalized. MATLAB was used for all data analysis. Spectrograms and time courses in individual power bands were computed for the EEG data to examine the time course of cortex activity. The spectrograms for each of the four conditions all show a higher power in the rest period before movement compared to the stabilization period in the Beta band (13 - 26 Hz), which is consistent with the phenomenon known as event related desynchronization (ERD). The ERD occurred within 500 ms of the movement cue, during the point to point movement and was sustained throughout the entire stabilization period for all four conditions. Analysis of the individual power bands showed an enhanced ERD during the stabilization period for the Co-contraction and Voluntary conditions versus the Stability condition versus the Point to Point condition. These results suggest Beta band power may be associated with error processing due to the varying amount of error in each condition.

Disclosures: D.B. Snyder: None. **S.A. Beardsley:** None. **B.D. Schmit:** None.

Poster

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Topic: D.17. Voluntary Movements

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BMBF Bernstein Center for Computational Neuroscience 01GQ1005C

DFG CRC-889

Title: Graded neural selectivity in sensorimotor cortex reflects reward-independent preferences during rule-based reach goal selection in monkey

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Abstract: Neurons in dorsal premotor cortex (PMd) and parietal reach region (PRR) can simultaneously encode two alternative motor goals when monkeys have equal preference to choose either goal. This is true even if goals are selected based on two competing spatial transformation rules, i.e. without a spatial target stimulus marking the potential motor goal locations. (Klaes et al, 2011). Here, we test if graded preferences for such rule-based reach goals are reflected in graded spatial selectivity of individual neurons during decision making, and if preference encoding occurs independent of reward expectancy. A rhesus monkey had to determine the correct reach goal from two instructive cues. A pre-cue consisted of two differently colored triangles which appeared at one of the four cardinal directions from the center (e.g. top) and which pointed to two opposite directions (e.g. left and right). The pre-cue indicated, first, the two possible goals in a given trial, located at 90^o clockwise (CW) and 90^o counterclockwise (CCW) relative to its own position (rule). Second, the triangle sizes could differ and represented the probability with which the rule corresponding to each triangle would be instructed later. After a delay, a spatially neutral color cue was presented. In instructed trials the cue was equal-colored to one of the pre-cue triangles and indicated the valid rule. In interspersed free-choice trials the color was neutral and both potential goals were randomly rewarded with equal amount and probability. Preliminary results show that the pre-cue successfully induced a reward-independent graded preference in the monkey: The response alternative which was more likely to be instructed at the end of the trial according to the size of the pre-cue, was chosen more often and faster even in free-choice trials when there was no objective advantage from freely selecting this alternative. The spatial selectivity of individual PMd and PRR neurons during motor planning was modulated in accordance with the degree of later choice bias, i.e. showed weak spatial selectivity in balanced trials and increasingly stronger selectivity with increasing bias of the animal. We conclude that in a rule-based motor goal

selection task, the probability with which a rule is instructed induces a reward-independent subjective preference in free-choice behavior. The degree of behavioral preference is reflected in the degree of spatial selectivity of individual neurons in PRR and PMd corroborating the relevance of both areas in rule-based motor goal selection. Klaes et al. (2011) *Neuron 70*:536.

Disclosures: L. Suriya-Arunroj: None. **A. Gail:** None.

Poster

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Topic: D.17. Voluntary Movements

Support: NIH, NINDS R21NS075944

Title: Vestibular contribution to arm movement planning

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Abstract: Multiple studies have demonstrated the brain's ability to build internal representations of environmental and musculoskeletal dynamics, allowing efficient planning of limb movements. We have recently reported that both humans and monkeys optimally utilize the gravitational force when reaching in the vertical plane. Furthermore, vestibular signals are thought to allow the macaque brain to compute an internal model of the gravity force (Angelaki et al. 1999, 2004). The present study aimed at probing the potential role of vestibular signals in planning arm movements in macaques. Using a virtual reality system, three macaques were trained to perform single degree of freedom reaching movements between sets of two targets. The task consisted of shoulder flex/extension or shoulder ab/adduction (20 degrees amplitude). Kinematic and electromyographic (fine wire intra-muscular electrodes) signals were recorded from the right arm. After extensive training, two monkeys underwent a bilateral labyrinthectomy (ablation of the vestibular organs) and daily recordings were performed starting 24h post-surgery. Before surgery, we observed directional asymmetries on the kinematics of arm movements performed in the vertical plane but not in the horizontal plane (as previously reported). Precisely, the time to peak acceleration was shorter and the acceleration peak was larger for upward than for

downward movements. These asymmetries result from specific patterns of muscle activations that (along with theoretical simulations) demonstrate the optimal integration of the gravitational force into the planning processes of reaching movements. After surgery, we observed specific modifications of movement kinematics in the vertical plane but not in the horizontal plane. First, spatial errors that differed between upward (undershoot) and downward (overshoot) movements were observed early after surgery (first 3 sessions). Second, the above-mentioned directional asymmetry disappeared or even slightly reversed; i.e. the time to peak acceleration being longer and the peak acceleration being smaller for upward than for downward movements. This effect lasted for about ten days and slowly vanished such that vertical movements progressively returned to normal (after 20 days). Overall, our results unveil the contribution of vestibular signals to the planning of reaching movements: monkeys behaved as if the gravity force was absent immediately after labyrinthectomy. Furthermore, these results suggest that remaining sensory cues (visual, somatosensory) allowed the monkeys to progressively re-optimize the planning of vertical arm movements after labyrinthectomy.

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Poster

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Topic: D.17. Voluntary Movements

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Pennsylvania Department of Health

Title: Stochastic motor control as probabilistic inference in spiking neural networks with noise

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Abstract: The organizational principles of motor control in biological organisms are still largely unknown. Several recent publications, based on behavioral data and abstract models, have

proposed that probabilistic inference provides a suitable theoretical framework for understanding salient aspects of biological motor control [1,2]. Bayesian inference is a special case of such probabilistic inference (where one focuses on the role of priors and applications of Bayes' theorem). While these approaches reproduce many features of actual movements, it has been difficult to relate these abstract models to the biological substrate of neural computation and motor control in the central nervous system, i.e., to networks of spiking neurons. Therefore it has not been possible to test the validity of these models against neural recordings. Here we propose a model that implements stochastic motor control by solving a probabilistic inference problem with networks of spiking neurons. Our approach is based on recent work that has shown that the stochastic dynamics of networks of spiking neurons can approximate probabilistic inference through sampling [3]. This finding provides a solution to an important subclass of probabilistic inference problems and allows us to solve behaviorally relevant motor control task directly with spiking neurons, even with biologically realistic substantial amounts of noise. Furthermore, the architecture of our network allows the control problem to be inverted to predict the outcomes of motor acts. We demonstrate the viability of our model in a simulation of a standard arm reaching task, and show that the model readily reproduces a number of experimental results. Specifically, (1) we show that the network is able to compensate for perturbations applied during task execution, (2) we find that the strategies exploited by the model for corrective movements are similar to those exploited by behaving monkeys, and (3) we show that the experimentally observed trial-to-trial variability of our model neurons mimics recordings from motor cortex. This trial-to-trial variability enables the network to exploit redundancies in the task representation in order to explore alternative solutions. Our work thus provides a missing link between theoretical models of motor control and their biological implementation. **References:** [1] Botvinick M, Toussaint M (2012) Planning as inference. Trends Cogn. Sci. [2] Doya K (2006) The Bayesian Brain: Probabilistic Approaches to Neural Coding. MIT Press. [3] Buesing L, Bill J, Nessler B, Maass W (2011) Neural dynamics as sampling: A model for stochastic computation in recurrent networks of spiking neurons. PLoS Comp. Biol.

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Poster

251. Reaching Control: Movement Selection and Strategy

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Topic: D.17. Voluntary Movements

Title: Sport fencing as a model system for studying complex, highly trained reaching movements

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Abstract: Neural strategies and mechanisms underlying reaching movements have received intense study in both animals and humans. In most previous multi-joint reaching experiments, subjects were instructed to move their arm to a specific target. Similarly, in fencing the goal of the fencer is to move the arm to move the tip of blade to a specific target - the opponent's body. In foil fencing, the tip must not only hit the target but also compress in its axial direction by 500g. Although most fencing actions involve direct extension of the arm/blade toward the target, the "flick", an advanced action, requires the fencer to rapidly decelerate the blade base to produce curvature that is difficult for the opponent to parry. The flick requires precise kinematic amplitude and timing to control both the resulting location and axial pressure. The specific aim of this study was two-fold: first, to determine the kinematic strategy employed by high level fencers; second, to determine if the flick is a useful model system to study complex, multi-joint movement requiring precise trajectory control. Fencers were instructed to stand "en garde" and then flick at a simplified model of the top of the shoulder (horizontal plate), a common target of the flick. Scoring success was determined by a standard scoring box. Movement was recorded with a high speed camera (650 fps) positioned perpendicular to the plane of movement. Markers were placed on the tip and base of the blade to determine its location and orientation. Markers on the arm allowed measurement of shoulder, elbow, wrist and "finger" joint angles. The results revealed that two kinematic factors - horizontal distance of the hand from the target at impact and the peak rotational finger velocity, had the greatest effect on scoring. Interestingly, hand height at impact, commonly singled out by coaches, was not significant. Several aspects of the flick suggest it may be a useful model system 1) the movement requires precise control, presumably of joint trajectories, to achieve success. The success rate of nationally ranked fencers was about 50%. 2) The flick movement is confined to a plane, simplifying video recording. 3) The fencing community can provide highly trained fencers that have taken years to learn the flick.

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Poster

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Title: Competing costs determine the dynamical structure of inter-trial variability in a shuffleboard task

Authors: *J. P. CUSUMANO[1], J. M. MAHONEY[1], J. B. DINGWELL[2]
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Abstract: The statistical variability universally observed in repeated trials of goal-directed tasks is a fundamental characteristic of the human perception-action system. In response to fluctuations that arise from intrinsic multiscale physiological noise, performers make adjustments in each trial in an attempt to perfectly execute a movement task. We describe a model-based data analysis approach that views motor variability as arising dynamically from this process of inter-trial error correction. Our approach combines simple stochastic optimal control models with the concept of goal equivalent manifolds (GEMs). The GEM arises from task redundancy, and contains all possible body states resulting in perfect task execution (i.e. zero goal-level error): it is defined using a minimal body-level state variable that determines the outcome of an individual trial. Using this approach, we analyze inter-trial fluctuations observed in experiments with two versions of a virtual shuffleboard task, for which the 2D body state is given by the position and velocity of the shuffleboard puck at release. We show that the structure of observed variability arises from a regulation process that seeks to minimize interacting costs that include, but are not limited to, goal-level error correction. The goal of each task is to release a puck with initial position and velocity so that it stops on a target line. In the first version, the coefficient of friction along the virtual court is constant. The second version includes a patch of virtual "ice" (with zero friction) after the target line: this penalizes overshooting the target. We analyze both tasks using the dynamical GEM framework to characterize the local geometric stability of repeated trials. Then, an optimal inter-trial controller with error, bias, and ergonomic terms is defined, and its weight parameters are estimated from the experimental data. We find the local stability properties are not distinguishable between the two versions. We also show that an optimal controller minimizing only error at the target does not capture the empirically-observed behavior, but a controller with competing bias and ergonomic costs can replicate both the dynamic and steady-state behaviors. Thus, while goal-level error is a dominant cost minimized by the human perception-action system during goal-directed movements, it is not the only one. In particular, our work suggests that relatively small additional costs, such as those stemming from ergonomic, physiological, and/or psychological factors, must be considered in combination with

error minimization in order to completely characterize experimentally-observed motor variability.

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Poster

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Wellcome Trust

Human Frontiers Science Program

Title: Co-optimization of multiple competing action plans

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Abstract: We often encounter situations in which there are multiple objects that can be acted upon. Several influential cognitive theories propose that in such situations, we represent multiple potential actions plans in parallel prior to one of the plans being executed. A commonly held, but as yet untested, assumption is that such action plans are independent from one another. Here we show that competing potential actions not only interact, but are in fact co-optimized. Participants viewed two potential rectangular targets of varying orientations and, when one of the targets was cued, were required to rapidly place the rectangular tip of a hand-held tool on the cued target. Some target orientations uniquely required either supination or pronation of the wrist to align the tool with the target, whereas other "ambiguous" target orientations equally afforded either pronation or supination. We found that on trials in which an ambiguous target was cued, participants were more likely to supinate or pronate the wrist when the competing potential target necessitated supination or pronation, respectively. Moreover, choosing the compatible orientation resulted in a reaction time advantage. Our results demonstrate that individuals

integrate task-relevant features across multiple objects so as to co-optimize plans to act on these objects.

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Poster

251. Reaching Control: Movement Selection and Strategy

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Topic: D.17. Voluntary Movements

Support: Sloan-Swartz Foundation, 2012–9

NIH, R01EY007492

Title: Inactivation of Parietal Reach Region (PRR) affects reach but not saccade choices in spatial decisions

Authors: *V. N. CHRISTOPOULOS[1], I. KAGAN[2,1], R. CHO[1], R. A. ANDERSEN[1]
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Abstract: A growing body of neurophysiological studies challenges a long-held theory that views decision-making as a distinct cognitive process from the neural systems for perception and action. Recent findings suggest that many action decisions emerge through a continuous competition between populations of neurons within the same brain regions that plan and guide action execution. The main line of evidence is the existence of decision-related neural activity in brain regions that are involved in sensorimotor processing. However, it could be argued that the activity is not "genuinely motor", but instead is related to spatial attention or visual salience. One approach to establish whether particular regions are involved in the decision process is to temporarily perturb these regions and observe the effects on decision making. We studied whether PRR inactivation causes deficits on attention and/or on decision-making. We reversibly inactivated part of PRR by locally injecting the GABA-A agonist muscimol, while a monkey performed memory-guided reach or saccade movements either to a single target (instructed trials) or selected between two targets presented simultaneously in both hemifields (free-choice trials). Reaches were performed by means of a 2-D joystick positioned between the legs of the animal sitting in an upright position. In the reach trials, the animal used the arm contralateral to

the inactivated hemisphere. Additionally, the animal was required to keep eye fixation on a central point during the reaching sessions. The results showed that the animal exhibited a spatial decision bias towards the ipsilesional targets after PRR inactivation in the free-choice trials, but only for reaching. On the contrary, the inactivation did not affect the saccadic choices. We also found no significant effects on the reaching/saccadic movements to single targets presented in either hemifield. These results cannot be accounted for as deficits in spatial attention, since the "lesion" had an impact only on the reaching and not on the saccade choices. Rather, the results support PRR being causally involved in decision-making for selecting between reach actions. These findings provide a new conceptual advance in understanding neglect-like disorders as a deficit of the decision-making process rather than sensory attention. Additionally, we developed a biologically plausible computational framework that explains how PRR inactivation affects reaching but not saccade choices.

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Poster

251. Reaching Control: Movement Selection and Strategy

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Topic: D.17. Voluntary Movements

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Title: Effects of age and fear cognitions on motor behavior in a step and reach task with varying support surface conditions

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Abstract: The purpose of this study was to determine the effects of age and fear of falling on movement time and joint excursions used to complete a step and reach task under varying surface conditions. Specifically, the coefficient of friction was systematically reduced via the application of a commercial cooking spray (i.e., PAM) on the linoleum support surface on which participants were standing barefoot. Twenty-one healthy male and female participants (11 ages 19-24, 10 ages 55-62) were instructed to step and reach with their right hand to a high or low target in the mid-sagittal plane. Participants reached three times to each target as fast as possible.

Targets were located such that the subject could reach them, in theory, with a forward step equal to ½ of their hip height, elbow extended with the shoulder flexed to 90 degrees, and trunk flexed to 15 degrees (high target) or 60 degrees (low target). Floor conditions for this study varied as follows 1) No Pam (NP); 2) Pam sprayed under just the left/plant foot (PP); 3) Pam under just the right/land foot (PL); and 4) Pam under both feet (PB). Prior to each change in surface condition, participants rated their concern of slipping or falling during the task using a 10 cm visual analog scale (VAS) where zero indicated "no concern," and 10 indicated "extremely concerned." Participants wore a safety harness that allowed free movement, but prevented an impending fall by resisting rapid accelerations of the participant's body toward the floor. Whole body kinematics were recorded using a 7-camera Vicon MX-13 System and peak-to-peak 3D joint excursions were determined using custom software. Fear of falling as assessed by the VAS increased from 1.19 cm (± 2.0) for NP condition to 6.9 cm (± 2.9) for PB condition ($p<0.05$). However, there were no age-related differences in these assessments. Three-way mixed-model ANOVAs revealed that movement time increased on average from 687.0 ms (± 47.0) to 869.2 ms (± 49.3) when the support surface was made more slippery ($p<0.05$). There was a trend for older subjects to have a MT about 134 ms greater than younger subjects ($p=0.065$). Older subjects used less lumbar flexion compared to younger subjects ($p<0.05$) and there was a surface condition by age interaction ($p<0.05$) showing that older subjects used increased lumbar rotation across surface conditions. The results show that varying surface conditions increased fear; in parallel, older participants altered movement strategy by decreasing lumbar flexion and increasing lumbar rotation. Further research is needed to assess the influences of fear cognitions on changes in motor planning in older cohorts.

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Poster

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Support: Wellcome Trust Award no 091806

Title: Are there shared resources for motor planning?

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Abstract: Different characteristics of human perception and memory can be described successfully with a resource model. In particular, resource models describe the decrease in precision with which individual items can be represented if the total number of items increases. Neurophysiological data suggests that the representations of items and movements towards these items are closely related. Therefore, we examined if a resource model could also describe the precision with which movement plans are represented. If this is the case, the precision of individual movements plans should decrease if the number of movement plans increases. In the first experiment, we examined if preparing one movement resulted in less movement planning variability than preparing two movements. Participants made speeded reaching movements with their left or right hand towards visual targets, in response to a go-signal. To manipulate the number of movement plans, we used a delayed response cueing procedure that allowed the participant to limit planning to a single hand or required that they plan potential movements with both hands. In the early cue condition, the required reach hand was cued at the same time as the target appeared, 1200 ms before the imperative. In the late cue condition, the hand cue was specified at the same time as the imperative, 1200 ms after target onset. We expected that with the early cue, participants would prepare a movement only with the instructed hand, while with the late cue, participants would prepare two movements to the target, one with each hand. Variability in initial movement direction was higher in the two-plan condition than the one-plan condition, demonstrating a cost associated with planning multiple movements, consistent with a limited resource. To test if the effect is due to an increase in movement planning variability, or due to a more general preparedness of the hand in the early cue condition, we varied the timing of the target presentation in two more experiments. In the early target condition the target was cued 1200 ms before the go-signal and in the late target condition the target was cued at the same time as the go-signal. In experiment 2 the hand was always cued early (1200 ms before the imperative), and in the experiment 3 the hand was always cued late (at the imperative). The results showed that initial movement variability was only decreased if the movement trajectory was fully specified. We conclude that movement planning variability increases if the number of plans increases, consistent with a limited resource model. Our results indicate that alternative movements cannot be planned independently even when they involve different limbs.

Disclosures: L. Oostwoud Wijdenes: None. **P.M. Bays:** None. **R.B. Ivry:** None.

Poster

251. Reaching Control: Movement Selection and Strategy

Location: Halls A-C

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Program#/Poster#: 251.27/KK1

Topic: D.17. Voluntary Movements

Support: NSERC Discovery Grant (LS)

Title: The neural correlates of dissociating the spatial directions of eye and arm movements

Authors: *D. J. GORBET, L. E. SERGIO
Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: Typical visually-guided arm movements involve first making a saccade to an intended target location and then a reach toward the direction of our gaze (i.e. “standard” visuomotor mapping). However, many increasingly common tasks require us to move our eyes in one direction while reaching in a different direction (i.e. “non-standard” visuomotor mappings, such as using a computer mouse). Yet most of what we know about the neural activity underlying coordinated eye-hand movements comes from examination of tasks where either the eyes or the hand are held in a fixed position as the other effector is moved. In a previous behavioural study, we demonstrated that spatially dissociating the directions of eye and arm movement results in changes to the kinematic properties of both effectors, even when participants are equally proficient at both tasks. This result suggests that the neural control of standard and non-standard visuomotor mappings differ (Gorbet & Sergio, 2009). These putative differences in brain activity are relevant not only for our fundamental understanding of visuomotor control but also have potential clinical implications for diagnostic and rehabilitative approaches. In the study presented here, we used fMRI to compare the neural correlates of standard eye-hand mapping where both effectors moved to the same cued target location and a non-standard mapping where the eyes moved to the cued target location but the hand moved 180 degrees in the opposite direction. An instructed-delay event-related imaging paradigm was used to facilitate examination of preparatory-related brain activity prior to motor onset. Female participants were used exclusively to avoid potentially confounding sex-related differences in brain activity associated with visually-guided reaching (see Gorbet and Staines, 2011). Whole brain comparisons of the two conditions reveal significantly greater preparatory brain activity associated with the non-standard task in the dorsal premotor, posterior parietal, and dorsolateral prefrontal cortical regions. Multivoxel pattern analyses within independently localized task-related regions suggest that regions of the prefrontal cortex are integral to the ability to spatially dissociate the directions of eye and arm movements. These regions correspond with our groups previous neurophysiological and clinical findings. Taken together these data characterize a network of regions crucial to the decoupling of a movement from its guiding sensory information. Refs: Gorbet & Sergio, 2009,Br.Res.; Gorbet & Staines, 2011, EBR.

Disclosures: D.J. Gorbet: None. **L.E. Sergio:** None.

Poster

251. Reaching Control: Movement Selection and Strategy

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 251.28/KK2

Topic: D.05. Visual Sensory-motor Processing

Support: DM102524

Title: Parietal activation during vergence eye movements: Implications for attentional control of reaching behavior

Authors: *C. W. TYLER, L. T. LIKOVA, S. C. NICHOLAS
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Abstract: Introduction. No mechanism for the control of near-far direction of vergence has been reported, although the frontal eye field (FEF) regions of cortex have been associated with both lateral (saccadic) and depth (vergence) eye movement control. We used event-related fMRI to dissociate convergent (nearwards) from divergent (farwards) eye movement activation throughout the human cortex. Methods. Auditory (verbal) signals triggered convergent and divergent eye movement in an event-related paradigm with eyes closed while recording whole-brain fMRI BOLD activation on a 3T Siemens scanner. Results. Significant vergence direction specificity was seen in the region of the precentral gyri (Brodmann 6) often designated as the human homolog of the FEF, with the nearward movement eliciting much stronger activation than the farward movement (including a separate island of activation in the most inferior part of area 6, PMVr, which was only activated for nearward movement). In parietal cortex, there was see-saw modulation between the superior parietal lobule (nearward) and the angular gyrus (farward). These are brain regions involved in reaching behavior and the spatial awareness of the body, respectively. Despite the absence of visual input, there was also differential activation in anterior hMT+ (human MST), which encodes motion vector fields. There was no activation in the retrosplenial cortex, and area often associated with perceived self-motion in depth. Conclusion. Vergence eye movements in the dark activate the parietal regions involved in reaching behavior, suggesting that this activation may be related not just to the arm movements per se but also to the eye movements associated with the reaching behavior, or even to the attentional control of changes in spatial distance involved in both tasks. In general, spatiomotor tasks are accompanied by eye movements for visual guidance and feedback, unless specifically controlled for. Eye movements themselves involve both visual and attentional guidance, and spatial representation

of target location and movement. All these factors need to be taken into account in interpreting cortical activation in complex motor tasks, which needs to be broken down into its component elements before the brain networks involved can be properly understood.

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Poster

251. Reaching Control: Movement Selection and Strategy

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 251.29/KK3

Topic: F.02. Animal Cognition and Behavior

Support: NIMH 5T32MH019524-20

Katowitz/Radin NARSAD Young Investigator Award

Title: Anticipation modulates sound representation and behavioral performance

Authors: *I. CARCEA[1], M. N. INSANALLY[1], R. C. FROEMKE[2]
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Abstract: Anticipation represents a form of attention directed towards an upcoming stimulus in order to facilitate its processing. This type of attentional control significantly increases arousal and contributes to states of high engagement during behavioral tasks, likely by preparing dedicated brain regions for sensory processing and motor execution. Frontal cortical areas contribute to attention-dependent modulation of stimulus representations in sensory cortices, and to performance on sensorimotor tasks. We identified a circuit mechanisms that orchestrates neuronal activity across distinct brain structures and across defined temporal domains during attentional states. Here, we used two variants of an auditory-based operant conditioning go-no go task in rats to examine how anticipatory recruitment of attentional resources dynamically impacts behavioral performance and sound representation in the auditory cortex. In one task variant the trials were randomly initiated, whereas in the other variant the animal initiated the trials. The latter self-initiated task variant included a defined anticipatory period preceding stimulus onset. Rats performed significantly better on self-initiated trials (random initiation d'=1.4±0.9, self-initiation d'= 2.3±1.1). This improvement in performance negatively correlated with the duration of the anticipatory period. Using single unit recordings in behaving rats, we found that

anticipation bi-directionally modulates sound evoked-firing rates in the auditory cortex, while conserving mean evoked activity at the population level. Self initiation also increased the reliability of sound-evoked neuronal spiking in the auditory cortex. To investigate if adjustments in baseline activity contribute to the above neuronal dynamics, we analyzed pre-stimulus firing in the auditory cortex and in a reciprocally connected frontal area, the medial agranular frontal cortex (maFC). We found a progressive and significant suppression of activity in both these regions during the anticipatory period. In maFC, the amount of spontaneous firing rate suppression was the best predictor of behavioral performance. We are now examining how activity, both measured and optogentically controlled, relates to frontal and auditory cortical neural dynamics. Together, our findings contribute new insights into the circuit mechanisms of attention by advancing the knowledge on the interaction between frontal and sensory cortices during behavioral engagement.

Disclosures: I. Carcea: None. **M.N. Insanally:** None. **R.C. Froemke:** None.

Poster

252. Neuroprosthetics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 252.01/KK4

Topic: D.18. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (B6453R, B6459L, A6779I)

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NICHD (RC1HD063931)

Doris Duke Charitable Foundation

MGH-Deane Institute

Katie Samson Foundation

Title: Advancing stability and performance of point-and-click cursor control by people with tetraplegia using an intracortical brain-computer interface

Authors: *A. A. SARMA[1,5,2], D. BACHER[1,2], C. BLABE[6], M. L. HOMER[3,2], B. JAROSIEWICZ[4,5,2], E. MATTESON[1], T. MILEKOVIC[4,2], C. PANDARINATH[6], J. SAAB[1,2], J. D. SIMERAL[5,1,11,2], B. SORICE[11], K. V. SHENOY[7,8,9,10], J. M. HENDERSON[6], J. P. DONOGHUE[5,4,1,2], L. R. HOCHBERG[5,1,11,12,2]
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Abstract: Point-and-click control of a computer could restore independence and communication for people who are unable to move or speak. Our ongoing BrainGate pilot clinical trial has previously reported accurate real-time decoding of motor intent in people with tetraplegia. We have previously demonstrated target acquisition times of 5-8s in a standardized task, and continue to pursue faster and more reliable control to create a practical assistive technology. Here, we report methods that have yielded a substantial improvement in speed (2-3s acquisition times) while maintaining the same target acquisition rate. Furthermore, they reduce the need for supervised recalibration tasks with explicitly defined targets, which take time away from practical BCI use. One challenge for point-and-click control using an intracortical BCI is nonstationarity in the recorded neural signals. Without recalibration, this can cause neural control to degrade over the course of minutes or hours. Continuously- and recursively-computed normalization of firing rate means and variances, particularly during periods of rest (between "blocks"), improves control by increasing the stationarity of neural features extracted from nonstationary recordings. In an online comparison of cursor control with a fixed Kalman filter, adaptive normalization outperformed fixed normalization by a difference of 1.7s per target acquisition (35.7% improvement, $p<0.001$). Importantly, this method stabilizes extracted signals even in periods of rest, during which calibration data cannot be collected. This suggests that underlying neural information about movement intent recorded from intracortical arrays may be more stable than previously reported once we account for drift in mean and variance. When combined with unsupervised recalibration of the Kalman filter during practical BCI use (see Jarosiewicz, et al, SFN 2014), these and other adaptive approaches may allow for long-term use of a BCI without the need for disruptive supervised calibration tasks. Related work by Nuyujukian, et al, and Pandarinath, et al, SFN 2014, discusses progress towards high-performance communication using point-and-click typing.

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Poster

252. Neuroprosthetics

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Program#/Poster#: 252.02/KK5

Topic: D.18. Brain-Machine Interface

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Doris Duke Charitable Foundation

MGH-Deane Institute

Craig H. Neilsen Foundation

Title: Collaborative, multi-institutional intracortical bci research and the key role of the clinical neurotechnology research assistant

Authors: *C. H. BLABE[1], B. SORICE*[6], D. BACHER[7,8], V. GILJA[1,7], M. HOMER[9], B. JAROSIEWICZ[10,11,8], T. MILEKOVIC[10,8], P. NUYUJUKIAN[1,2,3], C. PANDARINATH[1,3], J. A. PERGE[7,11,8], J. SAAB[10], A. A. SARMA[7,11,8], K. V. SHENOY[3,2,4,5], J. D. SIMERAL[11,7,8], J. P. DONOGHUE[8,10,7], J. M. HENDERSON[1], L. R. HOCHBERG[11,7,6,12,8]
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Abstract: The BrainGate pilot clinical trials represent an important, ongoing collaborative effort to translate fundamental neuroscience and neuroengineering into a powerful assistive device for people with tetraplegia due to cervical spinal cord injury, stroke, muscular dystrophy, or motor neuron diseases including amyotrophic lateral sclerosis. There are four actively enrolling clinical trial sites: Massachusetts General Hospital (coordinating center), Providence VA Medical

Center, Stanford Medical Center, and Case Western Reserve University. Researchers from those institutions, as well as at Brown University, also play a central role in the analysis of neural data, the creation of novel decoding algorithms, and the integration of the volitionally controlled neural signals. There have been nine participants enrolled thus far, with more than 6000 intracortical array implant days across those participants. Since January 2013, two active participants have been engaged in research sessions at Brown/MGH/PVAMC and Stanford. Of note, all BrainGate research sessions occur primarily in the research participant's place of residence. This ensures that the investigational BrainGate system is being developed and tested in the environment where such an assistive device must function for its future users. Through April 2014, there have been 123 research sessions run at Stanford University and 71 sessions run at Brown/MGH/PVAMC with the current two participants, both of whom have ALS. Sessions begin with a 20-30 minute setup period and usually last 2-4 hours, depending on the energy level, engagement, and daily schedules of the participants. Sessions involving neural cursor control typically consist of 10-20 blocks of data collection with 20-200 trials in each block (approximately 2-10 minutes). Clinical Neurotechnology Research Assistants (CNRAs) at both sites conduct sessions with a consistent style of interaction and "participant first" priority while enabling multiple scientific and engineering questions to be posed. The simultaneous enrollment of these two participants creates new opportunities and challenges in terms of both validating and deploying research sessions. The ability to test scientific hypotheses simultaneously with multiple participants has proven useful, allowing us to quickly iterate the development of advanced decoding methods for communication and multi-dimensional control of external devices.

Disclosures: **C.H. Blabe:** A. Employment/Salary (full or part-time):; Stanford University. **B. Sorice*:** A. Employment/Salary (full or part-time):; Massachusetts General Hospital. **D. Bacher:** None. **V. Gilja:** None. **M. Homer:** None. **B. Jarosiewicz:** None. **T. Milekovic:** None. **P. Nuyujukian:** None. **C. Pandarinath:** None. **J.A. Perge:** None. **J. Saab:** None. **A.A. Sarma:** None. **K.V. Shenoy:** None. **J.D. Simeral:** None. **J.P. Donoghue:** None. **J.M. Henderson:** None. **L.R. Hochberg:** None.

Poster

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Topic: D.18. Brain-Machine Interface

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NICHD (RC1HD063931)

Doris Duke Charitable Foundation

MGH-Deane Institute

Title: Progress toward a self-calibrating, practical intracortical BCI for people with tetraplegia

Authors: *B. JAROSIEWICZ[1,3,4], D. BACHER[2,4], A. A. SARMA[2,3,4], N. Y. MASSE[1,4], E. D. BERHANU[6], B. SORICE[6], E. M. OAKLEY[6], K. NEWELL[6], C. H. BLABE[7], C. PANDARINATH[7], K. V. SHENOY[8,9,10,11], J. M. HENDERSON[7], J. D. SIMERAL[3,2,6,4], J. P. DONOGHUE[3,5,2,4], L. R. HOCHBERG[3,2,6,12,4]
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Abstract: Brain-computer interfaces (BCIs) promise to restore communication and independence for people with severe motor disabilities by translating decoded neural activity directly into control of a computer. However, nonstationarities in recorded brain activity can degrade the quality of neural decoding over time, and periodically interrupting ongoing use of the BCI to perform decoder recalibration tasks would be time-consuming and impractical. Previously, we showed that typing performance in a neurally-controlled communication interface can be maintained without disruptive recalibration routines by mapping neural activity to movement intentions that are inferred retrospectively based on the user's subsequent selections. In two individuals with tetraplegia using a neurally-controlled point-and-click communication interface, typing speed using an "unsupervised" decoder (calibrated using data acquired during neurally-controlled free typing) was equivalent to typing speed using a "supervised" decoder (calibrated using a task with pre-defined targets). The current study extends this finding to several more sessions with two additional participants, and demonstrates for the first time that these methods can keep the BCI calibrated over long periods of practical, self-paced BCI use without the intervention of a technician. Technical innovations that made this possible include automated calibration routines running in parallel with decoding software, adaptive

normalization of extracted neural features, click decoder recalibration in parallel with directional decoder calibration, residual bias suppression, and other innovations (see Sarma et al. and Simeral et al., SFN 2014, for details). By introducing the potential to maintain decoder performance during extended use of unsupervised point-and-click assistive applications, this unsupervised calibration approach advances the potential clinical utility and independent use of BCIs by individuals with severe motor disability.

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Poster

252. Neuroprosthetics

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NICHD (RC1HD063931)

Doris Duke Charitable Foundation

MGH-Deane Institute

Title: Integrating advances in signal processing and decoding into intracortical BCIs for people with tetraplegia

Authors: *J. D. SIMERAL[1,2,3,6], A. A. SARMA[2,1,3], B. JAROSIEWICZ[4,1], D. BACHER[2,3], M. L. HOMER[5,3], B. SORICE[6], J. SAAB[2,3], J. P. DONOGHUE[1,4,2,3], L. R. HOCHBERG[1,2,6,7,3]
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Providence, RI; [2]Sch. of Engin., [3]Inst. for Brain Sci., [4]Dept. of Neurosci., [5]Biomed. Engin., Brown Univ., Providence, RI; [6]Neurol., Massachusetts Gen. Hosp., Boston, MA; [7]Neurol., Harvard Med. Sch., Boston, MA

Abstract: A broad range of research aims to contribute methods for translating recorded cortical signals into commands for assistive devices. In the BrainGate trial, research activities performed at each participant's place of residence provide the unique opportunity and practical motivation to develop and evaluate neural signal processing and decoding techniques that enable real-time neural control of assistive devices and communication software. To date, technical complexity and the nature of ongoing research have demanded the active engagement of trained technicians during use of this intracortical brain-computer interface in the home environment (see Blabe et al.). Here we list recent advancements in neural signal processing and neural decoding approaches and the ongoing integration of these into a neuroprosthetic system that could one day enable reliable long-term in-home BCI use without technical oversight. We decode motor intent from combinations of spiking activity (spikes) and local field potentials (LFPs) recorded from one or more microelectrode arrays implanted in motor cortex of individuals with severe motor disability. Because isolating spikes manually or through any of numerous automated "sorting" methods is time consuming and impractical for independent use, we have instead used threshold crossing counts after non-causally filtering for spike band frequencies, which has been shown to improve the extraction of information about movement intent from spike band activity (Masse et al., in review). We have also extracted motor intent-related information from the power in various frequency bands identified in the literature for decades as having information pertaining to movement intention (particularly in able bodied monkeys). However, we find that in practice the optimal bands for information extraction vary substantially between individuals and even across days. Therefore, we have developed and evaluated a data-driven method for improved selection of LFP bands to use for real-time decoding. We also describe innovations in neural decoding within the Kalman filter regime, such as automatic unsupervised decoder re-calibration during practical BCI use (see Jarosiewicz et al., SFN 2014) and continuous re-normalization of extracted neural signals during both use and non-use (see Sarma et al., SFN 2014), and evaluate the efficacy of non-parametric decoding methods in achieving neural control. Additionally, we report progress towards a wireless, mobile, embedded platform for safe and convenient neural signal processing and decoding.

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Poster

252. Neuroprosthetics

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NINDS-Javits (R01NS25074)

Doris Duke Charitable Foundation

MGH-Deane Institute

Title: Watch, Imagine, Attempt: Context-dependent decoding of movement direction in human motor cortex using spike train similarity analysis

Authors: *C. E. VARGAS-IRWIN[1,2], J. FELDMAN[1,2], S. S. CASH[4,6], E. N. ESKANDAR[5], G. FRIEHS[7], K. NEWELL[4], L. R. HOCHBERG[8,3,4,6,2], J. P. DONOGHUE[8,1,3,2]
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Abstract: Motor cortex (MC) plays a central role in the generation of voluntary movement, yet increasing experimental evidence has shown that it is not simply a circuit dedicated exclusively to generating motor outputs. MC is engaged during action observation as well as execution, suggesting a cognitive role related to abstract representations of movement. However, the extent to which MC dynamics change depending on the task context, such as the cognitive involvement of the subject, remains largely unknown. Here, we apply a novel relational decoding and visualization technique to study how single cell and ensemble activity changes across three cognitive conditions in human motor cortex. Relational decoding uses similarity measurements describe the relationship between multiple data samples. Spike train similarity space (SSIMS) analysis builds upon this framework by combining spike train metrics with dimensionality reduction techniques, producing similarity measures between neural activity patterns that can be

easily quantified and visualized. This approach does not require an explicit parameterization of neural data with respect to external variables, minimizing assumptions about putative neural encoding schemes. People with long-standing tetraplegia who have implanted multielectrode arrays as part of the BrainGate pilot clinical trial viewed an animation of an arm making point-to-point movements to four evenly spaced targets from the first person perspective. Participants were instructed to watch, imagine, or attempt the viewed action. Our results show that the context change between Watch, Imagine, and Attempt conditions is differentially encoded by neural activity at a level comparable to movement direction. Direction-related information is evident across all conditions distributed over a substrate of overlapping neuronal populations. Direction selective neurons typically alter their firing properties across conditions, incorporating information about both cognitive set and movement direction. Our findings demonstrate that MC circuits encode information in a flexible, context dependent manner, building upon a perceptual base, and suggest that cortically-driven neuroprosthetic systems will need to incorporate mental engagement states in addition to those directly related to movement parameters.

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Poster

252. Neuroprosthetics

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NICHD-NCMRR (N01HD10018)

Doris Duke Charitable Fountation

MGH-Deane Institute

Title: State space modeling of local field potentials in motor cortex during observed, imagined, and attempted arm movement in humans with tetraplegia

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Abstract: Neural activity in primary motor cortex (M1) can be used as a command signal for neural interfaces for people with tetraplegia (Hochberg et al., 2006; 2012). Spiking and local field potentials (LFPs) in M1 are modulated by attempted movement (Shaikhouni, 2010) and cognitive state (Feldman et al., 2011; Menzer et al., 2012), and can be decoded to provide information on these variables. This study presents a novel method of analyzing LFPs in a state space framework. The method uses spectral power difference and dimensionality reduction to compare the LFPs recorded on different experimental trials. We compare the difference in power on all pairings of trials of LFP recorded during the experiment separately for the 1-5, 15-30, 100-150, and 150-200 Hz frequency bands. Next, dimensionality reduction is used to represent the data in a 3D state space that allows for clustering analysis. We use this novel LFP State Space (LFPSS) approach to analyze the relationship between cognitive state and modulation of M1 LFPs during observed, imagined, and attempted arm movement (Watch, Imagine, Attempt; WIA task). In this task, participants view the identical computer-generated animation showing an arm performing center-out reach movements. LFPs were recorded from multielectrode arrays implanted in M1 of three people with tetraplegia enrolled in the pilot clinical trial of the BrainGate (IDE) Neural Interface System. We recorded 96 LFP channels simultaneously in 3 sessions with two participants, and 2 sessions in the other. Analog LFP data were filtered (0.3-7,500 Hz) and digitized to 30,000 samples/s. LFPs were then low-pass filtered (1-200 Hz) and downsampled to 1,000 samples/s. As reported previously (Menzer et al., 2012), LFP power was elevated in the 1-10 Hz and 60-200 Hz bands during arm movement compared to premovement. LFP power was also suppressed in the 10-40 Hz during movement vs. premovement. These power modulations were strongest during Attempt and weakest during Watch. The novel LFPSS procedure separates these data by cognitive strategy, especially during arm movement observation. This effect is strongest in the gamma frequency ranges > 100 Hz. This novel method is also used to evaluate the timecourse of changes in the relation between LFPs and cognitive state during the trials. These relationships between LFP modulation and cognitive state underscore the potential utility of M1 LFPs as a control signal for neural interfaces.

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Poster

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MGH-Deane Institute

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Title: Classifying movement types during an instructed delay task from motor cortex recordings in an individual with paralysis

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Abstract: Intracortical Brain-Computer Interfaces (iBCIs), including the investigational BrainGate Neural Interface System, are being developed to assist individuals with neurological injury or disease. Commonly, iBCIs record signals from motor cortical areas, decode continuous movements or classify movement types, and then send the resulting control signals to an assistive device. Decoders and classifiers are built on neural activity recorded while movements are attempted or imagined. In this study, we investigate the information available in neural activity occurring during the preparation of movement. We recorded activity within the arm area of motor cortex using two silicon multi-electrode arrays placed in participant T7, a 58-year-old man with amyotrophic lateral sclerosis. He performed an instructed delay task consisting of a

random sequence of four movement types: (i) elbow flexion, (ii) elbow extension, (iii) thumb flexion, and (iv) thumb extension. The delay period was of variable length so as to minimize the predictability of cues. We then applied an offline multi-class linear discriminant analysis to classify movement types using multiunit spiking activity prior to cue presentation, during the delay period, and during task execution (i.e. intended movement). As expected, prior to cue presentation, classification accuracy remained below chance-level (27% correctly classified movements). Accuracies then increased to above chance-level during the delay period (66%) and task execution (91%). These results indicate that movement goals can be predicted prior to task execution in the motor cortex of an individual with tetraplegia. As has been shown in non-human primate studies, using these predictions to weight real-time kinematic decoder outputs can reduce decoding errors and improve BCI control.

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Poster

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NIDCD (R01DC009899)

Title: Application of a high performance intracortical brain computer interface for communication in a person with amyotrophic lateral sclerosis

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Abstract: An important potential application for Brain Computer Interfaces (BCIs) is the control of computer cursors and keyboards for the restoration of communication. This report, combined with a paired report (Nuyujukian et al., SfN 2014), describes the development and application of one such BCI for use by a participant with amyotrophic lateral sclerosis (ALS), as part of the BrainGate2 FDA Pilot clinical trial. Our participant was achieved typing rates between 4.4-6 words per minute (22-30 net correct selections per minute), representing, to our knowledge, the highest reported communication rates of any human BCI. Participant T6 is a 51 yr old woman with declining motor function due to slowly-progressive ALS. She was implanted with a 96-channel electrode array (Blackrock Microsystems) in the hand area of dominant motor cortex. The data presented here were collected more than 13 months post-implantation. Spiking activity and high-frequency local field potential power (150-450Hz) were extracted for use as control signals. The ReFIT Kalman Filter (Nuyujukian et al., SfN 2014, Gilja*, Nuyujukian*, et al., Nat Neuro 2012) was used for continuous 2-dimensional cursor control. To achieve full "point-and-click" control of the computer interface, we added, in parallel, an algorithm for detecting transitions between movement and click-states: the Hidden Markov Model (HMM; Nuyujukian et al., SfN 2012). The participant generated a volitional click signal by attempting to squeeze her non-dominant hand (ipsilateral to the implanted array). This caused a suppression of neural firing, which could be detected as an intention to click. At each time step, the HMM estimated the state likelihoods based on a multivariate Gaussian model of the neural data, the previous likelihoods, and the prior probability of state transitions. Performance of the combined point-and-click interface was measured using two tasks - a grid task, in which the participant acquired randomly presented targets on a 6x6 square grid, and a keyboard task, in which the participant typed phrases using a QWERTY-keyboard layout without, using word completion or error correction. In the grid task, the participant was acquired targets with less than 10% false click rate and a bitrate between 2.2-3 bits per second. In the keyboard task, the participant was able select characters at speeds between 22-30 net correct selections per minute (4.4-6 wpm). These results demonstrate the successful translation of high-performance BCI methods for continuous control and discrete selection. This promising point-and-click interface could serve as a practical method to restore communication for persons with severe paralysis.

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Poster

252. Neuroprosthetics

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Title: Design of a high performance intracortical brain computer interface for a person with amyotrophic lateral sclerosis

Authors: *P. NUYUJUKIAN[1,2,3], C. PANDARINATH[1,3], V. GILJA[1,7], C. BLABE[1,3], J. A. PERGE[8,11,9], B. JAROSIEWICZ[10,11,9], L. R. HOCHBERG[11,8,12,13,9], K. V. SHENOY[4,5,6,3], J. M. HENDERSON[1,3]
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Brown Univ., Providence, RI; [11]Ctr. for Neurorestoration and Neurotechnology, Rehab. R&D Service, Dept. of VA Med. Ctr., Providence, RI; [12]Neurol., Massachusetts Gen. Hosp., Boston, MA; [13]Neurol., Harvard Med. Sch., Boston, MA

Abstract: One important potential application of brain computer interfaces (BCIs) is the restoration of communication for people with paralysis. Here, along with a paired report (Pandarinath, et. al., SfN 2014), we describe the development and application of an intracortical BCI decoder in the BrainGate2 FDA pilot clinical trial (NCT00912041). On a free-paced task using a traditional qwerty keyboard layout, our participant achieved typing rates of 4.4-6 words per minute (22-30 net correct selections per minute), representing, to our knowledge, the highest reported communication rates of any human BCI. Participant T6 is a 51 year old woman with declining motor function due to slowly-progressive ALS who was implanted with a 96-channel electrode array (Blackrock Microsystems) in the hand area of her dominant motor cortex in Dec 2012. Data presented here were collected from ongoing research sessions 13 months post implantation. Both spiking activity as well as high-frequency local field potential power (100-450Hz) were extracted for use as inputs to the neural decoder. Initial kinematic training data was collected using a data glove (Fifth Dim. Tech.) that mapped index finger flexion to the X dimension and thumb flexion to Y. A neural decoder was then generated from this behavioral training data. The decoder used for cursor control was the ReFIT Kalman Filter (Gilja*, Nuyujukian*, et. al., Nature Neuroscience 2012), which was previously shown to be faster and easier to use for this participant (Gilja, et. al., SfN 2013). Continuous cursor performance was evaluated with a center-out task and a random target Fitts task. On an 8 target center out acquisition task with 12cm target distance, 3cm diameter targets, and a 500ms hold time; T6 typically achieved success rates exceeding 95% and average aquire times under 1.5 sec. On a random target Fitts task with a 500ms hold time, where target distance and diameter would vary from trial to trial, T6 was able to consistently achieve a Fitts bitrate exceeding 1 bit/sec. In a typical experimental session, which would last two to four hours and span thousands of trials, participant T6 continued to move her fingers slightly during neural control blocks as she did during finger training blocks. As a control, she was asked to limit her finger movements and place her hands flat on a table while controlling the cursor, yielding comparable levels of control under this paradigm as well. These findings confirm that the ReFIT-KF algorithm successfully translates to a human participant from the healthy, intact, animal models in which it was originally developed and reported. These results may help make BCIs more suitable for the restoration of communication.

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Poster

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Title: Brain-machine interface performance is mediated by an internal model of decoder velocity gain

Authors: *S. D. STAVISKY[1], J. C. KAO[2], S. I. RYU[6,2], K. V. SHENOY[1,2,3,4,5]
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Abstract: Imagine moving a computer cursor to select an icon. If you use your own laptop, then you know how fast the cursor will move in response to your trackpad input; this allows you to select the icon quickly and precisely. If you instead reach for an unfamiliar laptop with a higher cursor velocity gain, you will move the cursor too quickly and overshoot the icon. Conversely, if the velocity gain is less than you expect, you will move the cursor too slowly. This example illustrates the control theory-inspired view that the motor system learns an internal model to predict the resulting sensory consequences when a physical plant (e.g. the arm) is driven by its motor commands. There is considerable evidence of internal model use when the motor system moves the body, but the field is just beginning to ask whether similar computational strategies are used to control a brain-machine interface (BMI) (Golub et al., 2012). Whereas that study manipulated the mapping between neural activity and cursor movement direction, we instead changed the BMI's velocity gain and observed how a subject's performance depends on whether they are given the opportunity to learn this gain. Two macaques were implanted with 96-channel arrays in both primary motor and dorsal premotor cortex. They first performed a 2D point-to-point cursor task with their hand. We used these data to train a velocity Kalman filter that decoded multiunit spikes to move the cursor. We varied the decoder by scaling its output velocity by 0.5 ("slow"), 1 ("normal"), or 2 ("fast") while the monkey performed the task. During the blocked condition (BC) a given decoder gain was presented for prolonged use.

During the interleaved condition (IC), one of the three gains was randomly chosen for each trial with no overt cue as to which gain was being used. We predicted that performance using each gain would be better during BC than IC trials in a manner akin to the example of using a familiar versus a frequently changing laptop cursor. We found performance differences between IC and BC trials indicative of the monkeys having a better internal model of the velocity gain during BC than IC BMI use. Times to target were 9% (12%) faster on BC compared to IC trials for monkey R (J) using the slow decoder, and 22% (17%) faster when using the fast decoder. Mean cursor speeds in the first 300 ms of fast decoder trials were 9% (11%) faster in IC than BC trials, leading to 74% (88%) further target overshoot distance. Conversely, the monkeys drove the slow decoder 9% (13%) slower in IC compared to BC trials. These results suggest that BMIs can be a platform for investigating internal model use in the motor system, and that adept BMI performance may be mediated by learning the properties of the BMI.

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Poster

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Title: A robust and high-performance brain-machine interface using a nonlinear recurrent neural network trained with years of neural data

Authors: *J. C. KAO[1], S. D. STAVISKY[2], D. SUSSILLO[1], S. I. RYU[5,1], K. V. SHENOY[1,3,4,2]
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Abstract: Clinically viable brain-machine interfaces (BMIs) must not only perform well, but should also be robust to across-days and within-day changes in the neural data. Linear techniques, including state-of-the-art Kalman filter-based decoders (e.g., Gilja et al., 2012) are not well-suited for dealing with non-stationary data because their modeling assumptions result in underfitting. Thus, although neural recordings from the previous months to years may be available, these decoder architectures do not adequately make use of the richness and variance of these datasets. Our goal was to overcome this by using nonlinear techniques (e.g., Sussillo et al., 2012). We present a novel nonlinear architecture for BMI use that is constructed to improve robustness: the multiplicative recurrent neural network (MRNN, Sutskever et al., 2011). The nonlinearities in the MRNN enable improved robustness by (1) leveraging multi-year training datasets and (2) training with explicitly perturbed neural data. We report both performance and robustness results superior to the current state-of-the-art. We implanted one monkey with 96-electrode arrays in both primary motor and dorsal premotor cortex and collected neural data across two years of reaching experiments. We trained the MRNN using a merged training set comprising approximately 120 datasets (60,000 trials) collected from 08/31/2012 until the day prior to experimentation in 03/2014. To train the decoder to be robust to unexpected neural changes, we perturbed the neural training data by randomly increasing or decreasing spike counts. We first evaluated if the MRNN could outperform an existing state-of-the-art BMI decoder, the FIT-KF (Fan et al., 2014), which was trained with data collected on the day of experimentation. The MRNN acquired targets faster than the FIT-KF (5.7% more targets per minute (tpm), $p < 0.05$). We next evaluated the robustness of the MRNN and FIT-KF to an unexpected loss of channels. The MRNN sustained better performance than the FIT-KF in the face of channel loss (52.1% more tpm across conditions, $p < 0.05$). Finally, we evaluated the robustness of the MRNN and FIT-KF to a natural sampling of neural non-stationarities. Both decoders were trained without access to the last four months of data and were then evaluated day after day, sampling naturally occurring neural differences between distant training days and the evaluation day. The MRNN substantially outperformed the FIT-KF (105% more tpm, $p < 0.05$). These results demonstrate that a nonlinear decoder that leverages a diversity of training data can substantially increase the robustness and performance of a BMI.

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Poster

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DARPA YFA

Paul G. Allen Family Foundation

Title: A brain-controlled spinal interface (BCSI) for reanimation of paralyzed limbs after spinal cord injury

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Abstract: Brain and spinal cord injuries often lead to severe motor impairments that limit individuals' daily activities and independence. Incomplete injury to the cervical spinal cord is the most common spinal cord injury diagnosis, and restoration of hand and arm function is the highest treatment priority for individuals with cervical spinal cord injuries. Recent advances in brain-machine interface and microstimulation technologies provide opportunities for the development of new devices that could restore motor function after central nervous system injury. We are developing a closed-loop brain-controlled interface that records movement intention from the brain and delivers task-specific real-time stimulation to the spinal cord using state-of-the-art neural recording and intraspinal microstimulation technologies. The primary goal of this Brain-Controlled Spinal Interface (BCSI) is to re-animate and restore function to the paralyzed hand and arm. We have tested early versions of this BCSI in a rat model of cervical spinal cord contusion injury. Animals' forelimb function was analyzed using standard behavioral assessments, and various aspects of forelimb function were quantified during alternating sessions with the BCSI engaged and deactivated to distinguish the immediate effects of the BCSI from those attributable to gradual rehabilitation. Our results indicate that BCSI operation enables improved forelimb function after spinal cord injury; for example, BCSI operation enabled a 5-fold increase in peak lever deflection and a 75% decrease in number of attempts preceding a successful lever press when compared with trials in which the BCSI was deactivated. These results support the potential utility of a BCSI in restoring function to the paralyzed hand and arm after spinal cord injury.

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Poster

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Support: National Science Foundation, EEC-1028725

Title: Novel electrocorticographic brain-computer interface framework for dexterous robotic control

Authors: ***D. SARMA**[1,2], J. WU[2], V. KUMAR[3], J. G. OJEMANN[4], R. P. N. RAO[3]
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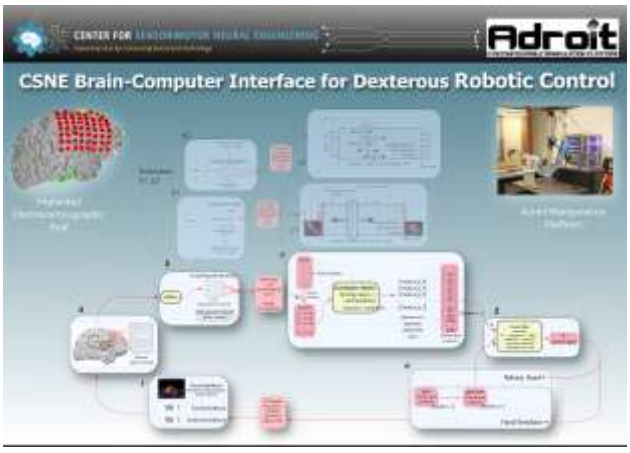
Abstract: An implanted chronic brain-computer interface (BCI) could improve the quality-of-life for individuals with severe neuromuscular deficits by providing a system to enact intuitive, volitional control of assistive devices. Electrocorticography (ECoG)-based control systems for dexterous upper-body robotic prostheses have shown promise to fulfill this role. However, current methods for analyzing and modeling electrocorticographic (ECoG) data are still relatively limited. Moreover, Brain-computer interfaces (BCIs) based on ECoG data still rely primarily on linearly translating power in single high-frequency bands (70-200Hz) to the movement of a cursor. To be effective in the long term, a BCI system for hand prosthetics must be developed such that it can replicate natural human control by taking advantage of motor primitives as represented in ECoG data. Ideally, these BCIs must be able to exploit the brain's natural abilities, including its remarkable plasticity, to accomplish fine manipulation tasks. Here we demonstrate preliminary techniques for dimensionality reduction of ECoG motor and sensory field potential recordings into synergistic motor primitives and present a framework to control a highly dexterous and adaptable robotic hand. Utilizing ECoG data recorded at different resolutions we are able to test a variety of strategies for robotic control as well as better understand the neural dynamics of innate human hand control. Leveraging recordings from 10 subjects, implanted with clinical (10mm spacing), medium (5mm spacing), and high-resolution (3mm spacing) platinum subdural ECoG grids (implanted as per separate epileptic clinical considerations), suggests the presence of synergistic movements of individual digit joints during coordinated grasping further establishing this type of dimensionality reduction as a robust set of

targets for control of the robotic systems. Through this system, we hope to significantly advance our understanding of the computational basis of human manipulation capabilities and enhance the utility of ECOG for hand prosthetics.



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Poster

252. Neuroprosthetics

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Title: Volitional modulation of optically recorded calcium signals during neuroprosthetic learning

Authors: *K. CLANCY[1], A. KORALEK[1], R. COSTA[2], D. FELDMAN[1], J. CARMENA[1]
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Abstract: Brain-machine interfaces (BMIs) have recently emerged not only as a promising treatment for paralyzed patients, but also as a powerful technique for investigating neuronal network dynamics during learning. While past work BMIs has demonstrated striking network reorganization with cell-specificity during learning, the majority of BMI research to date has been performed with chronically implanted microwire arrays, which offer limited spatial resolution for monitoring network reorganization during learning. We operantly trained mice to modulate spike-related calcium signals in individual neurons recorded with two-photon imaging through a chronic cranial window. Mice successfully controlled an auditory cursor to earn reward using neural activity in both motor and somatosensory cortices, exhibiting performance improvements across- and within-sessions. The learned modulations were sensitive to reward contingency, suggesting that they are goal-directed rather than habitual. Learning was accompanied by changes in the network correlation structure and sparsening of task-relevant activity modulations on fine spatial scales (tens of microns). Mice could control a single neuron to drive task behavior. Together, these results demonstrate spatially precise network alterations during learning and introduce a powerful new paradigm for dissecting the local network mechanisms of neuroprosthetic control.

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Poster

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Title: Decoding dexterous finger movements from peripheral neural signals using machine learning algorithms

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Abstract: Peripheral nerve implant based motor prostheses have the potential to provide dexterous control of individual digits to the user. Intrafascicular microelectrode arrays were implanted in the median and ulnar nerve of two human subjects who had undergone trans-radial amputations. Neural signals from the microelectrode arrays were recorded when subjects performed instructed volitional phantom finger movements, e.g. as guided by a virtual robotic hand. Discrete (individual finger movement) and continuous (movement trajectories) components of neural signals are required for robust control of a neural prosthesis. We achieve this by employing a Multiclass Support Vector Classification for identifying individual finger movements with 12 DOF on one day of data. Neuronal firing rates during the time of movements were extracted as input to the SVM. The classification accuracy for all 12 degrees of freedom was 76.8%, while an example finger, i.e. little finger flexion, abduction and extension, the classification accuracy was 91.76%. Additionally, the movement trajectories for the little finger were decoded using Support Vector Regression with a mean squared error of 14.83%. We also demonstrate an experimental design for a peripheral nerve implant of a microelectrode array in a healthy nonhuman primate. Neural signals will be recorded from an intrafascicular Utah slanted electrode array during a finger movement task performed with a mechatronic manipulandum which continuously records both position and force for the thumb, index and middle fingers. These movement parameters will be estimated from the neural signals, while the data recorded from the manipulandum provide a control. A chronic implantation in a nonhuman primate will allow for investigation of the long-term reliability of neural control signals from the peripheral system while allowing for greater control of experimental parameters than is possible with sub-chronic human implantations. A chronic peripheral implantation will allow for future work comparing the efficacy of linear, e.g. Kalman filter, and nonlinear, e.g. SVM & SVR, decoding algorithms when applied to both continuous force and position parameters. The application of nonlinear Machine Learning algorithms to peripheral neural signals may enable improved dexterous control of motor prostheses.

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Poster

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MEXT, SRPBS

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KAKENHI 26282165

Title: ECoG-based neural decoding and prosthetic control in a completely paralyzed ALS person

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Abstract: Background Severely disabled people, especially patients suffering from amyotrophic lateral sclerosis (ALS) have great difficulty in daily movements and communication. Brain-machine interfaces (BMI) enable such disabled people to control machines and to communicate with others through the direct use of brain signals. In this study, we investigated neural decoding and prosthetic hand control using electrocorticograms in a completely paralyzed ALS person. Methods The patient was completely paralyzed except for slight eye and mouth movements. We implanted a patient-specific 96-channel grid electrode fitting to the sensorimotor cortices for three weeks. Four types of hand and arm imagery movements were decoded using a support vector machine algorithm, and prosthetic hand control was performed using a real-time, continuous decoding and control algorithm. Safety and efficacy including classification accuracy of upper arm movements and performance of prosthetic hand control (grasping and releasing a soft ball) were evaluated. Results Subdural hematoma was found and surgical evacuation was performed 5 days after electrode placement, but there were no neurological deficits later than 10 days after the implantation. High gamma band activities and cortical potentials were clearly induced by imaginary hand movements in the hand knob in the precentral gyrus. Classification accuracy of hand and arm movements was 77.7% (50% by chance). Regarding prosthetic control, averaged required times were 7 s for grasping a ball and 2 s for releasing it. Conclusion This is the first report that demonstrated that a completely paralyzed ALS patient was able to control a prosthetic hand in a real time.

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Poster

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Fondation Motrice

Fondation Nanosciences

Fondation de l'Avenir

Title: Brain Computer Interface human platform to control a 4-limb exoskeleton based on the ECoG-recording implant WIMAGINE® : preliminary results

Authors: *C. MESTAIS[1], G. CHARVET[1], F. SAUTER[1], N. ABROUG[2], N. ARIZUMI[1], S. COKGUNGOR[1], T. COSTECALDE[1], M. FOERSTER[1], B. MORINIERE[2], J. PRADAL[1], D. RATEL[1], N. TARRIN[1], N. TORRES-MARTINEZ[1], A. VERNEY[2], A. YELISYEYEV[1], T. AKSENOVA[1], A.-L. BENABID[1]
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Abstract: The goal of CLINATEC® Brain Computer Interface Project is to improve tetraplegic subjects' quality of life by allowing them to interact with their environment through the control of effectors with multiple degrees of freedom after training. Thanks to a long-term wireless 64-channel ECoG recording implant WIMAGINE® (Wireless Implantable Multi-channel Acquisition system for Generic Interface with NEurons) and an innovative signal processing, the subject should be able to control a 4-limb exoskeleton EMY (Enhancing MobilitY). The ECoG signals from the subject's brain will be recorded and wirelessly transmitted to a base station by the WIMAGINE® implant. This implant is composed of an array of 64 biocompatible electrodes, a hermetic titanium case which houses electronic boards, biocompatible antennas for wireless transmission of the data, and a remote power supply. Qualification tests are in progress to demonstrate compliance to the European Directives for Active Implantable Medical Devices. Innovative ECoG signal decoding algorithms will allow self-paced control of the exoskeleton by decoding the subject's brain activity. The neuronal signal processing approach is based on a tensor data analysis. It allows simultaneous treatment of the signal in several domains,

(frequency, temporal, and spatial). Before applying the BCI platform to patients, a set of preclinical experiments are carried out on male Macaque Rhesus. Ethical approval was obtained from ComEth in accordance with the European Communities Council Directive of 1986 (86/609/EEC) for care of laboratory animals. As the WIMAGINE® implant (designed to be implanted in patient's skull) is too large for implantation in primate's skull, a silicone-platinum cortical electrode array was epidurally implanted over the motor cortex and connected to the recording electrodes of the implant WIMAGINE® by means of a transcutaneous connector and a specially designed test assembly. The primate was trained to reach an exposed target using the right hand. The hand movements were recorded by an optical motion capture system Vicon (Motion Systems, Oxford, UK). During the calibration stage, the primate's ECoG data were correlated with the hand position data to identify a prediction model. This model was applied to the ECoG data on the second stage to generate control commands for the arm prosthesis. High performance decoding of the continuous three-dimensional hand trajectory from epidural ECoG signals of the primate's brain allows reproducing the arm movement by the exoskeleton arm (EMY) in real time.

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Poster

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Support: National Science Foundation grant EFRI-M3C 1137267 (JMC)

Defense Advanced Research Projects Agency contract N66001-10-C-2008 (JMC)

Title: Spike-by-spike control using an adaptive optimal feedback-controlled point process decoder improves BMI performance

Authors: *M. M. SHANECHI[1,2], A. ORSBORN[2], H. MOORMAN[2], S. GOWDA[2], J. M. CARMENA[2]
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Abstract: Closed-loop decoder adaptation (CLDA) methods fit the decoder parameters during closed-loop BMI operation based on the neural activity and inferred user velocity intention. Combining Kalman filters (KF) with CLDA has resulted in the high-performance ReFIT-KF decoder (Gilja et al. 2012). Here we demonstrate proficient, robust, and generalizable spike-by-spike BMI control enabled by a novel CLDA algorithm, termed an adaptive optimal feedback-controlled (OFC) point process filter (PPF). In addition to providing a different mathematical encoding model, PPF allows users to issue neural commands and receive feedback of the consequence of such commands at a much faster rate (every 5ms) than the KF (typically every 50-100ms). Moreover, it allows the decoder parameters to be updated on a fast spike-by-spike time-scale compared with the currently used time-scale of minutes. In addition to using the point process model, adaptive OFC-PPF models the brain in closed-loop BMI operation as an infinite-horizon optimal feedback-controller (Shanechi et al. 2012) to infer velocity intention during adaptation, in contrast to current intention estimation methods. We recorded multi-unit activity from the primary motor cortex of two rhesus macaques over tens of online BMI sessions. We investigated the advantages of adaptive OFC-PPF both during CLDA and at steady-state. During CLDA, we found that spike-by-spike adaptation resulted in faster convergence in decoder parameters compared with current batch-based techniques, and was robust to initialization. Moreover, the OFC intention estimation resulted in a PPF with higher performance compared with a PPF obtained using current intention estimation techniques. We then compared the steady-state performance of adaptive OFC-PPF with that of a KF that was trained using SmoothBatch CLDA (Orsborn et al. 2012) and the CursorGoal method (Gilja et al. 2012). In both monkeys, OFC-PPF outperformed the trained KF in a self-paced center-out movement task with 8 targets. This improvement generalized to a multi-curvature obstacle avoidance task. To understand the fundamental reasons behind this improvement, we designed novel experiments to dissociate the effects of the three components that are different in a PPF compared with KF. These are the mathematical encoding model, the increased control rate, and the increased feedback rate. Our experiments showed that each of these three components improved BMI performance in both monkeys. Hence our results identify fundamental factors that facilitate the brain's ability to achieve proficient BMI control.

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Poster

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Title: Reconstruction of hand trajectories from MEG recording for BCI application

Authors: *T. AKSENOVA, N. ARIZUMI, M. C. SCHAEFFER, C. PÖSCHL, T. COSTECALDE, E. LABYT, N. TARRIN, C. MESTAIS, A.-L. BENABID
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Abstract: The goal of the Brain Computer Interface project at CLINATEC®, CEA, Grenoble is to allow a tetraplegic subject to control external effectors, such as a 4-limb exoskeleton EMY (Perrot, et al., 2013) using ElectroCorticoGram (ECoG) recording through WIMAGINE® implants (Charvet, et al., 2013). Within the framework of the project the multi-way decoding algorithm (Eliseyev & Aksenova, 2013) was developed and tested in preclinical experiments for upper limb real movement trajectory reconstruction from ECoG recordings of nonhuman primates. In the present work, to ensure future clinical applications, algorithms were tested in humans, in MEG experiments for real and virtual (imagined) movements. The performance of real and virtual movement trajectory reconstruction was studied. During the experiments, predefined movements were performed after a visual stimulus at randomized time moments. Trajectories of predefined movements were registered with the same operators outside the MEG session using VICON movement tracking system and accelerometer. During MEG session two types of movements (reach-down-back and reach-left-back) were mixed in both cases of real and virtual tasks. In case of real movements, trajectory information was registered with a MEG compatible accelerometer in parallel to neuronal activity. For performance validation recordings were split in training and test sets. Training recordings were used for decoding model calibration. MEG and VICON recordings were synchronized according to accelerometer in case of real movements. In case of virtual movements they were synchronized (fixed and restricted variation in starting point and duration) in a way to obtain the best decoding performance. Restricted set of MEG sensors located in the neighborhood of motor region was specified by experts. Preliminary results (2 operators, 2 and 1 session respectively) of reconstruction: the correlation coefficients between real/virtual and predicted trajectory are respectively CC = 0.68 (CCx = 0.67, CCy =

0.61, CCz = 0.75), and CC = 0.52 (CCx = 0.50, CCy = 0.46, CCz = 0.61). The most informative frequency bands were 6-12Hz and 60-80Hz for real and virtual movements. Perrot, Y., et al. (2013). EMY: Full-body Exoskeleton. ACM SIGGRAPH Emerging Technologies. Anaheim, US. Charvet, G., et al. (2013) WIMAGINE®: 64-channel ECoG recording implant for human applications. Engineering in Medicine and Biology Society (EMBC), 35th Annual International Conference of the IEEE. Eliseyev, A., & Aksenova, T. (2013). Recursive N-way partial least squares for brain-computer interface. PLoS One, 8(7), e69962.

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Poster

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Support: Strategic Information and Communications R&D Promotion Programme (SCOPE) no. 121803027 of The Ministry of Internal Affairs and Communication in Japan

Title: Thresholding the discriminant output improves the reliability of communication using visual P300 speller brain-computer interface (BCI) for physically disabled persons

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Abstract: Brain-computer interface (BCI) enables the most severely disabled persons even to express their thoughts. Visual P300-based BCI decodes user's attention to a particular letter in a matrix from visually evoked potentials. The accuracy of 70% or better in decoding the users' intended letters is commonly considered good enough. However, the message with 30% errors in the spelling often becomes ambiguous to interpret. In most BCI systems that utilize P300 responses, the stimulus that scores highest with a given discrimination method automatically becomes the output of the decoder. Although there are studies using a dictionary and grammar to counter the errors, the process may add another layer of ambiguity, which could hinder a human reader from inferring an intended message correctly. In this study, another approach was tested

whether inhibiting the decoder output from statistically low-reliable trials may increase the overall accuracy of the output characters and aid communication. [METHODS] Patients who were on mechanical ventilation due to amyotrophic lateral sclerosis (ALS), as well as neurologically normal subjects participated in the study. 8 to 15 channels of EEG data were collected with a V-Amp (Brainproducts, Germany). The subjects were instructed to mentally count the number of flashes of an indicated character in a matrix. A modified P300 Speller of BCI2000 (Wadsworth Center, Albany, NY) was used in repeated offline simulation analyses with different thresholding for the same EEG data sets. The scores of the discriminator output were standardized and thresholded, and the inferred characters were produced only when the scores were higher than the threshold. This study has been approved by the internal research ethics committees of the respective affiliated institutions of the authors. [RESULTS] With a proper value of the threshold, almost all wrong inferences were eliminated from the output if the accuracy of the unthresholded inferences was relatively good. However, it did not improve the accuracy if the unthresholded performance was less than 70%. [CONCLUSIONS] A method to improve the accuracy of visual P300 speller BCI with output thresholding is proposed. The improvement observed here suggests that the performance of BCI may not be uniformly stochastic for the subjects with a relatively good overall BCI speller performance. By detecting and rejecting the output of low reliability trials, the overall BCI speller could be made more reliable, which should help improve the communication with the people in the locked-in state.

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Poster

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Topic: D.18. Brain-Machine Interface

Title: Evaluation of fractal dimension estimation of eeg signals for features extraction in motor imagery based bci

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Abstract: A Brain Computer Interface (BCI) is a tool that enables a direct communication between a brain and a computer; it provides a non-muscular channel for sending commands or messages to the external world. The BCI translates brain activity into computer commands using

preprocessing, feature extraction and classification methods. Feature extraction is an important step because it has the effect to improve on the classification accuracy and speed. Features extraction is the process of simplifying the representation of the data by reducing its dimensionality; get in its relevant characteristics. The fractal dimension is a statistical measure that shows the complexity of an object, signal or quantity, which is self-similar over some region of space or time interval. The brain signals are self-similar and we can consider them fractal pieces to obtain their features with some method like Higuchi, DFA, Katz and Hurst exponent algorithm. There are several of fractal dimension estimation methods, but some are not applicable to all types of data that have fractal proprieties. The objective of this work is using the fractal dimension to evaluate the features extraction in motor imagery for detect sensorimotor rhythms (mu 8-12hz and beta 18-26hz) marked by right and left hand motor imagery with the fractal dimension. Based on motor imagery is possible to detect this activity with only three main channels C3,C4 and Cz using the 10-20 international system for EEG. The classification method was performed by Neural Networks with good results. The algorithms were tested with the signals of two persons (each person with different session). The sessions were recorded in a EEG with 14 channels but for this work only 3 channels were used C3,C4 and Cz. The sampling rate was 256Hz and the protocol settings were based on model proposed by Pfurtscheller for the discrimination of two mental states. The task of the patient was to imagine and execute movements of the right and left hands depending on a visual guide displayed in the screen of a computer; in the entire task, the patient was kept with open eyes. In this work, it was observed that the faster algorithms Hurst Exponent go and the slower the Higuchi algorithm is, and about the accuracy the best method is the Katz algorithm and the worst is the Hurst exponent. With the Katz method good accuracy was obtained with only two electrodes (C3 and C4); and it was faster than the Higuchi algorithm that it had the second best accuracy This project was supported in part by CONACYT

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Poster

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Topic: D.18. Brain-Machine Interface

Support: Det Obelske Familiefond

Title: Plasticity following skilled learning and the implications for BCI performance

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Abstract: During use of a brain computer interface (BCI) the user is trained to fit into the performance of the BCI governed by a classifier or a detector. However, any design of a BCI must address the fact that the brain is undergoing continuous adaptation through learning (Sanes & Donoghue, 2000) and hence consider the principles related to how the brain acquires, improves and maintains its natural function. Here we investigated if a commonly used signal extracted from the electroencephalogram, (EEG) the movement related cortical potential (MRCP) is affected by motor learning. Eight healthy, volunteers (25-43 years) with no history of neurological conditions participated. Ten monopolar EEG channels (FP1, Fz, FC1, FC2, C3, Cz, C4, CP1, CP2 & Pz) were recorded (sampling frequency of 256 Hz). The learning task comprised a six randomized figures each sketching a different series of combinations of ankle movements. Subjects were instructed to these as displayed on a computer screen by controlling the activation level of their tibialis anterior (TA) muscle. A single training run lasted for 4 min followed by a 2 min rest period. A total of eight training runs (32 min of training) were completed. The signals were band pass-filtered (0.05-10 Hz, 2nd order Butterworth filter) and data divided into epochs of 4 s (from 2 s before to 2 s after the onset of EMG in the TA). The main time of interest for the MRCP was prior to the peak negativity as this is where the movement is conventionally detected for device control. The average EEG signal from -2 to -1 s prior to task onset and its associated SD were extracted from the EEG data. Results showed that during motor learning, the variability of the EEG activity within 1-2 s prior to movement onset (the SD within the time window), increased significantly while the averaged MRCP amplitude showed no difference. Results demonstrate that the trial to trial variability of the MRCP in the time prior to the peak negative phase was significantly greater during the learning of a motor task. This has important implications in the design of any MRCP-based BCI for online detection of movement. Online detecting relies on threshold values for the period prior to movement onset, thus the findings here suggest that BCI performance will decline with skill acquisition unless the algorithm is adapted to the new level of activity.

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Support: National Science Foundation under Grant ECCS-1126707

Title: The need to be me: Influence of participant specific instructions on mu-based BCI performance

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Abstract: Brain-computer interface (BCI) technology is a growing field, becoming an increasingly viable aid for individuals who have lost normal neural motor output. The mu rhythm, a sensorimotor rhythm that is suppressed when one imagines motor activity, has been particularly utilized in BCIs because of its potential for diverse applications. While many novel implementations of mu BCIs have been developed, little work has been conducted investigating how to improve the neural signal coming from participants. Previous research from our laboratory suggested that specific instructions for imagined movement and relaxation improve overall strength of mu rhythms (Corbit et al., 2013). Improved mu power was hypothesized to improve performance on a mu-based BCI device. However it is important that sustained control over mu power, rather than maximum strength of mu rhythm is attained in order to successfully operate a BCI device. The current study aimed to improve participants' mu BCI performance by giving them participant specific instructions for imagine motor behavior for improved control over sustained mu power. Based on previous research from our lab participants were able to successfully produce mu rhythms in response to imagined motor or relaxing behavior in a single trial. Building upon these methods participants were provided with instructions (non-specific, specific, or participant specific instructions on how to imagine motor behavior) and the ability to control the strength of the mu rhythm was analyzed. The BCI system algorithm calculated a bilateral mu power value from two electrodes positioned over the left and right sensorimotor cortices and compared to a participant's baseline value to determine if the power increased or decreased; this difference corresponded to feedback shown on a computer screen. Preliminary evidence suggested there is a significant difference in control over mu power based on the type of instructions provided to the participant. These data may suggest that the success of an individual using a mu-based BCI device may depend on the type of instructions provided. Decreasing training time, increasing BCI literacy, and enhancing control over mu-based BCI devices will make this type of BCI device more accessible to individuals with impaired motor behavior.

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Title: EEG change through long term brain-computer interface training in focal hand dystonia

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Abstract: An electroencephalogram (EEG)-based brain-machine interface or brain-computer interface (BCI) has originally succeeded to control robotic arm and home electronics by extracting features in EEG, and thus has expected to be a tool to compensate lost motor functions in patients with amyotrophic lateral sclerosis or spinal cord injury. Recently, some research groups succeeded in showing another possible use of BCI, that is, as a tool to promote neural plasticity causing functional recovery from stroke. The number of clinical applications of such BCI-based neurorehabilitation is expected to increase in the near future. Focal dystonia is a disorder of movement characterized by involuntary, sustained muscle contractions, frequently causing twisting and repetitive movements or abnormal postures of a body part. Writer's cramp (WC) is an example of task-specific focal hand dystonia. WC was once believed to be a purely psychological problem, but more recently is understood to be due to more specific neural dysfunction, including that of the basal ganglia. The current study employed a BCI paradigm to provide visual feedback of ongoing EEG features that represents the exaggerated excitability of the sensorimotor cortex during hand movement, and assessed neurological and behavioral changes through 30-day use in a WC patient as a clinical pilot study. Each recording day, a participant was requested to perform the following: (1) tonic contraction of the right extensor muscle (tonic motor task) for 60 s; (2) right hand extension with rhythmic auditory cue for 40

times, one every 2.5 s (repetitive motor task) for 40 times; and (3) write Japanese characters with a pen and digital pen tablet(writing task). The patient completed biweekly one-hour training for over 30 times without any adverse effects (for totally over a year). In first 10 times of training, significant decrease of the beta frequency component (26-34 Hz) during handwriting was confirmed, and was associated with clear hand wringing improvement. In last 10 times of training, the hand did not show comparatively large hand writing change, but the average of pen pressure was significantly decreased ($P<0.01$) by the training. Our results suggest that there is another application of therapeutic BCI for patients with exaggerated cortical activity associated with involuntary muscle contractions, such as a dystonia patient, using visual feedback of abnormal EEG activity.

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Title: Identifying hand poses using common spatial patterns in human electrocorticographic signals

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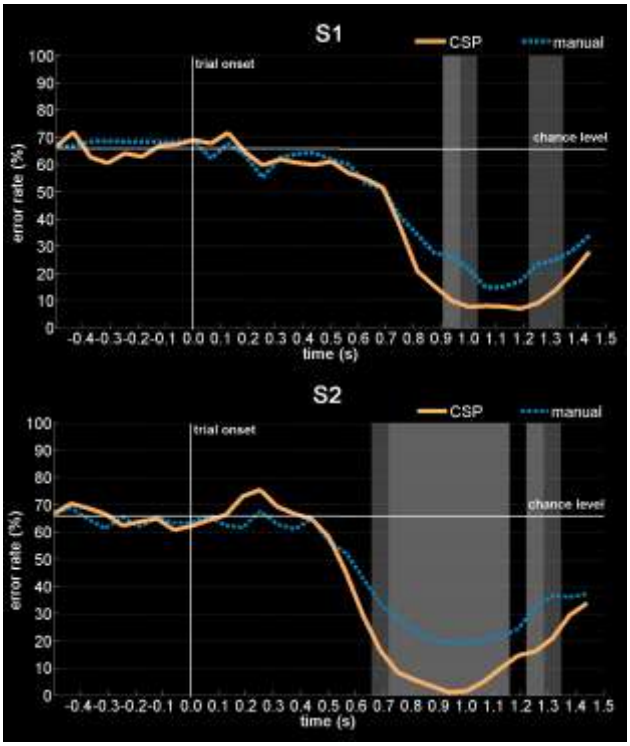
Abstract: Decoding brain activity of corresponding high-level tasks may lead to an independent and intuitively controlled Brain-Computer Interface (BCI). Most of today's BCI research focuses on analyzing the electroencephalogram (EEG) which provides only limited spatial and temporal resolution. Derived electrocorticographic (ECoG) signals allow the investigation of spatially highly focused task-related activation within the high-gamma frequency band, making the

discrimination of individual finger movements or complex grasping tasks possible. Common spatial patterns (CSP) are commonly used for BCI systems and provide a powerful tool for feature optimization and dimensionality reduction. This work focused on the discrimination of (i) three complex hand movements, as well as (ii) hand movement and idle state. Two subjects S1 and S2 performed single ‘open’, ‘peace’ and ‘fist’ hand poses in multiple trials. Signals in the high-gamma frequency range between 100 and 500 Hz were spatially filtered based on a CSP algorithm for (i) and (ii). Additionally, a manual feature selection approach was tested for (i). A multi-class linear discriminant analysis (LDA) showed for (i) an error rate of 13.89 % / 7.22 % and 18.42 % / 1.17 % for S1 and S2 using manually / CSP selected features (see Fig. 1), where for (ii) a two class LDA lead to a classification error of 13.39 % and 2.33 % for S1 and S2, respectively. Such a BCI system can be used to control a virtual or physical avatar just by brain activity and the study showed successfully that hand hand-poses can already be decoded to be transferred to avatar movements in real-time.



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Poster

252. Neuroprosthetics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 252.26/KK29

Topic: D.18. Brain-Machine Interface

Support: National Key Basic Research Program of China 2013CB329506

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NSF of China 31371001

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Title: Real-time artificial hand posture control by using electrocorticographic signals from human sensorimotor cortex

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Abstract: Brain-machine interfaces (BMIs) build a direct communication pathway between brain and external devices, which provide a hope for restoring lost motor function for the disabled. Electrocorticographic (ECoG) signal from sensorimotor cortex has successfully been used in classification of hand grasp types or arm movement directions and in prediction of hand trajectories and muscle activities because it is less invasive than microelectrode and can offer higher spatial resolutions than EEG. However, investigations on real-time robotic prosthesis control using ECoG are lacking, despite its potential in practical neuroprosthesis and neurorehabilitation technology. In this study, we recorded ECoG signals from subdural macro-electrodes covering sensorimotor cortices of two patients who were undergoing inpatient monitoring for diagnosis and treatment of intractable epilepsy. Participants performed three hand postures following visual cues: Rock-Paper-Scissors. Movement instruction alternated with rest where the participant was explicitly instructed to rest hand for a random time. The three hand postures with an additional resting state were classified asynchronously using a fuzzy k-nearest neighbor model, and an artificial hand was controlled online using a shared control strategy. The results showed that the high gamma power (70-120 Hz) of ECoG signals from 8 and 13 of electrodes was responsive to hand movement in two participants respectively. The offline classification performance of ECoG for three hand postures was as high as 97.1% on the average. In the online demonstrations, the instantaneous status of hand postures could be extracted from ECoG signals recording from only 5 macro-electrodes and successfully to control the artificial hand with averaged accuracy of 76% and 85.1% respectively. Our pilot studies demonstrated that ECoG signals from human sensorimotor cortex could be used to decode information about hand postures and be interpreted as real-time control commands for artificial hand, suggesting its potential application in practical BMIs.

Disclosures: **S. Zhang:** None. **D. Wang:** None. **Y. Li:** None. **Q. Zhang:** None. **J. Zhu:** None. **X. Zheng:** None.

Poster

252. Neuroprosthetics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 252.27/KK30

Topic: D.18. Brain-Machine Interface

Title: Arm prosthetic control through electromyographic recognition of leg gestures

Authors: ***K. R. LYONS**, S. S. JOSHI
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Abstract: Use of surface electromyography (sEMG) has shown promise as a method of controlling prosthetic devices, but current control paradigms may suffer from at least one of two main problems: non-intuitive input and invasiveness. A typical approach to the problem is to recognize gestures by recording EMG signals from multiple muscle sites near the amputation and analyzing these signals with a classification algorithm to detect a discrete user intention (e.g. close hand, rotate wrist, etc.). Here, we introduce the idea of recording EMG from the lower leg as the control input to a prosthetic arm. The user performs gestures with the lower leg or foot which map relatively intuitively to forearm or hand movements, requiring essentially no training. A completely noninvasive system for recording, processing, and transmitting commands from the leg to a prosthetic arm could be reduced to a sleeve design with embedded electronics. Although the musculature of the lower leg is somewhat different from that of the forearm on which most EMG-based gesture recognition research is performed, we show that many leg and foot gestures, which have directly analogous arm and hand movements, can be recognized with pattern recognition techniques.

Disclosures: **K.R. Lyons:** None. **S.S. Joshi:** None.

Poster

252. Neuroprosthetics

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 252.28/KK31

Topic: D.18. Brain-Machine Interface

Title: Altered spatial representation of event related desynchronization during finger and shoulder motor imagery

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Abstract: Scalp electroencephalogram (EEG) based Brain-Computer Interface (BCI), can facilitate motor learning by explicit representation of motor-related cortical activity, and has been proposed as a rehabilitative measure for stroke hemiplegia. BCI rehabilitation systems often use event-related desynchronization (ERD) of the 8-13 Hz component in the parietotemporal EEG, which is known as a cortical sensorimotor excitability marker, but a little is known about its somatotopical characteristics observed during distal and proximal muscle contractions in the upper extremity. Functional anatomy has revealed that proximal muscles tend to be innervated bilateral corticospinal paths rather than distal muscles, thus ERD may be distributed bi-hemispherically. In this study, we assessed spatial distribution of ERD with various types of upper limb movement and imagery. 128 channels of scalp EEGs were recorded from three healthy individuals. The monitor was placed in front of the participants to give a task cue of Rest (4s) - Ready (2s) - Task (5s). During the task period, the participants were asked to image either finger pinching or shoulder elevation movement in a random order. Noted here that visual feedback of the amplitude of ERD derived from different hemispheres was given for each. The tasks required were thus as follows; right finger pinching imagery with ipsilateral ERD feedback, right finger pinching imagery with contralateral ERD feedback, right shoulder elevation imagery with ipsilateral ERD feedback, and right shoulder elevation imagery with contralateral ERD feedback. As results, contralateral ERD during right finger pinching imagery was found regardless of contralateral or ipsilateral side of feedback. On the other hand, bilateral ERD during right shoulder elevation imagery was found. Larger ERD was seen in the hemisphere at which feedback signal was derived. These results implies that motor imagery with distal muscles recruited contralateral sensorimotor cortex dominantly, whereas motor imagery with proximal muscles recruited bilateral hemispheres. From these findings, we expected that feedback with the ipsilateral ERD to the imaged hand may lead activation of the ipsilateral cortex, and would contribute to establish a pathway-dependent neurorehabilitation for stroke in future.

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Poster

252. Neuroprosthetics

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Program#/Poster#: 252.29/KK32

Topic: D.18. Brain-Machine Interface

Support: DARPA Contract N66001-10-C-4056

Title: Proprioceptive feedback modulates motor cortical activity during the control of an intracortical microelectrode brain-machine interface

Authors: *D. M. RAGER[1,2], J. E. DOWNEY[4], J. L. COLLINGER[4,5,6], D. J. WEBER[4,5], M. L. BONINGER[5,6], V. VENTURA[2,3], R. A. GAUNT[4,5]
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Abstract: Loss of proprioception is known to severely impair motor control. Previous research by Ghez et al. demonstrated that people lacking proprioceptive input show significant deficits in reaching tasks performed without visual guidance. People with proprioceptive loss are also known to be unable to walk without visually attending to their legs. However, the neural mechanisms by which proprioception aids in the planning and execution of visually guided movements are not well understood. In this study, we explore the neural and behavioral impact of providing proprioceptive feedback to a subject using a prosthetic arm controlled by an intracortical microelectrode brain-machine interface (BMI). A single subject with tetraplegia and intact sensation was implanted with two 96-channel microelectrode arrays in primary motor cortex (M1). The subject made both visually-guided and blinded reaching movements with the BMI-controlled prosthesis with and without proprioceptive feedback, which was provided by moving the subject's limb in coordination with the prosthetic arm. The subject was specifically instructed to move the prosthetic arm back and forth across two lines in the horizontal plane as many times as possible during each one minute trial. Poor task performance in the no feedback condition was associated with a decrease in the mean firing rate of recorded M1 units and an increase in inter-channel spiking correlations as compared to any other feedback condition. Task performance was dependent on the paradigm used to train the BMI decoder; performance degraded significantly when proprioceptive feedback was added to a decoder trained using visual feedback only. This behavioral result was accompanied by significant changes in the preferred

velocity-tuning direction of the majority of recorded M1 units. Paradoxically, proprioceptive feedback resulted in better task performance than no feedback, but the mean path length per line-crossing and variance of movement in the non-task-relevant dimension were significantly smaller in the visual feedback/visual-trained-BMI condition than in the visual+proprioceptive feedback/visual+proprioceptive-trained-BMI condition. Performance differences between the visual and visual+proprioceptive feedback conditions were marked by different patterns of neural population activity, characterized by inter-channel spiking correlations. These findings suggest that neural tuning and dynamics change dramatically under various visual and proprioceptive feedback conditions, and that existing BMI decoding algorithms may not be suited to interpret the neural code resulting from proprioceptive inputs to M1.

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Poster

252. Neuroprosthetics

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Program#/Poster#: 252.30/LL1

Topic: D.18. Brain-Machine Interface

Support: DARPA N66001-10-C-4056

NIH R01EY015545

Boswell Foundation

Title: Brain-Machine interface using human parietal cortex: Control of prosthetic devices from anterior intraparietal area and Brodmann's area 5

Authors: *S. KELLIS[1], C. KLAES[2], T. AFLALO[2], B. LEE[3,2], Y. SHI[2], K. PEJSA[2], K. SHANFIELD[5], S. HAYES-JACKSON[5], M. AISEN[5,4], C. HECK[4], C. LIU[3,6,2], R. A. ANDERSEN[2]
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Abstract: The posterior parietal cortex (PPC) processes sensory inputs, develops motor plans, and projects outputs to motor and pre-motor areas. In both human and nonhuman primate work,

subregions of parietal cortex have been implicated specifically in planning reach movements (parietal reach region and Brodmann's area 5) and grasping (anterior intraparietal area, AIP). Neuronal activity, recorded from PPC of non-human primates, shows coding of different movement types, e.g. specificity for reach movements of the contralateral and ipsilateral limb and grasp postures of the hands. Moreover, PPC represents both kinematic trajectories as well as goals for reach movements. Local field potentials recorded from PPC manifest with stronger power than in other areas of cortex, and encode information about movement planning. These high-level, cognitive encodings of movements suggest that PPC would be an ideal target for a neural prosthetic to assist paralyzed patients. Based on these findings, we initiated a clinical trial to evaluate a brain-machine interface using signals recorded from the PPC of a tetraplegic human subject. To secure representation of both grasp and reach, we implanted Utah electrode arrays in the presumed anterior intraparietal area (AIP) and Brodmann's area 5 (BA5). The implantation sites were selected based on functional magnetic resonance imaging of the subject when he imagined reaching or grasping. In the first year after implantation, there have been no device-related adverse events in the course of our study, and neural signal recordings have been very stable. We have found strong evidence for the important qualities of PPC that motivated our study, including encoding for multiple effectors, goal and trajectory decoding, representation and online control of grasp, and the utility of local field potentials. The participant has demonstrated brain control of a physical robotic limb and virtual reality scenarios that include control of virtual limbs and 3D computer cursors. To our knowledge this is the first motor prosthetic demonstrated in a human with an implant outside motor or premotor cortex. The results demonstrate that the PPC is an excellent target for signals for prosthetic control. The high-level, cognitive nature of the signals provide for versatile and intuitive control of external assistive devices.

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Poster

253. Brain–Machine Interface

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 253.01/LL2

Topic: D.18. Brain-Machine Interface

Title: Potential of neural-network based modeling of bladder pressure from sacral dorsal root ganglia recordings

Authors: *S. S. RAJAGOPALAN[1], S. E. ROSS[2], T. M. BRUNS[2]
[1]Univ. of Michigan, Bloomfield Hills, MI; [2]Biomed. Engin., Univ. of Michigan, Ann Arbor, MI

Abstract: Automatic evacuation of the bladder in patients who have lost the ability can be achieved with medical devices that stimulate nerves to control the bladder. These devices, however, are typically unaware of the bladder state and are inefficient. Neural signals measured from sacral dorsal root ganglia (DRG) show significant correlation with bladder pressure and have high signal to noise ratio when compared to peripheral nerve locations. It is thus possible to estimate bladder pressure through the measurement of these neural signals. Previous research has shown the potential of data fitting techniques (using least squares), assuming a static relationship between the neural response and bladder pressure. Such techniques require offline pruning and sorting of channels and are inefficient in modeling dynamic systems. An alternative approach is offline system identification through artificial neural networks (ANN). The model can then be used for implementation in real-time neuroprosthetic bladder control. A catheter was inserted into the bladder to measure the pressure, while microelectrode arrays were inserted in sacral DRG of alpha-chloralose anesthetized cats. The measured pressure was filtered using a 4 Hz low-pass Butterworth filter, and used as target data during the model identification. Ninety neural recording channels from S1 and S2 DRG were used to calculate firing rates which were then used as inputs to the ANN. It was observed that a non-linear, auto-regressive moving average ANN provided the best input-output response. The ANN was first trained using "open-loop training" to obtain initial parameter guesses. In this method modeling errors at each time step are not propagated to the next prediction step as the measured pressure value is used instead. Next, "closed-loop training," which exclusively feeds back model estimated pressure at each step to predict the next output, was then applied thereby propagating modeling errors at every step. The trained network was then implemented in Simulink towards real-time bladder pressure estimation. Neural networks, trained separately using partial data from a given dataset, were able to model the same dataset with high accuracy (mean-squared error (mse) < 2 cmH2O), but did not fit other datasets at all. However concurrent training, using multiple data sets yielding promising results (mse < 10 cmH2O) across multiple datasets. Further refinement is necessary before implementation of a real-time ANN in a bladder neuroprosthesis.

Disclosures: S.S. Rajagopalan: None. **T.M. Bruns:** None. **S.E. Ross:** None.

Poster

253. Brain–Machine Interface

Location: Halls A-C

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Program#/Poster#: 253.02/LL3

Topic: D.18. Brain-Machine Interface

Title: Targeting sacral dorsal root ganglia for concurrent monitoring and control of bladder function via electrical stimulation

Authors: *S. E. ROSS, J. T. BENTLEY, T. M. BRUNS
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Abstract: Current strategies for bladder control using electrical stimulation typically do not incorporate feedback on bladder state, requiring continuous stimulation. We are developing a closed-loop strategy that targets sacral dorsal root ganglia (DRG), which contain the cell bodies of sensory afferent fibers innervating the lower urinary tract. These fibers include the pelvic and pudendal nerves, which carry information about the state of the bladder and the pelvic region. These DRG may provide an ideal interface location for a neuroprosthetic device for bladder control, as we can both monitor bladder state and stimulate sensory pathways that drive spinal circuits for bladder control. Recent work has shown that stimulation near 30 Hz in sacral DRG can elicit reflex bladder contractions and 5 Hz stimulation can lead to bladder relaxation. Penetrating microelectrode arrays of lengths 1.0 mm or 0.5 mm were inserted in S1 and S2 DRG in alpha-chloralose anesthetized male cats. Neural activity and bladder pressure (via a supra-pubic bladder line or urethral catheter) were recorded at different bladder volumes. Stimulation (200 microsecond pulsewidth, 7.5 - 37.5 microamperes, and 1 - 33 Hz) was delivered on individual and multiple electrode channels at different bladder states. We observed DRG stimulation-driven bladder excitation of up to 40 cmH20 and bladder relaxation of more than 30 cmH20. During these trials, we also monitored at least 9 neural recording channels that correlated to bladder pressure, each of which tracked the pressure during stimulation. This concurrent stimulation and bladder state monitoring demonstrates the potential for a closed-loop DRG neuroprosthesis for bladder control.

Disclosures: S.E. Ross: None. **J.T. Bentley:** None. **T.M. Bruns:** None.

Poster

253. Brain–Machine Interface

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Program#/Poster#: 253.03/LL4

Topic: D.18. Brain-Machine Interface

Title: A novel interface for visualizing and controlling stimulation on high-channel-count electrode arrays

Authors: *A. M. WILDER, M. A. FRANKEL, S. D. HIATT, E. L. BARCIKOWSKI, K. S. GUILLORY
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Abstract: The past two decades have seen tremendous advances in the use of high-channel-count electrode array technologies for electrophysiology research. Such arrays have spurred the development of a new generation of data acquisition instruments to amplify and sample hundreds of signals at very high rates. Accompanying software development efforts have produced a wealth of applications for visualizing and analyzing large numbers of signals in parallel—both off-line and in real-time. More recent electrophysiology instrumentation efforts have produced hardware devices capable of delivering distinct electrical stimulation patterns on hundreds of electrode channels concurrently. To realize the full potential of these high-channel-count stimulation platforms, new software programs and a host of new visualization and control paradigms are needed. We have developed a graphical interface for generating, arranging and executing stimulation patterns concurrently on hundreds of electrodes. The interface is based on the metaphor of music scoring and draws inspiration from a variety of graphical interfaces in the consumer electronics industry—specifically multi-track audio editing applications. The interface presents the user with a list of all stimulation channels provided by the hardware. The interface also presents a simple, form-based interface through which the user can, for each channel, design one or more stimulation patterns. Finally, the interface provides a graphical, timeline-like representation of a stimulation "score" to which the user can add and arrange any stimulation patterns he/she has defined. Individual patterns can be used multiple times in a single score, and they can be selected and modified as needed in between executions of the score. The time-base of the score visualization can be adjusted to allow the user to inspect details of a specific pattern or access an overview. The user can also scroll the visualization window in time to access various periods of the score. The software provides other useful options such as allowing the user to play a score an arbitrary number of times in a row (i.e. loop mode), and saving the score for use at a latter time. Though this software application represents a tiny fraction of the range of possible high-channel-count stimulation control paradigms, it is the first to tackle this difficult task.

Disclosures: **A.M. Wilder:** A. Employment/Salary (full or part-time):; Ripple. **M.A. Frankel:** A. Employment/Salary (full or part-time):; Ripple. **S.D. Hiatt:** A. Employment/Salary (full or part-time):; Ripple. **E.L. Barcikowski:** A. Employment/Salary (full or part-time):; Ripple. **K.S. Guillory:** A. Employment/Salary (full or part-time):; Ripple.

Poster

253. Brain–Machine Interface

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 253.04/LL5

Topic: D.18. Brain-Machine Interface

Title: The development and validation of a high channel count simultaneous stimulation and recording system

Authors: *E. L. BARCIKOWSKI[1], S. D. HIATT[1], D. M. PAGE[2], S. M. WENDELKEN[2], G. A. CLARK[2], K. S. GUILLORY[1]
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Abstract: Emerging fields in the study of neural motor systems and neural engineering are beginning to require both large electrode counts and the ability to record neural signals simultaneously from stimulation electrodes. Additionally, stimulation across a large number of electrodes needs to be performed concurrently and independently between channels. In order to record immediately after a stimulation pulse, recording amplifiers require a mechanism to settle quickly to remove stimulation artifacts. Current commercially available stimulation systems do not allow simultaneous recording and do not accommodate large numbers of channels or use only a few multiplexed channels for stimulation that do not allow the channels to be truly independent. We have developed a stimulation/recording front end to address these issues. Each front end can provides up to 32 channels of independently controlled stimulation and recording, and each front end is connected to our data acquisition system that can connect to 16 front ends, allowing for a total of 512 channels of simultaneous stimulation and recording. The recording amplifiers include a fast settle capability that allows for neural recordings free of stimulation artifacts 1 ms after a stimulation pulse. These amplifiers have high impedance (250 MΩ) and low noise (2.1 μV). The stimulation/recording front end is small, fitting on a 2”x1.3” circuit board that weighs 5.2 g, so it may be placed very close to an electrode site. On the bench top, this system was tested using a Utah Electrode Array (UEA) in saline. A 1 kHz signal was injected into the saline while producing stimulation in the UEA with a series of biphasic pulses of varying amplitudes and duration. The 1 kHz signal was recorded both with and without stimulation showing that the fast settle capability completely recovers the signal 1 ms after stimulation. *In vivo* validation was completed in neural tissue, recording from a UEA implanted in cat sciatic nerve. Action potentials were recorded from afferent sensory activity while the paw was moved at the ankle joint. Stimulation pulses were delivered on the UEA at 30 to 100 Hz across a range

of pulse amplitudes and durations while producing action potentials. The stimulation/recording front end fast settle allowed for successful recordings of action potentials 1 ms after stimulation.

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Poster

253. Brain–Machine Interface

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Program#/Poster#: 253.05/LL6

Topic: D.18. Brain-Machine Interface

Support: A*STAR Neurodevice Programme

Title: Changes in response properties in motor cortex neurons during brain-machine interface control

Authors: C. LIBEDINSKY[1], R. SO[2], Z. XU[2], C. GUAN[2], *S.-C. YEN[3,4]
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Abstract: Background: Over 60,000 people each year suffer from spinal cord injuries that paralyze all four of their limbs. Many of these patients indicate that the ability to control a wheelchair using brain-machine interface (BMI) would be among their top priorities. For this reason, considerable effort has been devoted to the development of BMI systems that would enable self-motion. Multi-electrode arrays chronically implanted in primary motor cortex can be used to collect activity from multiple neurons simultaneously. Since single neuron signals have a high bandwidth, decoded information from these signals can be used to control machines with several degrees of freedom. Methods: Two monkeys were trained to move a robotic platform towards a target positioned in different locations of a room using a joystick. After training, both animals were implanted with multi-electrode arrays in the hand/arm area of primary motor. Their arms were then restrained, the joystick removed, and they were trained to control the robotic platform using single unit activity recorded from the implanted electrodes. Brain signals were used to determine movement initiation and direction in real-time and in a free-running mode. Results: Single neuron analysis of response profiles during BMI control revealed the existence of three distinct populations of cells: (1) Motor-BMI neurons, which maintained their selectivity

profiles consistent with that found during joystick control, (2) Motor-only neurons, which lost the selectivity present during joystick control, and (3) BMI-only neurons, which acquired selectivity that was not present during joystick control. Discussion: Activity of Motor-BMI neurons appears to reflect intended movement direction, while activity of motor-only neurons seems to be related to actual arm-movements. On the other hand, activity of BMI-only neurons may reflect the learning process that the monkeys undergo to control the platform using BMI.

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Poster

253. Brain–Machine Interface

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Program#/Poster#: 253.06/LL7

Topic: D.18. Brain-Machine Interface

Support: NSF SMA-0835976

Title: Decoder error detection in a saccade brain-computer interface

Authors: *A. SALAZAR-GOMEZ[1,2], S. L. BRINCAT[6], N. JIA[2,3], M. PANKO[1,2], E. K. MILLER[6], F. H. GUENTHER[2,4,5]
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Abstract: Brain-computer interfaces (BCIs) aim to provide a means of communication to the severely paralyzed by recognizing changes in brain signals and translating (decoding) them into commands for operating computers and other devices. Despite recent improvements, BCIs are prone to errors, and most of them still rely on knowledge of the "ground truth" to improve their performance, which is not feasible for actual clinical applications. In this project we characterize error-related signatures in local field potentials (LFPs), and assess their potential for automatic performance monitoring (decoder error detection), binary-choice selection, and unsupervised adaptive decoding. We recorded from three 32-channel Utah arrays implanted in the supplementary eye field (SEF), frontal eye field (FEF), and dorsolateral prefrontal cortex (PFC) of two macaque monkeys performing a brain-controlled 6-choice delayed saccade task. During online brain-computer interaction, intended saccades were decoded from delay-period multi-unit

activity, and feedback was given to the subjects via discrete cursor movement and reward (contingent on the classifier decoding the correct location but independent of any overt movement). Offline analysis of correctly and incorrectly decoded trials showed a characteristic time domain LFP waveform for trials with decoder errors (error-related potentials, or ErrPs), which were similar within each array, but different across them. ErrP amplitude was modulated by the distance between the true target and the decoded one -the closer the decoded target to the true location, the smaller its amplitude. Finally, offline automatic decoder error detection was carried out using post-feedback ErrPs and a linear classifier. Errors were correctly detected on 97% of trials on average for monkey 1, and 96% of trials for monkey 2. Our results suggest high levels of accuracy for decoder error detection that could be useful as a BCI control signal or as a supervisory signal for adaptive decoding, and in the long term for more reliable BCIs.

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Poster

253. Brain–Machine Interface

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 253.07/LL8

Topic: D.18. Brain-Machine Interface

Support: DARPA REPAIR (N66001-10-C-2010)

NIH NEI (EY015679)

Title: Smooth degradation of behavior with ICMS electrode silencing

Authors: *M. C. DADARLAT, P. SABES
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Abstract: Smooth and effortless control of neural prosthetic devices will require somatosensory feedback, in particular proprioception_the sense of the body's position in space. We have previously shown that multi-channel intracortical microstimulation (ICMS) can provide the real-time feedback needed for non-human primates to perform a reaching task. Specifically, we used patterned stimulation across eight electrodes to encode the distance and direction from the monkey's current hand position to that trial's reach target. The ICMS pulse rates of the eight electrodes followed a cosine tuning in direction, with equally spaced "preferred directions," and

a mean rate that scaled with distance. Monkeys were able to perform reaches guided by ICMS alone and, if both vision and ICMS inputs were present, would integrate the artificial sensory input with natural vision to form a minimum-variance estimate of position. These results established that ICMS can be used to provide artificial sensory information. Nevertheless, the monkey's strategy in interpreting the ICMS signal is not known. The ICMS encoding scheme creates redundancy across electrodes: in principle the animal could determine the instantaneous distance and direction from just two of the eight stimulating electrodes. In order to determine how the animals make use of this redundant information, we selectively "silence" sets electrodes (zero their stimulation amplitude) during a given trial. Behavioral sessions consisted of interspersed paired vision and ICMS trials and ICMS-only trials. For ICMS-only trials, we silenced either zero, two, four, or six of the eight stimulating electrodes. We found that behavioral performance (as measured by percent correct trials, total number of movement sub-segments, and total movement time) degraded smoothly with the number of silenced electrodes. In particular, the monkey showed difficulty completing reaches to the parts of space represented by the silenced electrodes, but not elsewhere. Furthermore, attempted reaches in these directions proved noisier, as measured by the variance in errors of initial reach angles.

Disclosures: M.C. Dadarlat: None. **P. Sabes:** None.

Poster

253. Brain–Machine Interface

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 253.08/LL9

Topic: D.18. Brain-Machine Interface

Title: Decoding hand gestures using high-density ECoG in somatosensory cortex

Authors: *M. P. BRANCO[1], Z. V. FREUDENBURG[1], M. G. BLEICHNER[2], M. J. VANSTEENSEL[1], E. J. AARNOUTSE[1], N. F. RAMSEY[1]
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Abstract: Brain-Computer Interfacing (BCI) is an emergent technology, which enables paralyzed patients to restore or replace motor function. One of the chief challenges is to increase the number of translational degrees-of-freedom. Electrocorticography (ECoG), which has very good spatial and temporal signal resolution, opens the possibility of distinguishing between complex and fine movements based on sensory-motor cortical activity, which could provide a

new multi-channel communication device for locked-in patients. Bleichner showed that four hand gestures could be anatomically distinguished with 74-97% accuracy for two subjects [1]. The anatomic (spatial) classification was based on the different patterns across channels produced by the mean gamma (70-125 Hz) power of the gestures. One of the questions is whether decoding can best performed from primary motor cortex M1 or from somatosensory cortex. In paralyzed patients, the execution of the movement is compromised, leaving motor imagery or attempted movements as the only decodable cognitive activity. It is not clear whether motor imagery activates primary motor cortex: Hermes ea [2] showed with fMRI that in healthy volunteers motor imagery failed to activate M1, but did activate somatosensory areas. This was further supported by ECoG where imagined movement failed to generate activity in M1. Thus, motor imagery might be better decoded from somatosensory cortex. Here we test whether from sign language gestures can be decoded from somatosensory cortex. We investigate the decodability of four gestures using high-density ECoG grids (3 mm center-to-center electrode spacing) covering the somatosensory cortex near the hand region. We employed decoding algorithms to classify the gestures using spatial and temporal information, limited to the power in the 70-125 Hz range and to electrodes on the somatosensory cortex. Results are reported for five epilepsy patients. Hand gestures were decoded with a mean classification rate of > 77 %, corroborating the proposition that spatio-temporal patterns of somatosensory activity may be an additional hallmark for specific gestures. Classification was significantly above chance level (mean 24%, determined by method validation with random values) and was validated using a leave-one-out cross-validation by means of Pearson correlation. The results suggest that somatosensory cortex may be a good candidate for motor imagery-based BCI. Furthermore, this work encourages the use of high-density ECoG grids as a robust and reliable BCI platform for fine movement decoding. [1] - Bleichner, M. G. (2014) Thesis. Utrecht University, Netherlands. [2] - Hermes et al (2011). JNE 8(2)

Disclosures: **M.P. Branco:** None. **Z.V. Freudenburg:** None. **M.G. Bleichner:** None. **M.J. Vansteensel:** None. **E.J. Aarnoutse:** None. **N.F. Ramsey:** None.

Poster

253. Brain–Machine Interface

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 253.09/LL10

Topic: D.18. Brain-Machine Interface

Support: State of Arizona

Arizona State Deans Office

Title: Stochastic facilitation of prosthetic derived sensory stimulation in primates

Authors: *J. C. TANNER[1], C. K. OVERSTREET[2], S. I. HELMS TILLERY[1]
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Abstract: Providing natural and practical sensory feedback in neuroprosthetics is a top priority in brain computer interfaces. This experiment seeks to provide tactile information from a prosthetic's environmental interactions and create repeatable, discriminable, and meaningful sensations via intracortical microstimulation (ICMS). In particular, we aim to provide robust tactile cues, such as the direction of slip. For our studies, we used a prosthetic fingertip known as the BioTac. To approximate environmental actions, the device uses impedance across electrodes embedded in a deformable skin filled with fluid; as pressure is applied to the fingertip, the skin deforms and the impedance adjusts accordingly. We derived ICMS stimulation trains by performing scanning motions in eight directions with the device (0o to 315o on 45o intervals) and choosing three impedance electrodes which modulated across those directions. Using the distinct patterns of these chosen electrodes, multichannel stimulation of somatosensory cortex was delivered to a NHP that had been trained on a two alternative forced choice discrimination task with pairs of visual, auditory, or ICMS stimuli. Trials were blocked by stimulus mode and randomized between same and different pairs of stimuli. Results from stimulation sessions (n=65 days) indicate that using this method of stimulation, and animal could achieve ~60% correct pattern discrimination, between stimuli separated by 90o. In order to increase the discrimination ability of the NHP, we introduced stochastic facilitation (SF, the introduction of a secondary noise signal) to the ICMS trials. SF can increase a system's response to small amplitude signals. We delivered randomized SF congruently with our derived patterns and saw marked improvement. Preliminary SF stimulation sessions (n=4 days) using a 10% SF elicits an increase in NHP's discrimination accuracy by ~20%. In order to better analyze the benefit we need to continue recording stimulation data, determine the ideal SF distribution, and refine the generation of BioTac derived stimulation patterns.

Disclosures: J.C. Tanner: None. **C.K. Overstreet:** None. **S.I. Helms Tillery:** None.

Poster

253. Brain–Machine Interface

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 253.10/LL11

Topic: D.18. Brain-Machine Interface

Support: DARPA

Title: Discrimination of electrical stimulation to primary somatosensory cortex

Authors: *S.-S. KIM, T. CALLIER, G. TABOT, F. TENORE, S. BENSMAIA
Univ. of Chicago, Chicago, IL

Abstract: One way to restore the sense of touch in upper-limb neuroprostheses is to electrically stimulate primary somatosensory cortex (S1) in the hopes of eliciting meaningful percepts. Our lab has been developing algorithms to convert patterns of sensor activation on the prosthesis into appropriate patterns of electrical stimulation of S1. In the present study, we probed the discriminability of electrical pulse trains and compared it to that of mechanical indentations of the skin. Specifically, we presented monkeys with pairs of stimuli (electrical or mechanical) that differed in amplitude and had them judge which of the two stimuli was stronger. On each trial, a standard stimulus, whose amplitude was constant across the experimental block, was compared to a comparison stimulus, whose amplitude varied from trial to trial. We could then characterize the relationship between the discriminability of the stimulus pairs - electrical or mechanical - as a function of the difference in amplitude between the standard and the comparison. From these functions, we could estimate amplitude discrimination thresholds for both mechanical and electrical stimuli. We could then investigate the degree to which the amplitude discrimination thresholds for electrical stimuli depend on other stimulation parameters, such as frequency. We found that the discriminability of electrical stimuli that differ in amplitude is relatively independent of other stimulation parameters and that, perceptually, electrical stimuli seem to scale linearly with amplitude while mechanical stimuli scale logarithmically.

Disclosures: S. Kim: None. **T. Callier:** None. **G. Tabot:** None. **F. Tenore:** None. **S. Bensmaia:** None.

Poster

253. Brain–Machine Interface

Location: Halls A-C

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Program#/Poster#: 253.11/LL12

Topic: D.18. Brain-Machine Interface

Title: Developing dry-electrode, minimal preparation BCI for the everyday user

Authors: *S. M. GORDON, B. KELLIHAN
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Abstract: Brain-Computer Interface (BCI) technologies represent a major advance in human-computer interaction (HCI). Whereas traditional BCI systems have focused on providing assistance to populations with physical or neurological disorders, emerging systems are being developed for healthy populations for applications such as monitoring cognitive fatigue and measuring both alertness and drowsiness levels. Furthermore, BCI is finding interest within the gaming industry as a potential new form of gamer interaction. Given the potential application and utility of such technology, modern BCI systems are poised to join the ranks of other popular, consumable, wearable electronic devices. Prior to mass distribution for the general public, however, BCI systems must be developed to 1) utilize dry electrode technologies, thus eliminating the need for messy gels that can potentially dry-out causing poor signal quality, 2) be easily donned/doffed, 3) have minimal-to-no calibration time, and 4) work for a large portion of the population. Here we describe our efforts and initial results in building such a BCI system. Starting from a Mindo 32S dry-electrode EEG system we were able to construct a 2-channel BCI system that was integrated within a comfortable softcap design. The system can be donned in less than one minute. Our first sample application was category selection using well-established evoked potential components, such as the P300. Using an unsupervised learning approach that focused on identifying outliers, initial results indicated that for over 70% of the subjects tested, we were able to achieve correct classification without a priori training. While it, of course, remains to be shown that other BCI approaches can be mapped to such dry-electrode technology and made to work with zero-training, we believe our system is an important step in demonstrating that BCI can be transitioned from the laboratory to the everyday user.

Disclosures: S.M. Gordon: None. **B. Kellihan:** None.

Poster

253. Brain–Machine Interface

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Program#/Poster#: 253.12/LL13

Topic: D.18. Brain-Machine Interface

Support: ARC Special Initiative 'Bionic Vision Australia'

Title: Eye-implanted microelectrode arrays and surrounding tissue assessed by contrast-enhanced microCT imaging

Authors: *A. J. WOOLLEY[1,3], T. T. HUNG[2], A. T. FUNG[4,5], G. J. SUANING[1], N. H. LOVELL[1], J. W. MORLEY[3]
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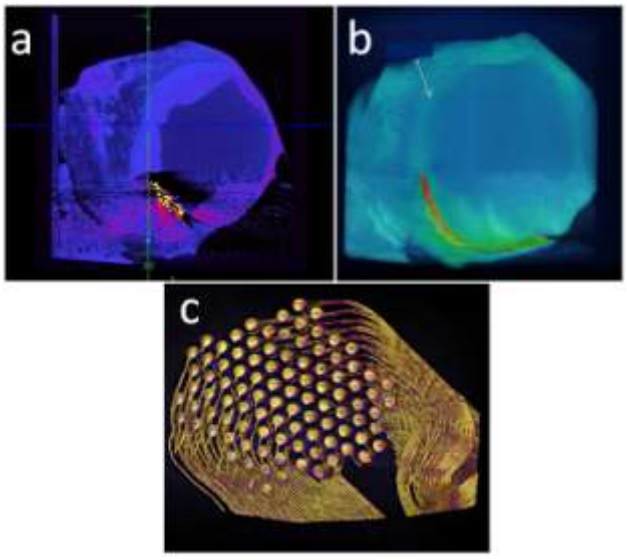
Abstract: Microelectrode arrays surgically implanted sub-scleral into the suprachoroidal space are being developed to electrically interface with neighboring nervous tissue of the retina to treat visual impairments. To simultaneously assess implanted arrays and their surrounding tissue, we implemented a contrast-enhanced microCT X-ray method to image enucleated ovine eyes which had previously been surgically implanted with arrays. Our method was developed to assess three important characteristics of the intact, post-implant eye: (1) 3D location of device components with respect to eye features, (2) physical damage to device interconnects and electrodes due to surgical techniques used, and (3) underlying pathology of tissue surrounding implanted device components. Our method involves collecting and fixing the eye with the implant remaining inside, treating with an X-ray contrast solution, imaging with a microCT system, and analyzing the collected data. Results demonstrated 3D imaging of both the metallic components (electrodes and interconnecting tracks, Fig.1c) and the contrast-enhanced soft tissue of the sheep eyes (single plane shown in Fig.1a; cross-section of 3D rendering shown in Fig.1b) using microCT imaging. Results of this work have informed the spatial design of the microfabricated array as well as the surgical procedure used to implant the device. Future applications of this method may inform the assessment of other metal implants in tissue, such as cardiac pacemakers and stents, deep-brain stimulation electrodes, and other implanted devices, especially ferromagnetic implants not compatible with microMRI imaging.



Disclosures: **A.J. Woolley:** None. **T.T. Hung:** None. **A.T. Fung:** None. **J.W. Morley:** None. **N.H. Lovell:** None. **G.J. Suaning:** None.

Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.01/LL14

Topic: E.02. Neuroimmunology

Support: SFI/12/RC/2273

02/CE/B124

07/CE/B1368

Title: Microbiota regulates microRNA expression in the hippocampus in a sex dependent manner

Authors: *G. MOLONEY[1], O. O' LEARY[1], L. DESBONNET[1], F. SHANAHAN[2], T. G. DINAN[3], G. CLARKE[3], J. F. CRYAN[1]
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Abstract: Accumulating evidence highlights that the gut microbiota communicates with the CNS to influence brain function and behaviour. Understanding how the gut microbiota achieves this influence is a new frontier of neuroscience. The use of germ-free (GF) animals has been pivotal in progressing research in this area. Studies in GF mice have described changes in gene expression, particularly in the hippocampus and we have previously shown that many of the molecular consequences of growing up germ-free in the CNS are more evident in males. MicroRNAs (miRNAs) are small, non-coding RNAs that are critical to cellular function and regulation of gene expression. However, the possibility that miRNAs in the brain might be under the influence of the gut microbiota and involved in the regulation of gene expression and associated downstream behaviours remains unexplored. The aim of this study was to assess the impact of an absent microbiota on the expression of miRNAs in the hippocampus of GF, and conventionally-colonised (CC) animals. Moreover, we sought to determine if any changes could be rescued by colonisation of the GF animals post-weaning (GFC) by removing mice from the GF unit after weaning and housed in the standard facility with faecal matter from their conventionally colonised counterparts to promote the development of the gut microbiota. Animals were euthanized and the brain removed, the hippocampus dissected and stored at −80 °C. RNA was extracted from hippocampi using the miRVana isolation kit and RNA quality was assessed. Microarray was performed as previously described, with the level of significance

set at $p<0.05$ and a fold change of 1.3. miRNAs were selected for follow up using qRT-PCR based on differences in expression between groups based on gender and colonization status. 16 miRNAs showed gender and microbiota specific alterations. There was a significant effect of gender and colonisation status on levels of miR294-5p with a significant increase in this microRNA in male GF mice compared with male CC mice. This alteration was reversed following colonisation and a significant decrease in miR294-5p was noted between GF and GFC mice. We show that the microbiota modulates expression of miR294-5p in the hippocampus of mice and that these alterations can be rescued following the introduction of a gut microbiota post weaning. These findings confirm our hypothesis that the gut microbiota can recruit microRNAs to potentially influence gene-expression at the level of the CNS and that this capacity is amenable to microbiota manipulation later in life. Further analysis of miR294-5p and its mRNA targets may reveal important molecular pathways for brain-gut microbiome signalling.

Disclosures: **G. Moloney:** None. **O. O' Leary:** None. **L. Desbonnet:** None. **F. Shanahan:** None. **T.G. Dinan:** None. **G. Clarke:** None. **J.F. Cryan:** None.

Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.02/LL15

Topic: E.02. Neuroimmunology

Title: Microbiome impacts vasopressin immunoreactivity in juvenile Swiss-Webster mice

Authors: ***N. V. PETERS**[1], M. J. PAUL[3], B. CHASSAING[2], A. GEWIRTZ[2], G. DE VRIES[1]
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Abstract: The microbiome is the collection of microorganisms that inhabit our bodies, including the gut, and plays a role in multiple biological processes. The gut microbiome is thought to influence the brain and behavior through the gut-brain axis, and has been implicated in the control of anxiety behavior, social behavior and immune function. Furthermore, the microbiome may affect the development of neurodevelopmental disorders such as autism spectrum disorders, which are characterized by deficits in social behavior and are frequently associated with gastrointestinal disorders. These conditions present themselves in juveniles. The neuropeptide vasopressin has also been shown to modulate anxiety, social, and sickness behaviors. We hypothesized that the microbiome impacts anxiety and social behaviors, in part, by affecting

vasopressin signaling. To test whether the microbiome can influence the expression of vasopressin in the juvenile brain, we compared levels of vasopressin immunoreactivity in three-week-old male and female Swiss-Webster mice that lack microbiota (germ-free; GF) with conventionally colonized (CC) mice. Preliminary analysis indicates that GF mice have higher vasopressin fiber densities in projection areas of the suprachiasmatic nucleus, including the subparaventricular zone of the hypothalamus and the paraventricular nucleus of the thalamus. This suggests altered output of the circadian system, perhaps affecting behavioral and/or physiological rhythmicity of GF animals. As vasopressin projections of the SCN also affect the HPA axis these changes may contribute to altered HPA function in germ-free mice.

Disclosures: N.V. Peters: None. **M.J. Paul:** None. **B. Chassaing:** None. **A. Gewirtz:** None. **G. de Vries:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: NIMH RO1 MH47538

NIDCH RO1 DC004562

Title: Toll-like receptor 2 and sex differences in SCN vasopressin expression

Authors: *C. FIELDS[1], A. KIM[1], J.-D. LI[2], G. DEVRIES[1]
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Abstract: Toll-like receptors are key players in the innate and adaptive immune system in vertebrates. These receptors register the presence of molecular patterns that signal the presence of infectious agents or aberrant physiological processes, and their activation leads to an immune response. By and large, their role in normal brain development and physiology remains elusive. In our facilities, TLR2-/- mice show high levels of anxious behavior (unpublished observations). We therefore investigated whether TLR2-/- mice display aberrant levels of vasopressin, a neuropeptide implicated in anxiety-related behaviors as well as in innate immune responses. Sections from brains of wildtype (WT) and TRL2-/- male and female mice were processed immunohistochemically for the presence of vasopressin. Vasopressin-immunoreactivity was

measured using gray-level thresholding in hypothalamic nuclei and hypothalamic and extrahypothalamic vasopressin projection areas. Expectedly, projection areas from the bed nucleus of the stria terminalis (BNST) showed an expected large sex difference in fiber density (males > females) but no overall effects of genotype, however the ventral septal area shows an interaction effect trending towards an elimination of the male-biased sex difference in vasopressin immunoreactivity. Unexpectedly, mice displayed an overall sex difference in vasopressin immunoreactivity in the suprachiasmatic nucleus (SCN; females > males). In addition, TLR2-/- mice displayed a higher density of vasopressin immunoreactivity in the SCN compared with their WT counterparts. In SCN projection areas, females showed greater levels of vasopressin immunoreactivity than did males. However, within the dorsomedial nucleus of the hypothalamus, TLR2-/- mice showed less vasopressin immunoreactivity than did WT, while in the paraventricular nucleus of the thalamus there was a trend towards a decrease in the female-biased sex difference in vasopressin immunoreactivity in TLR2-/- mice. No sex or genotype effects were observed in anti-vasopressin staining for the PVN or SON. Interestingly, vasopressin is one of most pronounced neuropeptide outputs of the SCN, and its expression shows a distinct circadian rhythm, which may underlie circadian rhythms in the immune system. SCN vasopressin production has also been linked to HPA activity; however, it is unknown whether TLR2 signaling affects those rhythms or the HPA axis. To our knowledge, this is the first study to show a sex difference in SCN vasopressin immunoreactivity in mice.

Disclosures: **C. Fields:** None. **A. Kim:** None. **J. Li:** None. **G. deVries:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: NIH R21 DA029491

NIH U54 AI065359

Title: Early expression of Osteopontin impacts Central Nervous System pathogenesis by controlling the Caspase-1-dependent Inflammasome and apoptosis during early West Nile virus infection

Authors: *M. MARCONDES[1], N. BORTELL[2], B. CONTI[2], H. FOX[3]
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Abstract: Osteopontin (OPN) upregulation is common to several Central Nervous System (CNS) inflammatory pathogeneses, and participates in the migration, survival, and activation of inflammatory cells. However, the mechanisms by which OPN modulates inflammatory pathways and neuropathogenesis are not clear. To understand the role of OPN in CNS viral infections, we used a mouse model of West Nile Virus (WNV) infection, where OPN levels in the brain increase significantly from day 3 post infection (pi). WNV infection in animals lacking OPN (OPN KO) increased mortality (94%) compared to C57Bl/6 wild-type (WT) mice (50%), in correlation with higher CNS viral load on day 3 pi, higher levels of type I/II IFNs and iNOS. The analysis of IFN-responsive innate immune genes on day 5 pi revealed increase of the Caspase 1-inflammasome components in OPN Kos compared to WT mice, which correlated with significantly higher levels of circulating mature IL1b. We also found that Caspase 8 was highly induced in the brains of infected WT, but not in KO mice. In In animals lacking OPN there was an increased expression of Caspase 3 and higher apoptosis in the brain tissue, compared to WTs. Together, these results indicate that OPN expression, particularly at early time points, provides protection against the spread of viral infections in the CNS by negatively controlling the type I IFN-induced, Caspase 1-dependent inflammasome, while promoting an alternative, Caspase 8-associated pathway for controlling the virus as well as by preventing the apoptosis of infected cells during CNS viral infections such as WNV.

Disclosures: **M. Marcondes:** None. **B. Conti:** None. **N. Bortell:** None. **H. Fox:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.05/LL18

Topic: E.02. Neuroimmunology

Support: Swedish Research Council

Title: Prostaglandins as central mediators of neuro-immune pathways

Authors: *P. REVATHIKUMAR[1], N. AGALAVE[2], E. LE MAÎTRE[1], C. I. SVENSSON[2], P.-J. JAKOBSSON[1], M. KOROTKOVA[1], J. LAMPA[1]
[1]Dept. of Med., [2]Dept. of Physiol. & Pharmacol., Karolinska Institutet, Stockholm, Sweden

Abstract: BACKGROUND Activation of cholinergic anti-inflammatory pathway (CAP) by vagus nerve stimulation (VNS) is known to regulate inflammatory conditions. However, the exact central mechanisms still remain unclear. We have recently shown that, prostaglandin (PG) E_2 inducing enzyme microsomal PGE synthase-1 (mPGES-1) is essential for CAP function (Le Maître, ARD 2012). Here, we intended to study the role of central PG system in neuro-immune regulation and the CAP. **MATERIALS & METHODS** Central PG-blockade: Male C57BL/6 mice (n=8) received lipopolysaccharide (LPS) (1.25 mg/kg; i.p) +/- diclofenac (10 µg) intrathecally and LPS + diclofenac i.p (5 µg). After 6 h, mice were sacrificed and cytokine content of KC-GRO, TNF and IL-10 in the brain and spleen was measured using a Mesoscale multiplex assay. Brain PG-inducing enzymes: Male C57BL/6 mice (n=7) were injected with LPS (2 mg/kg; i.p). After VN isolation, it was either electrically stimulated for 5 minutes (VNS) or left unstimulated (SHAM). After 6 h, mice were sacrificed and brains were collected. Expression of the rate limiting enzymes COX 1/2 and mPGES-1 was quantified by immunohistochemistry. *c-FOS* mRNA level was analyzed by *in situ* hybridization in Hippocampus (Hi), Hypothalamus (Hy), Periaqueductal gray (PAG), Cingulate cortex (CC) and Dorsal raphe nuclei (DRN). **RESULTS** Intrathecal PG blockade had no effect on KC-GRO or TNF production in the brain, but strongly suppressed the corresponding levels of KC-GRO in the spleen (317.53±31(LPS) vs. 82.07±24 (LPS + Diclofenac i.t), $p<0.05$), and also displayed a tendency to suppress splenic TNF cytokine content. Diclofenac i.p. did not affect KC-GRO nor TNF content in spleen, but up regulated these cytokines in brain, in line with previous data (Sacco et al, 1998). IL-10 was unaffected in both brain and spleen. After VNS, there was a significant up regulation of c-FOS expression in vagus related areas such as Hi (75.3±5.7 (SHAM) vs. 105.0±1.7 (VNS); $p<0.001$) and Hy (73.8±9.4 vs. 102.2±6.7; $p<0.05$). Moreover, the same areas displayed a VNS-induced increase in mPGES-1 protein, (Hi 0.60±0.09 vs 0.69±0.12; Hy 0.87±0.19 vs. 1.66±0.28 $p<0.05$). On the contrary, VNS did not affect induced COX1/2 expression. **CONCLUSION** Together with our previous data, these results suggest a key role for the brain PG system in neuro-immune communication between the brain and spleen. An involvement of PGE_2 in CAP activation is further suggested by the CAP-induced up regulation of mPGES-1 in vagus-related brain areas. Since COX1/2 was not affected in this context, mPGES-1 may constitute a future target in the development of pharmacological interventions for regulation of CAP-mediated immune suppression.

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Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

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Program#/Poster#: 254.06/LL19

Topic: E.02. Neuroimmunology

Support: NIH Grant AG000538

Title: IL-1beta sensitizes its own response in hippocampal neurons

Authors: *G. A. PRIETO, E. D. SMITH, M. NGUYEN, L. TONG, C. W. COTMAN
Inst. for Memory Impairments and Neurolog. Disorders, Irvine, CA

Abstract: Acute systemic inflammation can impair learning and memory, particularly in aging. Similarly, chronic brain inflammation contributes to cognitive decline in neurodegenerative diseases. These findings suggest that the aged brain may develop an increased sensitivity to inflammatory factors, thus rendering neurons vulnerable to immune challenges. BDNF plays an essential role in learning and memory. Previously we found that BDNF signaling is suppressed by acute IL-1beta in neurons. We hypothesized that chronic IL-1beta may sensitize its own on response and thereby contribute to age-related cognitive decline. We treated rat hippocampal neuronal cultures with IL-1beta for different times (1, 3, 6, 12 and 24 h) and evaluated the expression of IL-1 receptor-1 (IL-1R1), IL-1 receptor accessory protein (AcP) and AcPb, a neuron-specific AcP splice-variant involved in neuroprotection. IL-1beta increased the expression of IL-1R1 and AcP, but not of AcPb, thus affecting AcP/AcPb ratio. Concentration-response studies confirmed that IL-1beta upregulates AcP and IL-1R1 expression. Paralleling the increased expression of IL-1R1 and AcP, we found a boosted IL-beta response in neurons pre-treated with this cytokine. Specifically, we found an enhanced IL-1beta potency for the suppression of brain-derived neurotrophic factor (BDNF) signaling, as measured by Akt/mTOR phosphorylation. Sensitized IL-1beta response for inhibition of BDNF signaling was found to be dependent on both IL-1R1 and AcP, as demonstrated by experiments using the IL-1 receptor antagonist (IL-1ra) and knock down AcP using shRNA. Finally, the Toll/IL1 receptor (TIR) domain mimetic AS-1, a low-molecular weight and cell-permeable compound, confirmed that sensitized IL-1beta response depends on IL-1R1-AcP. Our data support the idea that inflammatory environments contribute to neuron dysfunction by IL-1beta-induced suppression of BDNF signaling, and this can be prevented by a TIR mimetic.

Disclosures: G.A. Prieto: None. **E.D. Smith:** None. **L. Tong:** None. **C.W. Cotman:** None. **M. Nguyen:** None.

Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.07/LL20

Topic: E.02. Neuroimmunology

Support: IVIC151

Title: Serotonin 2A receptors modified taurine transport in lymphocytes of rat treated with fluoxetine

Authors: *M. G. COLMENARES, L. LIMA
Inst. Venezolano De Investigaciones Científicas, Caracas, Venezuela, Bolivarian Republic of

Abstract: Serotonin (5-HT) is a monoamine implicated in the regulation of many physiological and pathological systemic events. Fluoxetine, a selective 5-HT reuptake inhibitor and antidepressant, modulates immune cell functions, such as mitogen-induced lymphocyte proliferation and natural killer cell cytolytic activity. The aim of this work was to determine the role of 5-HT2A receptors on lymphocyte taurine transport after treatment with fluoxetine. Male Sprague-Dawley rats were given fluoxetine, 10 mg/kg ip, or vehicle for 1 week. [3H]Taurine was explored *ex vivo* in the presence of the agonists of 5-HT2A receptors, (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI), 0.001, 0,01 and 1 μM. The antagonist ketanserin, 0.01, 0.1, 1 and 10 μM was also given. Capacity of transport (Vmax) increased in mononuclear cells of rats treated for 1 week with fluoxetine. Affinity (Kt) was significantly different at 1 week of treatment. In concordance with previous results, fluoxetine treatment affected Vmax of taurine transport, new evidences support that DOI significantly inhibited the elevation of taurine transport produced by fluoxetine treatment. Ketanserine, by itself, did not affect taurine transport. These results indicate that fluoxetine could play an important role in the regulation of taurine transport by allowing an autocrine effect of 5-HT trough 5-HT2A receptors in lymphocytes, and further encourage exploring other 5-HT receptors and their functional consequences during antidepressant treatment.

Disclosures: **M.G. Colmenares:** None. **L. Lima:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Program#/Poster#: 254.08/LL21

Topic: E.02. Neuroimmunology

Support: Japanese Grant-in-Aid Scientifi Research C

Title: Anti-inflammatory effect of remifentanil in the mouse brain after systemic lipopolysaccharide administration

Authors: *S. MAEDA[1], R. OHNISHI[2], Y. TOMOYASU[2], H. HIGUCHI[1], T. MIYAWAKI[2]
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Abstract: Inflammation, caused by infection, injury, surgery and autoimmune disease, is arranged by a lot of factors, including psychological condition. During the course of the inflammation, fatigue, high fever and pain are brought, and occasionally it can be a cause of death. Thus, a controlling inflammatory reaction is not only for an earlier recovery or an alleviating suffering, but also for a saving life. Opioids, which are often used against a surgical invasion under general anesthesia as well as a cancer pain, affect immune reaction, but a detail mechanism and a clinical significance remain unclear. Remifentanil is an intravenous opioid for general anesthesia, and can be used for induction and maintenance in most of all general anesthesia because it is metabolized quickly by non-specific esterase, context-sensitive half-life is short and is hardly affected by liver and kidney function. A purpose of our study is to clarify the anti-inflammatory mechanism of opioids, and in this study, we evaluated the effect of remifentanil against acute general inflammation induced by LPS in the brain as well as peripheral organs. We used acute general inflammation model generated by intraperitoneal injection of LPS into mouse. Remifentanil was continuously administered through osmotic pump implanted subcutaneously. Under deep anesthesia, liver and brain were removed. From the samples, total RNA was purified and cDNA was synthesized. Using the cDNA, level of inflammatory cytokine mRNA was evaluated with real-time PCR. Interleukin 6 mRNA levels in both the brain and liver were raised by LPS injection, and those were inhibited by continuous infusion of remifentanil in mouse. Especially, IL-6 reaction to LPS was totally inhibited by remifentanil in the hypothalamus while that in liver was significant but ambiguous. Pharmacological effect of remifentanil is mediated with opioid receptors, and a direct inhibition of inflammatory cytokine on immune cells has been suggested recently. On the other hand, main

targets of opioids, including remifentanil, are opioid receptors in the central nervous system, and clinical effect of remifentanil is suppression of reaction to invasion. In addition, the hypothalamus is the center of stress reaction. Thus, a suppression of IL-6 reaction in the hypothalamus by remifentanil is likely due to the inhibition of reaction to invasion, at least in part.

Disclosures: **S. Maeda:** None. **H. Higuchi:** None. **Y. Tomoyasu:** None. **T. Miyawaki:** None. **R. Ohnishi:** None.

Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.09/LL22

Topic: E.02. Neuroimmunology

Title: Tumor necrosis factor induces specific neurograms in the sensory vagus nerve

Authors: *S. S. CHAVAN, S. ROBBIATI, B. E. STEINBERG, H. SILVERMAN, T. TSAAVA, P. T. HUERTA, K. J. TRACEY
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Abstract: Peripheral neural networks project real-time information to the central nervous system about the body's physiological status through the afferent fibers of the vagus nerve. As a part of this network, peripheral neural circuits also continually monitor and sense the *immunological* status of host, and relay that information to the brain stem and hypothalamus that in turn activates an effector neural response. Here we have mapped the peripheral neural activity that occurred in response to tumor necrosis factor (TNF) by recording from the cervical vagus nerve of adult mice. Following 10 min of baseline recording, in which vagus showed baseline spike frequency of 7.6 ± 1.6 Hz, animals received intraperitoneal administration of cytokine. TNF induced dose and time dependent increases in vagus nerve activity with an activation peak at ~3 min and a response rate of 35.9 ± 5.2 Hz. Administration of trypsin-digested TNF failed to induce significant increases in the vagus nerve activity. Using TNF receptor knockout animals, we confirmed that TNF induced signals are mediated in TNF-receptor specific manner. Together, these studies demonstrate that TNF induces a ligand-specific, receptor-mediated, dose-dependent enhancement in the peripheral vagus neurograms. The identification of TNF-specific neural signatures provides mechanistic insight into the immunological sensory neural code. Supported by grant from DARPA (W911NF-09-1-0125) to KJT.

Disclosures: **S.S. Chavan:** None. **S. Robbiati:** None. **B.E. Steinberg:** None. **H. Silverman:** None. **T. Tsaava:** None. **P.T. Huerta:** None. **K.J. Tracey:** None.

Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.10/LL23

Topic: E.02. Neuroimmunology

Title: Characterization of LRRK2-linked signaling pathways in human lymphoblast

Authors: ***T. LI**[1], L. KONG[2], J. THOMAS[2], A. SAWA[3], W. SMITH[2]
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Abstract: Mutations in the leucine-rich repeat kinase-2 (LRRK2) gene cause autosomal-dominant Parkinson's disease (PD) and contribute to sporadic PD. Common genetic variation in LRRK2 modifies susceptibility to immunological disorders including Crohn's disease and leprosy. Previous studies have reported that LRRK2 is expressed in B lymphocytes and macrophages, suggesting a role for LRRK2 in immunological functions. In this study, we characterized the LRRK2 protein expression and phosphorylation using human lymphoblasts. Lipopolysaccharide (LPS), a preinflammatory agent, treated human lymphoblasts resulted in increases of LRRK2 expression and kinase activities in a time dependent manner. Moreover, increase of LRRK2 protein expression by LPS appeared to be associated with activation of Toll-like receptor 4(TLR4) signaling. Treatment with LRRK2 kinase inhibitor, LRRK2-in-1, reduced LPS-induced TNF-α secretion. These results suggested that LRRK2 is actively involved in preinflammatory responses in human lymphoblasts.

Disclosures: **T. Li:** None. **L. Kong:** None. **J. Thomas:** None. **A. Sawa:** None. **W. Smith:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: NIH Grant NS051709-06(JRB)

Title: Chronic thoracic spinal cord injury impairs peripheral T-cell immunity by inducing T-cell exhaustion

Authors: ***J. ZHA**[1], A. SMITH[2], S. ANDREANSKY[2,3], V. BRACCHI-RICARD[1], J. R. BETHEA[4]
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Abstract: Chronic spinal cord injury (SCI) induces immune depression in patients, which contributes to their higher risk of developing infections and poorer prognosis. Using a well-established influenza virus mouse model, we demonstrated that chronic SCI mice have deficits in viral clearance following influenza infection, with a reduction in the number of virus-specific $CD8^+$ T-cells, suggesting the peripheral T-cell immunity is affected by chronic SCI. To study the chronic effects of SCI on the peripheral T-cell immunity, we used a severe contusion SCI mouse model at thoracic level 9 and investigated the peripheral T-cells under pathogen-free condition. We found that chronic SCI down-regulated both $CD4^+$ and $CD8^+$ T-cell cytokine production, but did not affect the T-cell proliferative capability. The observed $CD8^+$ T-cell dysfunction correlated with increased expression of programmed cell death 1 (PD-1) exhaustion marker on these cells. Blocking PD-1 signaling *in vitro* restored the CD8+ T-cell functional defect. In addition, we showed that chronic SCI mice had higher levels of splenic norepinephrine (NE), which contributed to the T-cell exhaustion phenotype, as T-cells show higher PD-1 expression and dysfunction in cytokine production following sustained exposure to NE *in vitro*. Taken together, these results indicate that alteration of sympathetic acitivity following chronic SCI induces T-cell exhaustion, which in turn impairs T-cell function and contributes to immune depression. T-cell exhaustion pathway should be considered as a new therapeutic target for the treatment of chronic SCI-induced immune depression.

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Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: DARPA Grant W911NF-09-1-0125 to KJT

Title: Neuronal input modulates antigen flow through peripheral lymph nodes

Authors: *W. HANES[1], P. S. OLOFSSON[1], T. TSAAVA[1], Y. A. LEVINE[2], S. S. CHAVAN[1], K. J. TRACEY[1]
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Abstract: The lymphatic system serves an important role in immunity. Extracellular fluid drains into the lymph vessels in tissues and is subsequently filtered through lymph nodes, where immune responses to foreign antigens are initiated. This initial exposure to an antigen, by infection or vaccination, is a critical step in developing protection against subsequent infection. A better understanding of these early steps in the initial host response could inform improvements in vaccination strategies. Antigen injected into a mouse hind foot dorsum flows first through the popliteal lymph node, then to the sciatic lymph node, before continuing up into the major thoracic lymphatic vessels. Interestingly, in mice immunized to Keyhole-Limpet Hemocyanin (KLH), flow of IrDye-labeled KLH was restricted through the popliteal and sciatic lymph nodes. Examination of KLH concentration one hour after administration in the hind foot, revealed a significant decrease in sciatic and popliteal KLH levels in immunized mice compared to naïve animals. Because neurons have recently been described to respond directly to bacterial infection, and because lymph nodes are innervated with motor and sensory neurons, we reasoned that neurons may regulate antigen flow in peripheral lymph nodes. Blocking of neuronal activation with bupivacaine at the popliteal and sciatic lymph nodes in immunized animals resulted in restoration of antigen flow, with an increase in popliteal antigen. Conversely, direct activation of neuronal signals at the popliteal lymph nodes of naïve animals using monopolar electrical stimulation resulted in significant decrease of antigen trafficking compared to sham stimulated controls. Skin samples taken from the injection site of immunized animals showed colocalization of PGP9.5-expressiong neurons and antigen signal, suggesting a direct activation of sensory neurons by antigen. Taken together, these studies reveal that neuronal input plays a significant role in regulation of hind limb lymph node antigen trafficking.

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Poster

254. Neuroimmune System Pathways and Regulators

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Program#/Poster#: 254.13/LL26

Topic: E.02. Neuroimmunology

Support: Peter Deane Trust

Title: Circadian time of infection affects the progression and outcome of vesicular stomatitis virus-induced encephalitis

Authors: *K. GAGNIDZE[1], I. KARATSOREOS[3], P. D'AGOSTINO[1], Z. MASHEEB[1], K. BULLOCH[1,2]
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Abstract: Circadian rhythms are recurring patterns of behavioral, endocrine and physiological parameters that exhibit periodicities of approximately 24 hours. Furthermore, many immune parameters exhibit circadian rhythmicity. For example, immunoreactions such as rate of phagocytosis, lymphocyte proliferation, antigen presentation as well as levels of serum cytokine and cytokine receptors have been shown to display significant circadian variations. Likewise the immune responses to various pathogens also display striking circadian dependency. Intranasal (i.n.) infection with vesicular stomatitis virus (VSV) in mice is characterized by the development of viral-induced encephalitis. Based on the expression of CD45, CD11b and CD11c, we have recently described three distinct cell populations that accumulate in the olfactory bulbs (OB) of VSV-infected mice. Further phenotypic and functional analysis revealed a heterogeneous group of immune cells with complex immunological functions within each of these cell populations. Thus, VSV-induced encephalitis mouse model provides an excellent system to study proinflammatory responses within the brain. In the present study we used this model to evaluate the effect of circadian rhythms on the pathogenesis of the viral-induced encephalitis. Two groups of mice were maintained on the opposite 12h:12h light-dark cycle and were subjected to i.n. VSV infection at either (i) onset of light (rest) cycle (designated as zeitgeber time 0, ZT0); or (ii) onset of dark (active) cycle (designated as zeitgeber time 12, ZT12). The health and survival of animals were monitored daily for two weeks. We found that the disease progression was significantly slower in mice infected at ZT12 compared to mice infected at ZT0. Accordingly, the survival rate of mice in ZT12 group was significantly higher, indicating that animals infected at the onset of active cycle were more resistant to viral-induced encephalitis. In addition,

increased survival rate of ZT12 mice was accompanied by a significantly larger proportion of CD45+/CD11b+/CD11c+ cells, which we have shown to be peripherally derived monocytic dendritic cells (DCs) with antigen-presenting capability. These data indicate that the local cellular response to VSV immune challenge is under circadian (or at least diurnal) regulation. Thus, further studies are warranted to identify antiviral response genes as well as the lineage and migration pattern of CD11c+ DCs that are under the control of circadian clock. These studies will provide new mechanistic insight into the development and resolution of neuroinflammatory pathologies.

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Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: MEXT/Monbukagakusho

Title: Electrophysiological properties of thalamic relay cells are prone to neuro-immune modulation by Interleukin-1β, interleukin-6 and tumor necrosis factor-α

Authors: *V. N. SAMIOS, T. INOUE
Waseda, Tokyo, Japan

Abstract: The reciprocal communication between brain and body has long called attention of human civilization, especially regarding health and disease paradigms. Inflammatory processes seem to play a key role in that relation, so that currently it is possible to find a growing body of evidence reporting actions of major pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in the brain. Considering that pro-inflammatory cytokines may affect information processing in neurons through cellular and molecular actions, experiments were conducted in order to investigate such actions. For that purpose, relay neurons in the thalamic dorsal lateral geniculate nucleus in mouse brain slices were exposed to IL-1β, IL-6 and TNF-α and studied with the patch-clamp technique. IL-1β promoted hyperpolarization of the resting membrane potential (Vrest), decrease of input resistance (Rin), decrease of Ih rectification and decrease in action potential (AP) threshold. IL-6

promoted decrease of Rin and increased latency of low threshold calcium spike (LTS) burst. TNF-α promoted decrease in the count of APs and prolonged inter-spike intervals in LTS bursts. Computer simulations provided candidates for ionic conductance affected by those cytokines, suggesting that IL-1β may cause an increase in the membrane potassium leak conductance and a decrease in the Ih current. The results here presented demonstrate, for the first time, that IL-1β, IL-6 and TNF-α have modulatory effects over electrophysiological properties of thalamic neurons. These effects may have clinical relevance when considering inflammatory conditions, either central or systemic.

Disclosures: **V.N. Samios:** None. **T. Inoue:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Support: Korean Government (MEST) Grant 2012R1A1A2040338

National Research Foundation of Korea (NRF) Grant 2012R1A3A2048834

Title: Mechanism of Natural Killer cytotoxicity against peripheral sensory neurons *in vitro*

Authors: *A. J. DAVIES, J. CHOI, M. LEE, S. OH
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Abstract: Natural Killer (NK) cells are circulating lymphocytes that display innate cytotoxicity against transformed tumor and virus-infected target cells, however very little is known about the interactions between NK cells and the nervous system. Dorsal root ganglion (DRG) neurons cultured from embryonic (E15) mice were more susceptible to lysis by syngenic IL-2 primed NK cells than DRG neurons cultured from adult mice. We show that cytotoxicity against embryonic DRG neurons is functionally dependent on the activating NK cell receptor NKG2D and that the ligand RAE1 is expressed over 10-fold higher in embryonic DRG neurons compared to adult by real time PCR. IL-2 primed NK cells spontaneously produced IFN-y and the cytotoxic factor granzyme B. We found that co-culture of NK cells with either embryonic or adult DRG neurons potentiated levels of granzyme B in the culture supernatant as detected by ELISA while levels of IFN-y were reduced, suggesting a switch to a cytotoxic phenotype. Using live confocal Ca2+-

imaging we show that embryonic DRG lysis is preceded by a rapid, synchronous intracellular Ca2+ response. We also observe that adult DRG neurites are minimally susceptible to Ca2+-dependent degeneration by IL-2 primed NK cells. In conclusion, expression changes of natural killer ligands may drive NK cell cytotoxicity against peripheral sensory neuron targets.

Disclosures: **A.J. Davies:** None. **J. Choi:** None. **S. Oh:** None. **M. Lee:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: JSPS Grant 25462309

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Title: PACAP receptor expression of hematopoietic stem/progenitor cells in mouse bone marrow with special reference to sympathetic innervation

Authors: Z. XU[1], *S. TANAKA[2], H. OHTAKI[1], J. WATANABE[1], Y. HIRAIZUMI[3], S. NUMAZAWA[2], S. SHIODA[1]
[1]Dept. of Anatomy, Showa Univ. Sch. of Med., Tokyo, Japan; [2]Dept. of Pharmacol., Toxicol. & Therapeut.,, Showa Univ. Sch. of Pharm., Tokyo, Japan; [3]Dept. of Orthopaedic Surgery, Showa Univ. Sch. of Med., Tokyo, Japan

Abstract: Functional linkage between nervous system and immune system is essential for maintaining homeostasis. In this crosstalk, the pathway via hypothalamic-pituitary-adrenal (HPA) axis has received more attention than the direct innervation of the immune organs. Pituitary adenylate cyclase-activating polypeptide (PACAP) has been thought as a neurotransmitter and immune-regulator, but its role in immune organs is poorly understood. Therefore, the purpose of this study is to investigate the role of PACAP in the crosstalk between nervous system and central immune organ, bone marrow (BM). The sympathetic nerve fibers were detected in the BM by frozen sections with tyrosine Hydroxylase and neurofilament 200 antibodies, wrapped with GFAP positive Shwann cells. Gene expressions of three PACAP receptors (PAC1R, VPAC1R and VPAC2R) were recognized in the BM by RT-PCR, but few expressed of these ligand PACAP. Then, PAC1R was multiple-immunostained with

hematopoietic cell markers in BM smear sections. Strong expression of PAC1R was detected in larger size and light chromatin condensation cells which were co-expressed with hematopoietic stem/progenitor cell markers (CD34, CD117 and Sca-1). PAC1R-like immunoreactions were also merged with Gr-1 and F4/80, which were myeloid markers. These results suggest that innervation of sympathetic nerve to the mouse BM where PAC1R is expressed on hematopoietic stem/progenitor cells and myeloid lineages. The source of PACAP in the BM, neuronal or humoral, and its regulatory role in hematopoiesis are studied at present.

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Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: NIH Grant R01-MH082900

Title: Mechanisms by which estradiol (E2) suppress neuronal cox-2 gene expression

Authors: *W. STACEY[1], R. M. UHT[1,2]
[1]Univ. of North Texas Hlth. Sci. Ctr., FORT WORTH, TX; [2]Inst. for Aging and Alzheimer's Research, Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Mechanisms by which estradiol (E2) suppress neuronal *cox-2* expression Winfred N Stacey[1] Rosalie M Uht[1, 2] [1]Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX 76107, USA [2]Institute for Aging and Alzheimer's Research, University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107, USA ABSTRACT Data from culture and animal models indicate that 17β-estradiol (E2) deprivation increases susceptibility to neurodegenerative and neuropsychiatric diseases. E2 plays a key role in attenuating inflammatory response in the brain by suppressing expression of pro-inflammatory genes; however, the mechanisms by which E2 suppress neuronal pro-inflammatory genes are not well established. The pro-inflammatory cyclooxygenase-2 gene (*cox-2)* is selectively expressed in neuronal populations of the amygdala, hippocampus and cortex (Yamagata, 1993; Kaufmann, 1996). Although E2 downregulates *cox-2* expression in the periphery, E2 effects on neuronal *cox-2* expression remain uncharacterized. To characterize the

effect of E2 on *cox 2* in neuronal system, we used the AR-5 rat neuronal cell line (Mulchahey, 1999). This immortalized line is derived from the amygdala. We first determined that the cell line constitutively expresses COX-2 protein by Western blots and immunocytochemistry, and mRNA and hnRNA by RT-qPCR. Twenty-four hours of E2 exposure reduces neuronal COX-2 mRNA and hnRNA levels. To assess whether the E2 effect was mediated by ER-alpha (ER-α) and/or ER-beta (ER-β) we treated the cells with E2, Diarylpropionitril (DPN), and propyl-pyrazole-triol (PPT). E2 and DPN treatment led to suppression of COX-2 mRNA and hnRNA after 24hrs. In distinction, PPT had no effect. Collectively, the data indicate that E2 suppresses neuronal *cox-2* expression through ER-β. Keywords: E2, *cox-2*, Neuroinflammation Funding: This work was supported by National Institutes of Health Grant R01-MH082900 (to R.M.U.)

Disclosures: W. Stacey: None. **R.M. Uht:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Support: NIH/NIGMS grant T32-GM007507 (Neuroscience Training Program)

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Title: Multiple intracellular antigen specific T cells enhance chronic but not acute inflammation in EAE

Authors: *A. RAYASAM[1], B. D. S. CLARKSON[2], M. G. HARRIS[2], A. RITTER[2], L. STEINMETZ[2], J. KARMAN[3], M. SANDOR[2], Z. FABRY[2]
[1]Univ. of Wisconsin - Madison, Madison, WI; [2]Pathology, Univ. of Wisconsin-Madison, Madison, WI; [3]Genzyme Corp., Cambridge, MA

Abstract: Multiple sclerosis (MS) is a chronic demyelinating disease involving abnormal immune-mediated processes directed towards the central nervous system (CNS). MS is partly driven by myelin-specific autoreactive T cells that infiltrate the CNS. Whether the contribution of antigen specific T cells is more dominant in the acute or chronic phases of EAE is still not understood. In order to address this issue, we generated transgenic mice using the

oligodendrocyte specific promoter 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) to localize an EGFP-tagged fusion protein containing ovalbumin (OVA) antigenic peptides in oligodendrocytes (CNP-OP mice). Using this model, we tested whether anti-OVA peptide-specific sentinel OT-I (CD8+) and OT-II (CD4+) T cells contribute to disease progression in the early or late phases of experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. Previously, we reported that intracellular oligodendrocyte antigens are sampled by antigen specific T cells in the deep cervical lymph nodes under homeostatic conditions. However, activated OT-I and OT-II T cells are unable to access the CNS in the absence of CNS inflammation. These results imply that under steady state conditions, the efferent CNS immune response is restricted, but the afferent immune system is intact despite the lack of conventional lymphatics. During neuroinflammatory conditions such as those that arise in EAE, OT-I and OT-II OVA specific T cells infiltrate the CNS, but the contribution of these cells to disease progression is not known. Here we report that adoptively transferred OVA257-264 peptide specific, MHC class I-restricted OT-I T cells and OVA323-339 peptide specific, MHC class II-restricted OT-II T cells did not exacerbate the acute phase (day 12-15) of EAE. However, adoptively transferred OT-I and OT-II T cells significantly enhanced EAE clinical scores in the chronic phase (Day 25-30). Furthermore, these symptoms correlated with antigen specific T cell accumulation in the brain and spinal cord implicating that the presence of OVA antigenic peptides within oligodendrocytes provides a milieu favorable for OT-I and OT-II T cell maintenance that likely contributes to a toxic environment resulting in augmented demyelination, axonal degradation and cell death. Together these results indicate that autoreactive T cells directed toward intracellular brain antigens exacerbate EAE symptoms and suggest that therapies targeted towards brain antigen specific T cells deserve more attention for treating MS.

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Poster

254. Neuroimmune System Pathways and Regulators

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Vanderbilt Joel G. Hardman Chair in Pharmacology (R.B.E.)

Title: Reovirus-mediated induction of ADAR1 (p150) only minimally alters RNA editing patterns

Authors: *J. L. HOOD[1], M. V. MORABITO[7], C. R. MARTINEZ, III[2], J. A. GILBERT[3], E. A. FERRICK[4], G. D. AYERS[5], J. D. CHAPPELL[6], T. S. DERMODY[6], R. B. EMESON[3]
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Abstract: Transcripts encoding ADAR1, a double-stranded, RNA-specific adenosine deaminase involved in the adenosine-to-inosine (A-to-I) editing of mammalian RNAs, can be alternatively spliced to produce an interferon-inducible protein isoform (p150) which has been shown to be up-regulated in both cell culture and *in vivo* model systems in response to pathogen or interferon stimulation. In contrast to other tissues, p150 is expressed at extremely low levels in the brain and it is unclear what role, if any, this isoform may play in the innate immune response of the central nervous system (CNS) or whether the extent of editing for RNA substrates critical for CNS function is affected by its induction. To investigate the expression of ADAR1 isoforms in response to viral infection and subsequent alterations in A-to-I editing profiles for endogenous ADAR targets, we have employed a neurotropic strain of reovirus to infect neonatal C57BL/6J mice and quantify A-to-I editing in discrete brain regions using a multiplexed, high-throughput sequencing strategy. We analyzed established editing sites known to impact protein/cellular function including transcripts encoding the serotonin-2C receptor (5-HT_{2C}R), multiple ionotropic glutamate receptor subunits, the gamma-aminobutyric acid receptor (GABA-A) subunit alpha-3, and the Kv1.1 voltage-gated potassium channel, as well as recently identified editing substrates CYFIP2, BLCAP and FLNA. While intracranial injection of reovirus resulted in a widespread increase in the expression of ADAR1 (p150) in multiple brain regions and peripheral organs, significant changes in site-specific A-to-I conversion were quite limited, suggesting that steady-state levels of p150 expression are not a primary determinant for modulating the extent of editing for numerous ADAR targets *in vivo*. We present a detailed quantitative analysis of ADAR1 p150 induction in concordance with the editing profiles of 12 editing sites in frontal cortex, hippocampus, and cerebellum, both in control and reovirus-infected animals. This study represents, to our knowledge, the first demonstration of ADAR1 p150 induction in the brain and the lack of altered editing in response to this induction is an unexpected finding.

Disclosures: J.L. Hood: None. **M.V. Morabito:** None. **C.R. Martinez:** None. **J.A. Gilbert:** None. **E.A. Ferrick:** None. **G.D. Ayers:** None. **J.D. Chappell:** None. **T.S. Dermody:** None. **R.B. Emeson:** None.

Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.20/MM5

Topic: E.02. Neuroimmunology

Title: Characterization of hematopoietically-derived macrophages to the central nervous system in mixed chimeric mice

Authors: *T. D. WILLIAMS, M. W. DEFAZIO, Z. W. HEROUX, J. M. KURTZ
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Abstract: Migration of macrophages into the central nervous system (CNS) during a non-inflammatory state is an area of research with the potential for significant clinical implications. We have established a mixed hematopoietic chimerism mouse model that utilizes donor green fluorescent protein (GFP) bone marrow cells and allows for the study of peripheral monocyte engraftment into the CNS parenchyma. Chimerism of blood and brain tissue was assessed by flow cytometry at various weeks post bone marrow transplantation (BMT). CNS macrophages were initially identified as $CD45^{mid}/CD11b^{+}$ cells. Expression of immune markers MHC class II and Ly6C was measured on both donor GFP^{+} and recipient GFP^{-} cell populations. To assess CNS macrophage and microglia morphology, Iba-1 and GFP expression was visualized using immunofluorescence microscopy (IF). At 12 weeks post-BMT, results demonstrated 43.2 ± 8.2% of hematopoietically-derived infiltrating macrophages expressed MHC class II and 38.5 ± 13.4% expressed Ly6C, whereas non-donor derived brain macrophages showed no Ly6C expression and 2.1 ± 0.2% expressed MHC class II. In the donor blood monocyte population 0.9 ± .1% of the cells expressed MHC class II and 63.3 ± 14.9% expressed Ly6C. Additionally, IF imaging showed $Iba\text{-}1^{+}/GFP^{+}$ cells closely resembled the morphology of resident microglia. These data suggest bone marrow-derived macrophages infiltrate the CNS in a non-disease state, change their phenotype, and adopt a microglial morphology.

Disclosures: T.D. Williams: None. **M.W. DeFazio:** None. **Z.W. Heroux:** None. **J.M. Kurtz:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Program#/Poster#: 254.21/MM6

Topic: E.02. Neuroimmunology

Support: Foundation Olle Engkvist Byggmästare

Title: Expression of peptidoglycan sensing molecules during postnatal brain development and their regulation by the commensal gut microbiota

Authors: *T. B. ARENTSEN[1], T. FEMENIA CANTO[1], S. GKOTZIS[1], K. UDEKWU[1], H. FORSSBERG[2], R. DIAZ HEIJTZ[1]
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Abstract: Pattern-recognition receptors (PRRs) play a key role in the innate immune responses by recognizing conserved microbial molecules derived from a diverse variety of microbial pathogens. However, these motifs are not exclusive to pathogens and are abundantly produced by the indigenous gut microbiota. Recent studies in germ-free (GF) mice have demonstrated that soluble peptidoglycan released by non-pathogenic bacteria can translocate into circulation and mediate remote systemic priming of immune cells. These novel findings raise the possibility that a similar mechanism may be operating within the brain. To this end, we investigated the expression of PRRs that selectively detect peptidoglycan molecules and the levels of peptidoglycan during mouse postnatal brain development (i.e., postnatal days 1, 3, 5, 7, 14, 21 and 56) as well as the influence of gut microbiota on their expression. Gene expression analysis of peptidoglycan recognition proteins (i.e., Pglyrp1-4) and nucleotide-binding oligomerization domain-containing protein (Nod)-like receptors (i.e., nod1 and nod2) showed significant expression changes during postnatal development. Interestingly, some PRRs (Pglyrp2-4) are highly expressed only during the first few days of life, indicating that a sensitive period exists, in which microbial colonization of the gut can influence brain development. Next we determined whether peptidoglycans could be detected during postnatal brain development. Peptidoglycan molecules were detected in the mouse brain and the results revealed an age-dependent increase in peptidoglycan levels during postnatal development. Perturbation of the intestinal microbiota (e.g., by using GF mice and antibiotic treatment during perinatal life) resulted in reduced brain-specific expression of some PRRs during early postnatal life. Our results indicate that microbiota-derived peptidoglycan molecules may affect brain development through activation of PRRs within the brain.

Disclosures: **T.B. Arentsen:** None. **T. Femenia Canto:** None. **S. Gkotzis:** None. **K. Udekwu:** None. **H. Forssberg:** None. **R. Diaz Heijtz:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.22/MM7

Topic: E.02. Neuroimmunology

Support: Research Grant for Doctoral dissertation from Focus Program Translational Neuroscience

Title: Deficiency of Angiotensin II type 2 Receptors (AT2) results in increased brain damage whereas activation of AT2 does not affect lesion growth after experimental traumatic brain injury in mice

Authors: *S. CORONEL CASTELLO[1,2], E.-V. SCHAIBLE[1], A. GARCIA BARDON[1], A. S. SEBASTIANI[1], M. K. E. SCHAEFER *[1,2], K. ENGELHARD[1,2], S. C. THAL[1,2], R. TIMARU-KAST[1]
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Abstract: Introduction: Recent studies demonstrated reduced brain damage by Angiotensin II receptor type 1 (AT1) inhibition after experimental traumatic brain injury (TBI) [1]. Alongside direct blockade of AT1 mediated vasoconstriction and inflammation, reduced brain injury may be due to increased activation of Angiotensin II receptor type 2 (AT2) that is upregulated after TBI [1]. The activation of AT2 with its antiinflammatory and regenerative properties may contribute to the protective potential of AT1 inhibitors [2,3]. Aim of the present study was to investigate the role of AT2 in murine TBI.
Methods: Study A: AT2 deficient (AT2-/y) mice and their wild type (WT) littermates were subjected to controlled cortical impact (CCI). Study B: 30 minutes after CCI and then daily the selective AT2 agonist compound 21 in low (LD 0.03 mg/kg), high dose (HD 0.1 mg/kg) or vehicle solution (VEH) was applied in C57B6 mice. In both studies neurological outcome was assessed using Neurological Severity Score (NSS). In study A contusion volume was determined 1 and 5 days, in study B 5 days after CCI in Nissl stained sections. Statistics: Wilcoxon Mann Whitney rank sum test.

Results: In both studies NSS showed no difference between groups within the first 5 days after CCI. There was no volumetric difference of brain damage 1 day after CCI in AT2-/y (40.5±4.2mm³) and WT (39.2±7.7mm³). However, contusion volume in AT2-/y was increased 5 days after CCI compared to WT (18.4±2.8 vs. 13.2±3.8mm³; $p<0.05$). Contusion volumes of LD, HD and VEH showed no differences (12.7±2.5, 12.3±3.0 and 13.8±2.4 mm³).
Discussion: While AT1 inhibition leads to reduced neurological deficit and brain damage 24 hours after TBI [1] AT2 deficient mice show no difference to WT at this time point. This suggests that AT2 has minor influence in the early phase after TBI. However, increased brain damage in AT2 deficient mice after 5 days indicates that AT2 is beneficial. Gene expression of AT2 is low in healthy adult animals. It is increased after TBI [1] and, moreover, by AT1 inhibition [4]. In previous studies it was postulated that AT2 is a protective factor in cerebral insults [2,3]. However, in contrast to AT1 inhibition selective AT2 activation did not affect secondary brain damage expansion within 5 days after TBI. The present results indicate therefore, that the neuroprotective effect of AT1 inhibition is not due to increased AT2 activity in the first 24 hours. Additive protective effects of AT2 activation with simultaneous AT1 inhibition at later time points require further investigations.
References: 1. Timaru-Kast, Crit Care Med 2012; 2. Li, FASEB J. 2005; 3. Mogi, Hypertension 2006., 4. Bregonzio, Stress 2008

Disclosures: **S. Coronel Castello:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Focus Program Translational Neuroscience (FTN). **E. Schaible:** None. **A. Garcia Bardon:** None. **A.S. Sebastiani:** None. **M.K.E. Schaefer *:** None. **K. Engelhard:** None. **S.C. Thal:** None. **R. Timaru-Kast:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 254.23/MM8

Topic: E.02. Neuroimmunology

Support: FISM GRANT 2010

Title: IL-27 and IL-35 as gene therapy of neuroinflammation

Authors: *G. CASELLA[1], A. FINARDI[2], J. MELDOLESI *[2], R. FURLAN[2]
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Abstract: Interleukin 27 and 35 belong to IL-12 cytokine family, which also includes, IL-12, IL-23. Even if all cytokines, belonging to this family, present a structural homology, they mediate different functions. In particular, IL-12 and IL-23 are mostly pro-inflammatory cytokines, with, respectively, key roles in the development of TH1 and TH17 cells, which represent the crucial effector cells in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). IL- 27 and IL-35 are mainly immunomodulatory cytokines and principally act on immune cell-mediated response. Current literature supports IL-27 as an immunoregulatory cytokine that modulates TH1 and TH17 cells response; IL-35 is a potent inhibitory cytokine produced by thymus derived natural regulatory T cells (nTreg) that suppresses T cells proliferation by inducing cell-cycle arrest in G1 phase without inducing apoptosis.We propose here to investigate the role of IL-27 and IL-35, through their expression by third generation lentiviral vectors in form of a single polypeptide chain containing the two subunits bound by an appropriate linker. We administered their into C57Bl/6 mice affected by chronic EAE, using the ependimal route as injection method to transfer the vector in the central nervous system (CNS). Both these cytokines, *in vitro*, demonstrate an anti-inflammatory activity: IL-35 modulates TNF-α release from stimulated CD4+ T cells in a dose dependent way, while IL-27 modulates CD4+ T cells proliferation. Nevertheless, *in vivo* present different effects: IL-35 shows slight modulation of EAE clinical score, unlike IL-27 that shows a significant modulation. Molecular analysis of IL-35 show no modulation of both pro- and anti-inflammatory genes, with the exception of M2 microglia phenotype markers Arg1 and Ym1. Unlike of IL-35, IL-27 gene therapy shows a interesting modulation of principal pro-inflammatory gene, such as iNOS, IL-23, T-bet and up-regolation of the anti-inflammatory genes: YM1, Arg1 and chemokine CCl22. The same results are obtained from the CD11b+ cells analysis from the brain tissue of the EAE mice. Pathological analysis show a significant decrease of perivascular infiltrates, which may represent a decrease in the number of postcapillary venules that are permissive to inflammatory cells diapedesis. This may suggest an effect of IL-35 gene therapy on vessel's structure. We are concluding our experiments using IL-27 and IL-35 gene therapy in EAE model and we believe that these cytokine can contribute an improvement to EAE pathogenesis, in different ways, and it's better to study their capacity to induce on M1/M2 phenotype on macrophages and microglia.

Disclosures: G. Casella: None. **A. Finardi:** None. **J. Meldolesi *:** None. **R. Furlan:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: NIH grant HL096571

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Title: Regional and phenotypic differences in hematogenous cells repopulating the brain after irradiation or chemotherapy-based myeloablative approaches for bone marrow transplantation

Authors: *Y. SUGIYAMA, G. FARACO, C. IADECOLA, J. ANRATHER
Feil Family Brain and Mind Res. Institute, Wei, New York, NY

Abstract: Bone marrow (BM) transplant is a widely used method to study the trafficking of immune cells into the central nervous system. However, the use of irradiation to induce myeloablation has been questioned because of the associated blood brain barrier alterations and induction of pro-inflammatory cytokines. Therefore, myeloablation using the alkylating agent busulfan has been proposed as an alternative (PLoS One, 8:e58544, 2013). However, the impact of different myeloablative modalities on the regional distribution and the phenotype of blood-derived cells has not been clearly established. To address this issue, we transplanted GFP+ BM into lethally irradiated (9.5Gy 18 hours before BM transplant) or busulfan-treated (30μg/g, i.p., 3,5,7days before BM transplant) C57Bl/6 mice (n=3/group), and examined GFP+ cells 14 weeks later in brain sections by confocal microscopy. GFP+ chimerism was >90% and comparable in blood neutrophils and monocytes of mice treated with irradiation or busulfan ($p>0.05$), while GFP+ chimerism in lymphocytes was lower in busulfan treated (71%) than irradiated mice (84%; $p<0.05$). In both groups, GFP+ cells were observed in the nucleus tractus solitarius (NTS), area postrema (AP), and subfornical organ, in the somatosensory cortex. However, the number of GFP+ cells repopulating the paraventricular nucleus (PVN) was higher in busulfan-treated (3025±399) than in irradiated mice (936±254/mm3; $p<0.05$; mean±SE). GFP+ cells repopulating the brain could be phenotypically divided in two major subpopulations: (a) elongated cells, closely associated with blood vessels and expressing the perivascular macrophage marker CD206 and (b) stellate cells with dendritic ramifications resembling microglia, not associated with blood vessels and expressing the microglia/macrophage markers Iba1 and cyclooxygenase-1. The number of elongated cells did not differ between irradiation- or busulfan-treated mice. However, the number of stellate cells was larger ($p<0.05$) after busulfan treatment than irradiation in PVN (irradiation 685±285; busulfan 2554±390/mm3), NTS (irradiation 644±478; busulfan 2187±261/mm3), and cortex (irradiation 7.7±1.3; busulfan 97.7±3.2/mm3). The data suggest that busulfan does not uniformly alter the brain repopulation of BM derived cells,

compared with irradiation. In PVN, NTS, and cortex, busulfan increases GFP+ stellate cells with the phenotypic characteristics of microglia. These marked regional differences in the number and phenotype of the repopulating cells need to be considered when using busulfan-based myeloablative approaches for BM transplantation.

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Poster

254. Neuroimmune System Pathways and Regulators

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Program#/Poster#: 254.25/MM10

Topic: E.02. Neuroimmunology

Support: HHMI Grant 52007563

RCMI Grant G12MD007585-23

NIH Grant 5R25GM067592-11

Title: Role of Interferon gamma (IFN-γ) in protecting SH-SY5Y cells against cisplatin-induced toxicity

Authors: C. ANDREWS[1], A. HENDERSON[1], M. R. VASKO[2], *G. D. GRIFFIN[1]
[1]Dept. of Biol., Tuskegee Univ., Tuskegee, AL; [2]Pharmacol. and Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: A serious health risk of the chemotherapeutic agent cisplatin is peripheral neuropathy. This peripheral neuropathy is caused by neurotoxicity induced by cisplatin. To find agents that would alleviate the neuropathy produced by cisplatin, this work tested the hypothesis that cytokine interferon gamma (IFN-γ) decreased cisplatin-induced cellular toxicity. IFN-γ is a cytokine that displays both antitumor and anti-inflammatory activities. Given these roles, we tested the hypothesis IFN-γ protects neural-like cells from cisplatin-induced toxicity. SH-SY5Y cells (neuroblastoma cells) were treated with cisplatin (1, 3, and 5 ng/mL) for 24 hours. Trypan blue and MTT cell viability assays were used to measure the percentage of cells surviving cisplatin treatment. All the data were analyzed using Graphpad Prism 6. An ANOVA with the post-hoc Tukey's test was used to determine the statistical significance ($p<0.05$), if warranted. The trypan blue assay demonstrated that cisplatin (5 ng/mL) reduced cell viability by 60 % within 24 hours. Next, the MTT assay data showed that pretreatment of IFN-γ (1 ng/mL)

prompted an increase in viable cells in the presence of cisplatin ($p<0.05$). Lastly, Annexin V/Propidium Iodide labeling via flow cytometry showed that IFN-γ protects the SH-SY5Y cells from cisplatin-induced toxicity. This analysis revealed that IFN-γ (1 ng/mL but not 3, 5, and 10 ng/mL) caused a decrease of 55% in cells double-labeled with Annexin V and PI (compared to cells administered captopril alone). Altogether, these results indicate that IFN-γ reduces cisplatin-induced neuronal damage in a concentration-dependent manner. These data point to the cytokine IFN-γ as a therapeutic target to ameliorate peripheral neuropathy induced by chemotherapeutic agents such as cisplatin.

Disclosures: C. Andrews: None. **A. Henderson:** None. **M.R. Vasko:** None. **G.D. Griffin:** None.

Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

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Program#/Poster#: 254.26/MM11

Topic: E.02. Neuroimmunology

Title: Neuronal activity in the mouse brain during acute endotoxemia

Authors: *T. TSAAVA, B. E. STEINBERG, S. S. CHAVAN, K. J. TRACEY
The Feinstein Inst. For Med. Res., Manhasset, NY

Abstract: The central nervous system maintains body homeostasis and regulates organ physiology. When environmental challenges disrupt this equilibrium, the brain senses the perturbation and coordinates a neurophysiological response to regain homeostasis. Selective regions of brain are activated in response to specific challenges such as pain, tissue injury and inflammation seen during bacterial infection. The current study was designed to identify specific deep brain regions that are activated in response to systemic inflammation. We used lipopolysaccharide (LPS)-induced endotoxemia as a model for systemic inflammation, and immediate early gene *c-fos* expression as a neuronal activation marker to identify the deep brain structures activated by bacterial infection. Adult male BALB/C mice were habituated by daily handling to reduce background c-Fos expression prior to LPS challenge. Following habituation, animals received intraperitoneal administration of LPS (2 mg/kg), and brain c-Fos expression was analyzed after 90 min by immunohistochemistry. c-Fos immunoreactive cells were observed in nuclear groups of hypothalamus, medulla and pons. These findings demonstrate that short-term systemic inflammation results in activation of deep brain regions responsible for

modulating physiological responses during illness. The future work will generate a detailed map of the specific brain nuclei acutely activated by LPS and compare it to the activation of other infectious agents, such as viruses.

Disclosures: T. Tsaava: None. **B.E. Steinberg:** None. **S.S. Chavan:** None. **K.J. Tracey:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: NIH Grant 2R01 NS045727-07

Title: Functions of E prostanoid receptors in post-stroke inflammation

Authors: *P. B. LARKIN, Q. WANG, J. MENZIES, X. LIANG, R. JUELSGAARD, L. LIN, K. ANDREASSON
Dept. of Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA

Abstract: Stroke is the fourth most prevalent cause of death in the US and is a leading cause of disability. However, current treatment options are limited and do not help the majority of patients, primarily because these treatments target only the early pathological events that cause the initial stroke damage. To avoid this limitation, next generation therapeutics should target pathological events that exacerbate the initial injury or improve recovery after stroke. Specifically, inflammation occurs immediately after stroke and immune system activation can persist for several days or even weeks after stroke. Inhibiting inflammation by inhibiting cyclooxygenase (COX) enzymes improves stroke outcomes in animal models, even when COX inhibitors are given many hours after the stroke began. However, COX inhibition is not a viable therapeutic strategy in humans because of unacceptable side effects. Instead, we investigated the immunomodulatory E prostanoid (EP) receptors that are downstream mediators of the COX enzymatic cascade that are unlikely to contribute to the side effects of COX inhibition. The four members of the EP receptor family govern multiple distinct immunomodulatory functions in a variety of relevant cell types including neurons, endothelial cells and glial cells. Based on the role of inflammation in stroke and the function of these EP receptors in the immune system, we hypothesized that 1) the EP2 receptor mediates harmful immunomodulation and 2) the EP4

receptor mediates beneficial immunomodulation during post-stroke inflammation. We tested our hypotheses by using novel genetic and pharmacological tools to modulate either EP2 or EP4 signaling. Our genetic experiments employed conditional knockout mice that lack EP receptor expression in either neurons, endothelial cells or myeloid cells (including microglia). In contrast, pharmacological experiments that employed EP receptor agonists and antagonists allowed us to determine the effects of EP receptor inhibition in multiple cell types at both early and late time points. Our results are largely consistent with opposing roles for these two EP receptors and suggest that EP receptor mediated immunomodulation may be a viable therapeutic target in stroke.

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Poster

254. Neuroimmune System Pathways and Regulators

Location: Halls A-C

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Program#/Poster#: 254.28/MM13

Topic: E.02. Neuroimmunology

Title: Effect of endogenous neurotoxin salsolinol and nm-salsolinol on alpha-synucein aggregation in neuroblastoma sh-sy5y cells -a step forward to uncover the mechanism of parkinson's disease

Authors: *H. QING, F. WANG
Beijing Inst. Technol., Beijing, China

Abstract: Alpha-synuclein(α-syn) aggregation and its expansion in the central nervous system is one significant pathological feature in Parkinson's disease(PD). This progression process comes with a specific inflamed tissue environment. Little is known about the role of neuroinflammation and the adaptive immune system in aggregation of α-syn in PD brain. This study is designed to elucidate the effects of two endogenous neurotoxins i.e. 1-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline (Salsolinol, Sal) and 1(R),2(N)-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (N-methyl-(R)-salsolinol , NMSal) on aggregation of α-syn in wild type EGFP -SH-SY5Y(EGFP) cells, stable wild type α-syn overexpressed α-syn -EGFP-SH-SY5Y (α-syn-WT) cells and stable missense mutation A53T-α-syn-EGFP SH-SY5Y(α-syn-A53T) cells. Here, it is found that Sal and NMSal induced aggregation of α-syn in α-syn-WT and α-syn-A53T cells but not in EGFP cells in 24 hours. Meanwhile, Sal and NMSal induced α-syn

aggregation in EGFP cells when it was co-cultured with glioblastoma multiforme cell line U87 and human T-cell leukemia Jurkat cells for 24 hours but α-syn could not accumulated in EGFP cells when it was co-cultured with U87 cells. Aggregated α-syn did not cause apoptosis but induced autophagy with increased LC3 expression level in cells, furthermore, autophagy initiator rapamycin could deduce aggregation of α-syn in EGFP, α-syn-WT and α-syn-A53T cells. Results confirmed that Sal and NMSal could induce aggregation of stable over expressed wild type and mutant α-syn in SH cells, while peripheral lymphocyte Jurkat cells could accelerate the speed and progress of aggregation of endogenous α-syn in SH cells which treated by Sal and NMSal, and aggregated α-syn could induce autophagy with increased LC3 expression level.

Disclosures: H. Qing: None. **F. Wang:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Topic: E.02. Neuroimmunology

Support: FAPESP

CNPq

CAPES/PROEX

Title: Acute hypoxia increases proinflammatory cytokines in brain areas involved in autonomic regulation

Authors: *A. T. TAKAKURA[1], T. M. SILVA[2], R. C. SILVA[3], L. J. CHAAR[2], V. R. ANTUNES[2], N. O. CAMARA[3], T. S. MOREIRA[2]
[1]Dept of Pharmacology, Inst. of Biomed. Science, Univ. of Sao Paulo, Sao Paulo, Brazil; [2]Physiol. and Biophysics, [3]Immunol., Inst. of Biomed. Science, Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Prolonged and continuous exposure of mammals to a low oxygen environment (chronic hypoxia) elicits remarkable morphological and physiological adjustments. These include altered gene expression, increased peripheral chemosensitivity, enhanced respiratory drive and sympathoexcitation. The current study examines the hypothesis that acute hypoxia initiates an immune response elicited by an increased expression of inflammatory cytokines.

Male Wistar rats were subjected to acute hypoxia (AH: 8% O2, balanced with N2) or normoxic (21% O2) for 3 hours. AH increased the proinflammatory cytokine IL-6 in the heart (41 ± 29 vs. normoxia: 0.04 ± 0.02), lung (25 ± 9 vs normoxia: 3.3 ± 1), kidney (1.2 ± 0.2 vs normoxia: 0.04 ± 0.02) as well in brain areas such as the rostral ventrolateral medulla (RVLM) (2.0 ± 0.4 vs normoxia: 0.9 ± 0.2) and paraventriuclar nucleus of the hypothalamus (PVN) (2.1 ± 0.3 vs normoxia: 1.0 ± 0.04). AH also increased hypoxia-inducible factor - HIF-1α within the RVLM (1.43 ± 0.2 vs normoxia 0.75 ± 0.03). No changes were observed in IL-1 β and TNF values. Taking together these results suggest that immune system may be involved in the response to chemoreceptors activation during AH

Disclosures: **A.T. Takakura:** None. **T.M. Silva:** None. **R.C. Silva:** None. **L.J. Chaar:** None. **V.R. Antunes:** None. **N.O. Camara:** None. **T.S. Moreira:** None.

Poster

254. Neuroimmune System Pathways and Regulators

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Program#/Poster#: 254.30/MM15

Topic: E.02. Neuroimmunology

Support: CAPES

Title: Behavioral aspects of the LPS-induced sickness during lactation

Authors: *A. F. NASCIMENTO, SR[1], L. FREITAS FELICIO[1], M. BERNARDI[2], G. ALVES[1]
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Abstract: Maternal behavior (MB) in mammals has specific characteristics. The time period just after parturition is particularly sensitive to physiological changes that can modulate the expression of this important behavior. Behavioral changes observed in sick animals, are considered as sick behavior (SB). Exposure to LPS, an endotoxin derived from the wall of a gran negative bacteria, during pregnancy might cause mental diseases. In order to investigate, a possible relationship between MB and SB, animals were treated with LPS. For the study of MB and MB aggressive, 40 rats were divided in 4 groups, 2 control and 2 experimental groups. The experimental group received 100µg/kg LPS by ip, and control group the vehicle of endotoxin, after 48 hours of LPS administration the observations of SB began. For choice these days, 20 virgin and 20 lactating rats were divided in 4 groups, 2 control and 2 experimental. They received ip 100µg/kg. Body weight, water and feed consumption, and body temperature were

measured for 120h. Females in the control group were observed in the same way, but they were treated with vehicle of LPS. The results showed that: 1) In 48 hours after the treat with LPS, virgin and lactating rats showed temperature increase, loss of body weight, increase in water consumption and a decrease in consumption food, 2) In 48 hours after the treat with LPS, lactating rats showed reduced latency to rescue the first pup to the nest. In the offspring of mothers treated with LPS it was found that: 3) There were changes the vocalization pattern of pups on the 5th day of lactation from mothers exposed to LPS in 3th day of lactation; 4) Altered the Burst and phagocytosis on lactation 21th day of lactation. It is concluded that exposure of rats to LPS promoted changes in the in the interaction between mother and pups.

Disclosures: A.F. Nascimento: None. **L. Freitas Felicio:** None. **M. Bernardi:** None. **G. Alves:** None.

Poster

255. Thirst and Water Balance

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Topic: E.06. Thirst and Water Balance

Support: CIHR grant MOP9939

CIHR grant FRN82818

Title: Unique interweaved microtubule scaffold mediates osmosensory transduction via physical interaction with TRPV1

Authors: *M. PRAGER-KHOUTORSKY[1], A. KHOUTORSKY[2], C. W. BOURQUE[1]
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Abstract: The electrical activity of mammalian osmosensory neurons (ONs) is increased by plasma hypertonicity to command thirst, antidiuretic hormone release and increased sympathetic tone during dehydration. Osmosensory transduction is a mechanical process whereby decreases in cell volume cause the activation of transient receptor potential vanilloid type-1 (TRPV1) channels to induce depolarization and increase spiking activity in ONs. However it is not known how cell shrinking is mechanically coupled to channel activation. Using super-resolution imaging we found that ONs are endowed with a uniquely interweaved scaffold of microtubules throughout their somata. Microtubules physically interact with the C-terminus of TRPV1 at the

cell surface and provide a pushing force that drives channels activation during shrinking. Moreover, we found that changes in the density of these interactions can bi-directionally modulate osmosensory gain. Microtubules are thus an essential component of the vital neuronal mechanotransduction apparatus that allows the brain to monitor and correct body hydration.

Disclosures: **M. Prager-Khoutorsky:** None. **A. Khoutorsky:** None. **C.W. Bourque:** None.

Poster

255. Thirst and Water Balance

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Topic: E.06. Thirst and Water Balance

Support: CIHR Grant

Title: Effect of suprachiasmatic nucleus stimulation on organum vasculosum of the lamina terminalis neurons in rat hypothalamic slices

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Abstract: Increases in extracellular fluid (ECF) osmolality stimulate thirst by depolarizing and promoting action potential firing by osmosensitive neurons of the organum vasculosum of the lamina terminalis (OVLT). Although thirst is suppressed during sleep, this state is associated with a rise in ECF osmolality as water intake is suppressed, and body water is lost through evaporation and production of urine. The mechanism by which thirst is suppressed during sleep is unknown. In nocturnal rodents, neurons in the suprachiasmatic nucleus (SCN) are electrically silent during the dark (wake) phase and their electrical activity increases during the light (sleep) phase. Since the SCN is the master clock and thirst is suppressed during sleep, we hypothesize that SCN clock neurons inhibit OVLT neurons during sleep. To investigate this possibility we obtained whole-cell current clamp recordings from OVLT neurons in horizontal slices of adult rat hypothalamus. Low frequency electrical stimulation of the SCN (20-80 μA; 0.5 ms; 0.5 Hz) evoked a mixed fast synaptic response consisting of overlapping excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs). EPSPs were reversibly blocked by 3 mM kynurenic acid, whereas IPSPs were blocked by 10 μM bicuculline, indicating that they were respectively mediated by glutamate and GABA ionotropic receptors. In most cells the IPSP appeared to dominate the EPSP since a net hyperpolarization was observed after stimulation. We next

investigated the functional impact of stimulating the SCN at 10 Hz, a frequency corresponding to the firing rate of SCN neurons during sleep. In the absence of antagonists, repetitive stimulation for 30 s caused a reversible hyperpolarization associated with a pronounced inhibition of spontaneous firing by the OVLT neuron. This effect was accompanied by temporal summation of IPSPs and was reversibly blocked by bicuculline (10 μM), indicating the involvement of GABA-A receptors. Interestingly, the acute inhibitory effect observed during repetitive stimulation was commonly followed by a long lasting depolarization and increase in firing. Bath application of kynurenic acid and bicuculline together did not prevent this effect. However, application of the selective vasopressin V1a receptor antagonist SR 49059 (10 μM) significantly reduced the excitatory response of OVLT neurons caused by repetitive stimulation of the SCN. Although the acute inhibitory effect of GABA-A receptors observed during repetitive stimulation is consistent with our hypothesis that the SCN inhibits OVLT neurons during sleep, the functional significance of the V1a receptor mediated depolarization remains to be determined.

Disclosures: C. Gizowski: None. **C.W. Bourque:** None.

Poster

255. Thirst and Water Balance

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Topic: E.06. Thirst and Water Balance

Support: CNPq 302683/2009-0

Title: Central effect of IL-1β on sodium appetite: Participation of specific brain areas

Authors: *J. B. FREGONEZE[1], D. R. CERQUEIRA[1], A. I. R. NASCIMENTO[2], A. L. MOITEIRO[1], E. S. ARAÚJO[1], M. A. C. ALELUIA[1], H. S. FERREIRA[3]
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Abstract: Some data appoint to the reciprocal communication between the brain and immune system and interleukin-1β (IL-1β) seems to be the mediators of this communication. IL- 1β may modulate homeostatic functions including fever, feeding, drinking and cardiovascular control. Previously we observed that intracerebroventricular injections of IL-1β inhibit salt intake in sodium-depleted rats. In the present study we investigate the effect of IL-1β injections into the median preoptic nucleus (MnPO), the subfornical organ (SFO), the central amygdala (CeA) and

the medial amygdala (MeA), brain structures that are part of the central network regulating sodium appetite. Male Wistar rats (240-260g) rats were anesthetized with ketamine/xylazine (80/7 mg/kg) for guide cannula implant in the MnPO, SFO, CeA or MeA. Five days after the surgery sodium-depleted rats received injections of IL-1β (1.6 ng/0.2 µl) into the MnPO (n=10), the SFO (n=8), the CeA (n=7), or the MeA (n=6). The control groups received isotonic saline injections the same areas (SFO n=8; MnPO n=10; CeA n=9; or MeA n= 8). The data were submitted to one-way ANOVA followed by the post-test Student-Newman-Keuls test ($p<0.05$). The results shows that, in all brain areas studied, injections of IL-1β inhibit sodium appetite in sodium-depleted rats. The volume of hypertonic saline intake at 120 min after injection of IL- 1β into MnPO, SFO, CeA and MeA was 0.46 ± 0.2 ml, 3.95 ± 0.4 ml, 0.75 ± 1.4 ml and 1.71 ± 0.7 ml respectively. The amount of saline intake in the control groups was 6.62 ± 0.2 ml (MnPO), 6.6 ± 0.1 ml (SFO), 6.7 ± 0.1 ml (CeA) and 6.02 ± 0.1ml (MeA). Comparing the results of the different areas studied, the antinatriorexigenic effect induced by IL-1β injections into SFO was less prominent than the effects observed the other areas. The results suggest that SFO, MnPO, CeA and MeA are important brain sites for IL-1β actions modulating sodium appetite.
Keywords: IL-1β, sodium appetite

Disclosures: J.B. Fregoneze: None. **D.R. Cerqueira:** None. **A.I.R. Nascimento:** None. **A.L. Moiteiro:** None. **E.S. Araújo:** None. **M.A.C. Aleluia:** None. **H.S. Ferreira:** None.

Poster

255. Thirst and Water Balance

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Topic: E.06. Thirst and Water Balance

Support: CIHR

Title: Impact of activity pattern on presynaptic inhibition of excitatory neurotransmission via somato-dendritic release in rat supraoptic nucleus neurons

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Abstract: Magnocellular neurosecretory cells (MNCs) in the supraoptic nucleus can secrete peptides (oxytocin and vasopressin; OT, VP) from their axon terminals in the neurohypophysis, as well as from their soma and dendrites. Previous studies have shown that the firing rate and pattern of action potential (AP) discharge of MNCs can modulate neurosecretion from axon terminals. However it is unclear if patterning can also affect exocytosis from the somato-dendritic compartment. Here we investigated if spike clustering, a firing activity characterized by the occurrence of bursts of action potentials lasting between 0.3-2 seconds, can facilitate somato-dendritic release relative to a tonic firing activity when using the same number of action potentials (100 APs). We stimulated the organum vasculosum lamina terminalis during whole cell patch clamp recordings from MNCs in angled horizontal slices of rat hypothalamus to elicit evoked excitatory postsynaptic current (EPSC) in these cells. We found a significant difference between these two types of firing activity (clustering compared to tonic) on the depression of the evoked EPSC with a greater inhibition for clustering activity ($p<.05$) for both eGFP VP and RFP OT-identified cells. To determine if this activity-dependent inhibition act on a presynaptic locus, we analyzed paired pulse ratio (PPR) that showed a reduction of PPR following different train of stimuli elicited in MNCs. These preliminary results suggest that clustering and tonic activity differentially modulate the strength of presynaptic inhibition of glutamatergic neurotransmission.

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Poster

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Topic: E.06. Thirst and Water Balance

Support: FAPESP

CNPq

Title: Involvement of angiotensinergic mechanisms on sodium intake induced by central cholinergic activation

Authors: *C. F. RONCARI, R. B. DAVID, P. M. DE PAULA, D. S. A. COLOMBARI, L. A. DE LUCA JR., E. COLOMBARI, J. V. MENANI
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Abstract: Central facilitatory and inhibitory mechanisms are involved in the control of water and salt intake. Important inhibitory mechanisms for the control of water and sodium intake are present in the lateral parabrachial nucleus (LPBN). Intracerebroventricular (icv) injection of carbachol (cholinergic agonist) that usually induces thirst also induces NaCl intake if the inhibitory mechanisms are deactivated with injections of moxonidine (α2-adrenoceptor/imidazoline agonist) into the LPBN. In the present study, we investigated the involvement of central angiotensinergic mechanisms on water and NaCl intake in rats treated with carbachol icv combined with moxonidine into the LPBN. Male Holtzman rats (3 months old, n = 10) weighing 290-310 g with guide-cannulas implanted into the lateral ventricle and bilaterally into the LPBN were used. Moxonidine or vehicle was injected into the LPBN and atropine (muscarinic cholinergic antagonist), losartan (AT1 angiotensinergic antagonist) or saline was injected into the lateral ventricle (LV). Fifteen minutes later, carbachol was injected into the LV. Water and 0.3 M NaCl intake was recorded for 2 h starting immediately after carbachol injection. The experimental procedure was approved by Ethics Committee in Animal Use (CEUA) from the School of Dentistry - UNESP (CEUA-FOAr 01/2011). Moxonidine (0.5 nmol/0.2 µl) injected into the LPBN increased water (11.1 ± 3.6 ml/2 h, vs. vehicle: 5.0 ± 1.2 ml/2 h) and 0.3 M NaCl intake (16.6 ± 5.8 ml/2 h, vs. vehicle: 1.0 ± 0.2 ml/2 h) in rats treated with icv carbachol (4 nmol/1.0 µl). The pretreatment with icv injection of atropine (20 nmol/1.0 µl) or losartan (100 µg/1.0 µl) abolished water (0.9 ± 0.6 ml/2 h and 1.2 ± 0.6 ml/2 h, respectively) and reduced 0.3 M NaCl intake (2.4 ± 1.3 ml/2 h and 4.0 ± 2.0 ml/2 h, respectively) in rats treated with icv carbachol combined with moxonidine into the LPBN. The results suggest the involvement of central angiotensinergic mechanisms on water and NaCl intake induced by central cholinergic activation combined with the blockade of LPBN inhibitory mechanisms.

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Poster

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Topic: E.06. Thirst and Water Balance

Support: JSPS KAKENHI 23592743(K.I.)

JSPS KAKENHI 26462819(K.I.)

Title: Acetaldehyde induces thirst sensation through the renin-angiotensin system in rat

Authors: I. UJIHARA, S. HITOMI, K. ONO, Y. KAKINOKI, *K. INENAGA
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Abstract: In hangover, people frequently experience heavy thirst as well as headache, nausea, vomiting and dizziness. It has been thought that the latter symptoms are elicited by acetaldehyde (ACD), a metabolite of ethanol (EtOH) while thirst sensation is elicited by EtOH-induced urination. ACD has never been considered to be a thirst inducing factor in hangover. Studies have reported that ACD causes suppression of blood pressure and stretch receptors in afferent arterioles of the kidney are stimulated by the pressure drop, and then renin is secreted from the juxtaglomerular cells of the kidney. We hypothesized that ACD is a factor inducing thirst sensation in hangover. Male Wistar rats were used in the present study. Intraperitoneal injection of EtOH significantly increased water intake. Coadministration of the aldehyde dehydrogenase inhibitor cyanamide with EtOH increased both water and salt intakes further and earlier. ACD with cyanamide more rapidly elicited water and salt intakes. The elicited water and salt intakes were suppressed by intraperitoneal and intracerebroventricular injections of angiotensin receptor AT1 antagonist candesartan. Plasma renin activity was increased after ACD while plasma osmolality and Na^+ concentration were not changed. Urination was less found in the early stage even in the administration of ACD. When rats were allowed to drink water and salt solution, urine volume was increased only after drinking, suggesting that urination is not a main trigger for initiation of drinking behavior induced by ACD. Immunohistochemical study showed that ACD increased the number of c-Fos immunopositive neurons in the brain regions of thirst center. The increased number of immunopositive cells was suppressed by candesartan. Taken together, thirst sensation by ACD may be mediated through the renin-angiotensin system and induced by activation of the thirst center of rat brain. The present study suggests that ACD causes heavy thirst sensation in hangover.

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Poster

255. Thirst and Water Balance

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BBSRC - BB/J015415/1

CISBi-NAP/USP

Title: Effects of the salt loading during prenatal and postnatal period on the gene expression of the adulthood offspring

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Abstract: Several epidemiological and experimental studies demonstrated that pregnant rats, when exposed to adverse environments, permanently program the physiology and metabolism of their fetuses. Recently, several studies demonstrated that insults during ontogenic period program the profile of the genes expression in adult animals. We investigated genes expression profile of male rats from mothers (M) drinking 0.9% NaCl (MS) compared to mothers drinking water (MW) during the pregnancy-lactation period. After lactation, offspring (O) were divided into two groups, water (OW) or 0.9% NaCl (OS), until 60 days old. Thus, groups were Mother Water and Offspring Water (MW-OW), Mother Water and Offspring Saline (MW-OS), Mother Saline and Offspring Water (MS-OW) and Mother Saline and Offspring Saline (MS-OS). The transcriptome profile in the paraventricular (PVN) and supraoptic nucleus (SON) was performed using the RNA sequencing technology. The transcripts were aligned to Rattus novergicus rn5 UCSC reference genome sequence by TopHat program. Next, the alignment results were processed using Cufflinks for gene and transcript quantification at the Galaxy environment. The expression value of each transcript was calculated by FPKM. Differentially expressed genes were found by Cuffdiff program and screened out by each transcript expression in MW-OS, MS-OW and MS-OS groups compared to control group (MW-OW). Meanwhile, paired t-tests were performed to assess the difference significance. Transcripts with an value of log2 (foldchange) >= 1, log2 (foldchange) <= -1 and q-value <= 0.05 were considered differentially expressed. We identified 542 differentially expressed genes in the PVN and 171 differentially expressed genes in the SON between treatment and control groups. Among this genes, 49 were up-regulated in the MW-OS, 18 in the MS-OW and 32 in the MS-OS. Moreover, 25 down-regulated genes were found in the MW-OS, 26 in the MS-OW and 21 in the MS-OS at the SON comparison to MW-OW group. For PVN were identified 115 up-regulated genes in the MW-OS, 116 in the MS-OW and 143 in the MS-OS. Moreover, 17 genes were down-regulated in the MW-OS, 56 in the MS-OW and 95 in the MS-OS in the SON comparison to MW-OW group. These data suggest that a

high-sodium diet during pregnancy and lactation can be used as an animal model for further experiments and can provide valuable information of how changes in genes expression profile can generate diseases in adulthood by modifying the regulation of several systems as the neuroendocrine and hydroeletrolitic systems.

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Poster

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Topic: E.06. Thirst and Water Balance

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Title: Synergy of angiotensin II and deoxycorticosterone acetate during sodium depletion-induced salt appetite in old rats

Authors: *R. L. THUNHORST[1,2], S. CLAYTON[1], T. BELTZ[1], B. XUE[1,2], A. K. JOHNSON[1,2,3,4]
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Abstract: Old Brown Norway rats are notably deficient in expressing salt appetite after periods of sodium depletion. We hypothesize this is due to insufficient secretion of renin--with consequent diminished levels of circulating angiotensin II (Ang II)--and of the mineralocorticoid, aldosterone, in response to sodium deficit. Therefore, we tested if systemic delivery of Ang II and/or the synthetic mineralocorticoid, DOCA, will re-establish salt appetite in old (29 - 30 mo) rats. Rats were depleted of sodium by injections of the diuretic/natriuretic, furosemide. Some received DOCA (0.5 mg/kg bw, sc) after the diuresis. All had access only to sodium-deficient diet and water for 20 hrs. The next day, rats were infused for 3 hrs either with isotonic saline (10 µl/min) or Ang II (30 ng/min, iv) and provided access to water and hypertonic saline solution (0.3 M NaCl) for drinking. Intakes were recorded hourly. Depleted rats infused with isotonic

saline drank little saline solution, especially in the first hr of fluid access (i.e., 0.3 ± 0.2 ml). Depleted rats that also received sc DOCA or iv Ang II also drank trivial amounts of saline solution at this time (0.2 ± 0.2 ml and 0.2 ± 0.1 ml, respectively). However, depleted rats that received both sc DOCA and iv Ang II drank substantially more saline than the other groups (3.2 ± 0.7 ml). The amount of saline consumed by sodium-depleted rats receiving both sc DOCA and iv Ang II is the most ever observed by old Brown Norway rats in response to challenge. Non-depleted, control rats receiving either sc DOCA or iv Ang II separately drank little saline solution (0.1 ± 0.1 ml and 0.6 ± 0.3 ml, respectively). We conclude that old rats fail to express salt appetite after sodium depletion primarily because they fail to generate sufficient endogenous hormonal signal.

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Poster

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Topic: E.06. Thirst and Water Balance

Title: Activation of GABAA receptors in the lateral parabrachial nucleus induces ingestion of sodium bicarbonate in rats

Authors: *J. C. CALLERA[1], R. B. DAVID[2], C. A. F. ANDRADE[2], L. A. DE LUCA JR[2], J. V. MENANI[2]
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Abstract: We previously demonstrated that bilateral injection of muscimol, GABAA receptor agonist, into the lateral parabrachial nucleus (LPBN) induce strong ingestion of 0.3 M NaCl and water in rats (Callera et al., Neuroscience, 134, 725-735, 2005). In the present study we investigated if muscimol injections into the LPBN would induce the ingestion of other mineral solution like sodium bicarbonate (NaHCO3) in normohydrated rats or in cell-dehydrated rats. Male adult Wistar rats with bilateral stainless steel guide-cannulas implanted in the LPBN were used. Distilled water and 0.3 M NaHCO3 intake was measured in two-bottle tests at every 30 min during 210 min, starting 15 min after bilateral injections of muscimol or saline into the LPBN in normohydrated rats or in rats treated with intragastric load of 2 M NaCl (IG 2 M NaCl,

2 ml/rat). In normohydrated rats, bilateral injections of muscimol (0.5 nmol/0.2 µl, n=5) into the LPBN induced 0.3 M NaHCO3 intake (22.3 ± 9.3, vs. saline: 0.2 ± 0.2 ml/210 min) and a slight ingestion of water (4.4 ± 2.5, vs. saline: 0.2 ± 0.1 ml/210 min). Bilateral injections of muscimol into the LPBN in rats treated with IG 2 M NaCl also induced strong 0.3 M NaHCO3 intake (45.1 ± 9.0, vs. saline: 0.8 ± 0.3 ml/210 min), without changing water intake. These data show that muscimol injections into the LPBN also induce strong 0.3 M NaHCO3 intake in normohydrated or in hyperosmotic cell-dehydrated rats, suggesting that GABAA receptors activation in this area induces the ingestion of different mineral solutions containing sodium. Research supported by: FAPESP and CNPq

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Poster

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Topic: E.06. Thirst and Water Balance

Support: CNPq 475252/2011-0

Title: Effect o central administration of carvacrol on sodium appetite and blood pressure in spontaneously hypertensive rats

Authors: *A. S. BATISTA[1], C. F. PERRONE[1], J. M. ANDRADE[1], F. B. CARVALHO[1], H. S. FERREIRA[2], J. B. FREGONEZE[1]
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Abstract: Carvacrol is a monterpene constituent of essential oil of oregano (*Origanum dictamus L.*). Some studies have shown analgesic, antioxidant, anxiolytic effects of carvacrol. Also, it has been observed a hypotensive and bradycardic effect after intravenous injection of carvacrol in normotensive, anesthetized rats. Preliminaries data from our Lab show that intravenous injection of carvacrol present a hypotensive and bradycardic action. In the present study it was investigated the effects of carvacrol injections into lateral ventricle on the blood pressure and sodium appetite in spontaneously hypertensive rats (SHR). Male SHR (13-16 weeks) received a guide cannula implant in the lateral ventricule (VL) and a catheter was inserted into the carotid

artery to record mean arterial pressure (MAP) and heat rate (HR) 24 h before the experiments. Sodium depletion was achieved by subcutaneous injection of furosemide (20mg/kg) and low sodium diet 24h before the experiments. The results show that 60 mim after carvacrol injections into LV did not change the blood pressure in SHR (ΔMAP: 4.9 ± 3.2 mmHg) when compared to control group, vehicle-treated (corn oil) (ΔMAP: -6.0 ± 0.9 mmHg). Conversely, the salt appetite in sodium depleted SHR was inhibited by carvacrol injections into LV (39 ± 0.8 ml) when compared to control group, vehicle-treated (7.7 ± 0.3 ml). The data suggest that carvacrol do not influence the central control of blood pressure in SHR. However carvacrol presents an effective antinatriorexigenic action. **Keywords**: CARVACROL, SODIUM APPETITE, SPONTANEOUSLY HYPERTENSIVE RATS **Financial Support:** CNPq 475252/2011-0

Disclosures: **A.S. Batista:** None. **C.F. Perrone:** None. **J.M. Andrade:** None. **F.B. Carvalho:** None. **H.S. Ferreira:** None. **J.B. Fregoneze:** None.

Poster

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Topic: E.06. Thirst and Water Balance

Support: FAPESP

CNPq

Title: Involvement of serotonergic receptors in the NTS on water intake induced by cellular dehydration

Authors: *R. A. TOMEO, J. MENANI, P. DE PAULA
São Paulo State Univ., Araraquara, Brazil

Abstract: The nucleus of the solitary tract (NTS), the primary site of peripheral osmoreceptor and cardiovascular afferences, is suggested to receive important inhibitory signals involved in the control of water and sodium intake. It is not clear yet which are the neurotransmitters that mediate these signals in the NTS. Central serotonergic mechanisms are involved in the control of sodium and water intake and immunohistochemical studies have shown that serotonergic receptors are present in the NTS. Therefore, in the present study, we investigated the effects of DOI (a serotonergic receptor agonist) or methysergide (a serotonergic receptor antagonist)

injected into the NTS on water and 0.3 M NaCl intake in intracellular dehydrated rats. Male Holtzman rats (290-310 g, n=13-19) with bilateral stainless steel cannulas implanted into the NTS were used. Cellular dehydration was induced by an intragastric load of 2 M NaCl (2 ml/rat) administered 60 minutes before bilateral injections of DOI (2.5 μg/100 nl), methysergide (2 μg/100 nl) or vehicle into the commissural NTS. DOI injected into the NTS of hyperosmotic rats decreased water intake (6.4 ± 1.5 ml/2 h, vs. vehicle: 9.1 ± 1.2 ml/2 h, $p<0.05$), without changing 0.3 M NaCl intake (0.3 ± 0.1 ml/2 h, vs. vehicle: 0.7 ± 0.3 ml/2 h). However, bilateral injections of methysergide into NTS did not change water intake (9.6 ± 1.3 ml/2 h, vs. vehicle: 9.0 ± 1.3 ml/2 h, $p<0.05$) or 0.3 M NaCl intake (0.9 ± 0.5 ml/2 h, vs. vehicle: 0.3 ± 0.2 ml/2 h). The present results suggest that serotonin in the NTS may activate inhibitory mechanisms that participate in the control of water intake induced by cellular dehydration.

Disclosures: **R.A. Tomeo:** None. **J. Menani:** None. **P. de Paula:** None.

Poster

256. Central Pathways: Anatomy and Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 256.01/MM27

Topic: E.07. Food Intake and Energy Balance

Support: NIDA

Title: Feeding but not drinking from optogenetic stimulation of an LH to VTA pathway

Authors: *E. GIGANTE, R. A. WISE
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Abstract: Feeding and drinking can each be induced by electrical stimulation of the medial forebrain bundle at the level of the lateral hypothalamus. Two questions remain unresolved. First, is the stimulation effective because it activates the cells of the bed nucleus or because it activates fibers of passage? Second, do the two behaviors result from activation of a common mechanism or of similar but independent mechanisms that happen to be close to one another? We have found that optogenetic activation of ventral tegmental area fibers originating from cells of the lateral hypothalamus can induce feeding in sated animals. Here we determined whether activation of the same fibers would induce drinking. We established expression of the light-sensitive cation channel ChR2 in lateral hypothalamic cells by microinjection of a viral vector (AAV-CaMKII-ChR2eYFP). We activated the fibers from these neurons by projection of a

473nm laser beam through a fiber optics probe localized to the ventral tegmental area. We administered 1-min trains of 5msec pulses at 20Hz to sated rats in a small cage with either food or water available. As previously reported, the stimulation induced feeding with short latency, and continued for as long as the optical stimulation continued, and the feeding stopped as soon as the stimulation terminated. Similar stimulation failed to induce drinking in the same animals under the same circumstances. These data confirm the previous finding that activation of projections from the cells of the lateral hypothalamic bed nucleus make a likely contribution to the feeding response to electrical stimulation of the medial forebrain bundle, but fail to confirm that the same cells contribute to the drinking response to the same electrical stimulation.

Disclosures: E. Gigante: None. **R.A. Wise:** None.

Poster

256. Central Pathways: Anatomy and Development

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Program#/Poster#: 256.02/MM28

Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK081937 to AMK

NIH Grant GM109817 to AMK

UTEP Grand Challenges Award to AMK

Keelung Hong Graduate Research Fellowship to EMW

Title: The ventral tegmental area: Wide-field and confocal imaging of hypothalamic peptidergic afferents and their putative synaptic appositions

Authors: *E. M. WALKER[1], B. DE HARO[1], R. H. THOMPSON[2], A. M. KHAN[1]
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Abstract: Functional relationships between the hypothalamus and the ventral tegmental area (VTA) have been studied intensively, but the circuitry mediating the interaction remains unclear. This is largely due to a diverse chemoarchitecture coupled with a poorly differentiated cytoarchitecture, making comprehensive studies difficult. To better understand the organization of the VTA and its chemical transmitters we used epifluorescence to systematically image the VTA and surrounding region in combination with *camera lucida* drawings of adjacent Nissl

sections to precisely delimit the cytoarchitecture of the region. Parallel series of sections were collected and immunolabeled for tyrosine hydroxylase-immunoreactive (TH-ir) perikarya and fibers, and hypocretin/orexin (H/O)-ir and melanin concentrating hormone (MCH)-ir fibers individually and in all possible combinations with multiple fluorescence labels. The distribution of each transmitter was mapped onto the Nissl and then summarized on a series of standard atlas templates from Swanson (‘04). Images were also screened for potential transmitter interactions then reimaged as a series of optical slices using confocal microscopy and rendered in 3-D for analysis of putative synaptic contacts. Initial analysis has focused on a region in the lateral part of the rostral VTA with extremely dense TH-ir that progressively diminishes at more caudal levels. Notably, H/O-ir and MCH-ir fibers showed a similar distribution. The vast majority of H/O-ir and MCH-ir fibers formed appositions on intermixed but distinct populations of TH-ir cell bodies and neuronal processes, with H/O-ir contacts being more prevalent. These results suggest that both the H/O and MCH axonal projections to the VTA preferentially target TH neurons, but may contribute to different circuits because they interact with distinct populations of TH neurons. Although the H/O inputs onto TH somata far outnumber MCH inputs, the majority of the MCH axons are terminating on some other neuronal cell type. While GABAergic neurons are the most likely candidate, this remains to be shown directly. These results provide a framework for understanding the anatomical basis of communication between hypothalamic and midbrain networks controlling complex behaviors.

Disclosures: E.M. Walker: None. **B. De Haro:** None. **A.M. Khan:** None. **R.H. Thompson:** None.

Poster

256. Central Pathways: Anatomy and Development

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant DK52849 to RCR

NIH Grant GM109817 to AMK

NIH Grant DK081937 to AMK

UTEP Grand Challenges Award to AMK

Title: Close associations of α-MSH-immunoreactive fibers with traced vagal afferents, and catecholaminergic neurons in the rat hindbrain: high-resolution immunofluorescence studies

Authors: *S. D. CHENAUSKY[1], C. A. CAMPOS[2], R. C. RITTER[2], A. M. KHAN[1]
[1]Biol. Sci., Univ. of Texas At El Paso, El Paso, TX; [2]Washington State Univ., Pullman, WA

Abstract: Evidence suggests that the neuropeptide alpha melanocyte-stimulating hormone (αMSH) may control food intake by acting on hindbrain neural substrates, including a possible direct interaction with vagal afferents terminating in the nucleus of the solitary tract. To explore a possible anatomic basis for this interaction, we undertook studies examining the relations between αMSH-immunoreactive (-ir) fibers, biotinylated dextran amine (BDA)-traced vagal afferent endings, and dopamine-beta-hydroxylase (DβH)-ir catecholaminergic neurons in the hindbrain. We used multi-label fluorescence, wide-field imaging and confocal microscopy to examine the distribution of αMSH-ir, DβH-ir, and BDA-labeled vagal afferents. Wide-field images indicate that αMSH-ir fibers course throughout the nucleus of the solitary tract (NTS) and portions of the dorsal motor nucleus (DMX). Analyses of high-resolution optical sections show that the αMSH-ir fibers form appositions with BDA+ vagal afferents. In addition, other αMSH-ir fibers make contacts with neurons in the dorsal vagal motor nucleus and with DβH-ir cell bodies and neuronal processes. The data indicate that αMSH-ir fibers and endings are well positioned to influence distinct dorsal hindbrain targets known to participate in control food intake. We are now undertaking to map the terminations of the traced vagal afferents onto standard reference atlas plates of the region. Collectively, our data provide an anatomic basis for melanocortinergic modulation of hindbrain vagal afferent function and homeostatic control.

Disclosures: S.D. Chenausky: None. **C.A. Campos:** None. **R.C. Ritter:** None. **A.M. Khan:** None.

Poster

256. Central Pathways: Anatomy and Development

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 256.04/MM30

Topic: E.07. Food Intake and Energy Balance

Support: CONACYT Grant 129337

Title: Binge eating behavior induced by corn oil modifies preference for different corn oil concentrations

Authors: *W. ZEPEDA-RUIZ, C. RAMOS-LAZZARI, V. CHAVEZ-RODRIGUEZ, D. VELAZQUEZ-MARTINEZ
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Abstract: Binge is an eating disorder characterized by overconsumption of palatable food in brief periods of time; animal models allow us study some characteristics of this disorder. Most studies used Limited Access to induce binge; in this program subjects had two hours of access to sucrose for only three days per week under non-food deprived conditions. Binge eating had been induced with sucrose or shortening vegetable, but it has been reported differences in the consumption that depend on the presentation (solid or liquid) of fat. The objective of this work was to evaluate if liquid fat in the form of corn oil (30%, corn oil Mazola™ diluted with mineral oil) was useful to induce binge and if this binge can change the preference for different corn oil concentrations. Twenty-four male Wistar rats were divided in two groups: a) Control group; that have 24 hours seven days a week with access to corn oil (30%), b) Experimental group with access to corn oil (30%) on Tuesday, Thursday and Saturday 2 hours per day. In both groups food and water were freely available 24 hours seven days a week. In the first part of experiment we tested the most preferred concentration with a two bottle choice test, the concentrations evaluated were (7.5%, 30% and 60%) then we induced binge for five weeks and finally, a second two bottle choice test was carried out to evaluate if binge induced changes in the preference for different concentrations. Binge only was clearly induced in 8 subjects (Binge prone) while the four remaining subjects showed a modest increase in oil consumption (Binge resistant). There were significant differences between groups in the intake of standard food, but only in the first days of the experiment, and no differences in body weight between groups were found. Binge only decreased the preference for the lowest concentration (7.5%). Our results show that binge can be induced with corn oil; however it is important to consider that Wistar rats population is heterogeneous and that there are binge-resistant subjects. The decrease of the preference in the lowest concentration, but not in the highest, suggests that binge induced changes in the subjective value of the corn oil. However, to confirm that the lowest concentration becomes devaluated in its palatability more experiments are necessary.

Disclosures: W. Zepeda-Ruiz: None. **C. Ramos-Lazzari:** None. **V. Chavez-Rodriguez:** None. **D. Velazquez-Martinez:** None.

Poster

256. Central Pathways: Anatomy and Development

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Topic: E.07. Food Intake and Energy Balance

Support: NIH DK 040498

NIH DK 081546

Title: Lesions of hindbrain catecholaminergic projections to nucleus accumbens, bed nucleus of the stria terminalis, lateral parabrachial nucleus or locus coeruleus do not impair glucoprivic feeding

Authors: T. T. DINH, N. HUSTON, *S. RITTER
Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: We have shown previously that injection of the retrogradely transported immunotoxin, anti-dopamine beta-hydroxylase (DBH) saporin (DSAP), into the paraventricular nucleus of the hypothalamus (PVH) or arcuate nucleus, abolishes feeding in response to central or systemic glucoprivation. Since DSAP injection destroys DBH-expressing neurons with projections to the injection site, these results strongly implicate hindbrain catecholamine neurons as major mediators of glucoprivic feeding. In order to further define the essential circuitry underlying glucoprivic feeding, we injected DSAP into these additional sites: locus coeruleus (LC), accumbens shell (AcbSh), ventrolatersal bed nucleus of the stria terminalis (vlBNST) and lateral parabrachial nucleus (LPBN). These sites are innervated by hindbrain catecholamine neurons and some sites receive collateral innervation from PVH-projecting catecholamine neurons. Appropriate placement and volume for DSAP administration was determined by co-labeling of DBH-ir neurons with retrograde tracer injected into target sites. Lesions were confirmed by postmortem evaluation of DSAP injection site and by hindbrain catecholamine cell and terminal loss. We found that the feeding response to systemic glucoprivation was not significantly or permanently impaired by injection of DSAP into any of these sites. Based on our results to date, we tentatively conclude that direct projections from hindbrain catecholamine neurons to the LC, AcbSh, vlBNST and LPBN are not required for glucoprivic feeding. The hypothalamus appears to be the major recipient of direct innervation from catecholamine neurons required for glucoprivic feeding

Disclosures: T.T. Dinh: None. **N. Huston:** None. **S. Ritter:** None.

Poster

256. Central Pathways: Anatomy and Development

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant R01 NS029728

JDRF Grant 2-710-2008

Title: Hypoglycemia reduces c-Fos expression in the ventromedial hypothalamic nucleus

Authors: *N. FOSTER, S. AZAM, A. G. WATTS
USC, Los Angeles, CA

Abstract: A wealth of evidence indicates that the ventromedial nucleus of the hypothalamus (VMH) is critically involved in sensing cerebral glucose availability and organizing hormonal counterregulatory responses to hypoglycemia. Yet numerous studies have failed to detect hypoglycemia-associated changes in c-Fos expression in the VMH. We postulated that a high resolution analysis of c-Fos across the entire VMH may be more revealing. Male Wistar rats were injected ip with human insulin or normal saline. Blood glucose was sampled from the tail 0, 30, and 90 minutes later, followed immediately by anesthesia and perfusion. A 1-in-6 tissue series from each brain underwent immunofluorescence staining for c-Fos and NeuN for cytoarchitectonics. c-Fos-labeled neurons were tallied in each part of the VMH (anterior, dorsomedial, central, ventrolateral). We found significantly less c-Fos in the dorsomedial and central parts of hypoglycemic rats compared to euglycemic controls, with no differences in the anterior and ventrolateral parts. In these same animals we also assessed c-Fos in the medial parvicellular (neuroendocrine) part of the paraventricular hypothalamic nucleus (PVH). Here, hypoglycemia significantly increased c-Fos, which is consonant with our previous work examining PVH responses to hypoglycemia. The dorsal VMH appears to be closely associated with metabolic control as it possesses glucosensitive neurons and receptors for several metabolic hormones. Our results therefore suggest that a suppression of neuronal activity in this area may be a significant factor in the how the VMH contributes to the endocrine, autonomic, and behavioral responses to hypoglycemia.

Disclosures: **N. Foster:** None. **S. Azam:** None. **A.G. Watts:** None.

Poster

256. Central Pathways: Anatomy and Development

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant R037 DK35254 to TJB

Title: Central glucoprivation increases sympathetic drive to liver and some but not all white adipose tissue depots

Authors: *L. A. SZYMANSKI[1], J. EHLEN[2], T. J. BARTNESS[1]
[1]Biol., Georgia State Univ., Atlanta, GA; [2]Dept. of Neurobio., Morehouse Sch. of Med., Atlanta, GA

Abstract: Energetic challenges have differential effects on sympathetic drive, as measured by norepinephrine turnover (NETO), to brown and white adipose tissue depots in Siberian hamsters. Peripheral treatment with the glucoprivic agent 2-deoxy-D-glucose (2DG) increases sympathetic drive to inguinal (IWAT) and retroperitoneal (RWAT) white adipose tissue, but not epididymal (EWAT) white adipose tissue or interscapular brown adipose tissue (IBAT). Tract tracing studies in our lab have identified neural pathways from the paraventricular hypothalamus to white and brown adipose tissue via hindbrain areas that have been implicated in the control of glucoprivic feeding and hyperglycemia in other rodents. It is likely that in hamsters these hindbrain neurons modulate the increases in sympathetic drive to WAT after peripheral glucoprivic treatment. The current experiments test the hypothesis that glucose-sensing neurons in the hindbrain modulate sympathetic drive to adipose tissue. Adult, male Siberian hamsters were fitted with a cannula into the 4th ventricle (4V) in order to deliver the glucoprivic agent 5-thio-D-glucose (5TG) directly to the hindbrain. NETO was measured in IBAT, IWAT, EWAT, mesenteric white adipose tissue (MWAT), RWAT, and liver of animals treated with an acute 4V injection of either saline or 50 nmoles of 5TG. This dose of 5TG reliably induces the characteristic hyperglycemic response to acute central glucoprivation within 1 h of treatment. Another group of animals received the same dose of 5TG and changes in plasma concentrations of glycerol and free fatty acids, which provide another measure of increased fuel availability via lipolysis, were measured. Hamsters treated with 5TG showed significantly greater NETO in the liver, RWAT, MWAT, and EWAT compared to saline-treated hamsters. 5TG treatment did not induce greater NETO in IBAT or IWAT compared to saline-treated hamsters. This pattern of sympathetic activation differs from that seen in hamsters treated with 2DG or food-deprived hamsters, suggesting that changes in sympathetic output induced by decreased fuel availability are stimulus-specific. Plasma concentrations of glycerol increased significantly 4 h after central 5TG treatment, but there was not a significant increase in plasma concentrations of free fatty acids. The increases in plasma glycerol as well as the increases in NETO indicate that central glucoprivation is sufficient to increase sympathetic drive to adipose tissue. It is therefore likely that the hindbrain neurons

that have been identified as part of the pathway modulating sympathetic output to adipose tissue are glucose sensitive.

Disclosures: **L.A. Szymanski:** None. **T.J. Bartness:** None. **J. Ehlen:** None.

Poster

256. Central Pathways: Anatomy and Development

Location: Halls A-C

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Program#/Poster#: 256.08/MM34

Topic: E.07. Food Intake and Energy Balance

Support: NIH R01 DK089237

Title: Multifaceted effects of brain-derived neurotrophic factor on arcuate neurons

Authors: ***G.-Y. LIAO**[1], K. BOUYER[2], A. KAMITAKAHARA[2], C.-H. WANG[2], N. SAHIBZADA[3], R. B. SIMERLY[2], B. XU[1]
[1]Dept Neuro, Scripps Reserch Inst., Jupiter, FL; [2]The Saban Res. Institute, Children's Hosp. Los Angeles, Univ. of Southern California, Los Angeles, CA; [3]Dept. of Pharmacol. and Physiology, Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Brain-derived neurotrophic factor (BDNF) plays a critical role in energy homeostasis, in addition to neuronal survival, differentiation, and synaptic plasticity. The *Bdnf* gene produces two populations of transcripts with either a short or a long 3′ untranslated region (3′ UTR). We previously reported that the long 3′ UTR *Bdnf* mRNA was localized to dendrites for local translation and that $Bdnf^{klox/klox}$ mice lacking long 3′ UTR *Bdnf* mRNA developed severe hyperphagic obesity. To investigate whether the obese phenotype of $Bdnf^{klox/klox}$ mice is associated with alterations in arcuate neurons, we first examined the expression of TrkB, the receptor for BDNF, in the arcuate nucleus (ARC). We found that ~15% TrkB ARC neurons were colocalized with POMC neurons and another 15% TrkB ARC neurons colocalized with AgRP neurons. TrkB signaling in these neurons does not appear to impact cell number as we detected normal numbers of POMC neurons and AgRP neurons in $Bdnf^{klox/klox}$ mice. However, DiI labeling did reveal a drastic reduction in the projection of ARC neurons to the PVH in $Bdnf^{klox/klox}$ mice on postnatal day 12. In addition, fewer POMC axonal projections were found in the dorsomedial hypothalamic nucleus (DMH) in adult $Bdnf^{klox/klox}$ mice. This observation suggests that BDNF regulates axonal growth of TrkB-expressing ARC neurons, particularly in POMC populations projecting to the DMH and PVH. We employed immunohistochemistry and patch

recordings to examine the impact of BDNF deficiency on synapses onto POMC and AgRP neurons. We found that excitatory inputs onto POMC neurons were increased in *Bdnf*$^{klox/klox}$ mice, likely due to a compensatory response to the markedly hyperphagic phenotype displayed by the mutant mice. Interestingly, AgRP neurons in *Bdnf*$^{klox/klox}$ mice had normal number of inhibitory synapses on cell bodies; however, both frequency and amplitude of miniature IPSCs in these neurons were significantly reduced. This observation indicates that inhibitory inputs onto dendrites of AgRP neurons are impaired in *Bdnf*$^{klox/klox}$ mice. We propose that BDNF inhibits food intake in part by promoting development of TrkB-expressing ARC neurons and by enhancing inhibitory synaptic inputs onto AgRP neurons.

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Poster

256. Central Pathways: Anatomy and Development

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Program#/Poster#: 256.09/MM35

Topic: E.07. Food Intake and Energy Balance

Support: WSU Intramural Grant

Title: Diet-induced obesity alters the gut-brain communication and results in microglia activation in the hindbrain feeding centers

Authors: *K. CZAJA[1], A. C. VAUGHN[1], C. FLETCHER[1], L. A. BALLSMIDER[1], P. M. DI LORENZO[2]
[1]Washington State Univ., PULLMAN, WA; [2]Binghamton Univ., Binghamton, NY

Abstract: Because obesity itself is considered to be a state of chronic inflammation, we evaluated whether diet-induced obesity (DIO) alters gut-brain communication and results in microglia activation in the hindbrain feeding centers. Gastrointestinal signals that inform the brain of the quantity and quality of food being consumed during ongoing meals are important controllers of food intake. These signals contribute to the process of satiation, which results in meal termination. The nucleus of the solitary tract (NTS) in the caudal brain stem is the site at which the vagal sensory afferent fibers transmit gastrointestinal (GI) satiation signals and make their central synapses. GI-projecting motor neurons are located at the dorsal motor nucleus of the vagus (DMV). We used male Sprague Dawley rats (~ 470g body weight). Control rats were fed

Teklad F6 Rodent diet (3.1 kcal/g; 6.4% fat; RD) for the entire experiment. DIO rats received Teklad F6 Rodent diet for two weeks and then where switched to the Open Source D12492 diet (5.24 kcal/g; 34.9% fat; HFD) for four weeks. Body weight and food intake were monitored three times a week. Body fat composition was determined by a dual-energy X-ray Absorptiometry (DEXA) scan before introducing the high fat diet and four weeks later. At the end of the study the rats were transcardially perfused with 4% parafolmaldehyde. Hindbrains and nodose ganglia were collected, sectioned and stained using standard immunofluorescence methods. Primary antibody against Isolectin 4 was used to label vagal afferents. Microglia activation was determined by quantifying changes in the density of fluorescent staining with a primary antibody against the ionizing calcium adapter binding molecule 1 (Iba1). Results of the study showed a significant increase in the caloric intake and body weight in DIO rats compared to control rats. DEXA scan results revealed almost double the body fat percent in DIO rats compared to control rats (14.5% vs. 7.5% respectively). Immunofluorescent results showed a significant decrease in the density of vagal afferents in the NTS and DMV and an increase in microglia activation in the NTS. In conclusion, our results show that HFD induces obesity in male Sprague Dawley rats, triggers withdrawal of vagal afferents from the hindbrain feeding centers and results in microglia activation in the NTS. Similar alterations in the gut-brain communication after subdiaphragmatic damage to the vagus nerve, previously reported by our laboratory, suggest that HFD and/or DIO may trigger degenerative changes in vagal sensory neurons innervating the gut. However, further studies are needed to test this hypothesis.

Disclosures: K. Czaja: None. **A.C. Vaughn:** None. **C. Fletcher:** None. **L.A. Ballsmider:** None. **P.M. Di lorenzo:** None.

Poster

256. Central Pathways: Anatomy and Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 256.10/MM36

Topic: E.07. Food Intake and Energy Balance

Title: Suppressed Fos induction within the central nucleus of the amygdala corresponds with inhibited feeding in the presence of a fear-cue in male and female rats

Authors: *C. J. REPPUCCI, G. D. PETROVICH
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Our research examines environmental influences on feeding, and the brain substrates that mediate their competition with physiological drives. Here, we used a model of fear-cue induced inhibition of feeding in rats. In this preparation a tone that predicts footshocks is a fear-cue that halts feeding in hungry subjects. The first aim of the current study was to replicate the prior finding that this cessation of feeding extinguishes rapidly in males, but persists in females across repeated tests. The second aim was to extend prior lesion evidence that the central nucleus of the amygdala (CEA) is critical in this behavior, by assessing its recruitment across tests using Fos induction. Adult male and female rats were trained in alternating appetitive and aversive sessions conducted in two distinct contexts (A and B). During appetitive sessions in Context A, food-deprived rats consumed food pellets. During aversive sessions in Context B, half of rats (Experimental) received a total of four footshocks (1.0mA, 1sec) each signaled by a 60sec tone (75db, 2khz), while the other half (Control) received the same number of tones, but no shocks. Following training, food-deprived rats were tested for consumption in Context A. During each test rats received four presentations of the tone (fear-cue); no footshocks were delivered. Two additional control groups underwent an identical training regimen, except they did not have access to food during appetitive sessions or testing (No-Food Control, No-Food Experimental). Rats in the Control groups consumed substantial amounts of food during each test. Male and female rats in the Experimental groups, however, significantly inhibited food intake compared to the corresponding Control group during Test 1. Replicating prior findings, the females, but not males, in the Experimental groups also inhibited intake on Tests 2 and 3. Rats were sacrificed 90min after their final test (subsets of rats sacrificed after the 1st, 2nd, or 3rd test), and brain tissue was immunohistochemically stained to detect Fos. We found that during Test 1, rats in the Experimental groups had significantly less Fos induction in CEA than rats in the Control groups, while during Tests 2 and 3 males had significantly greater Fos induction than females. There was no difference in Fos induction between the two No-Food groups, and these groups showed lower Fos induction than all other groups. Fos induction in CEA significantly correlated with the amount of food rats consumed at test, irrespective of sex, group, or test day. Current results, combined with the prior lesion work, suggest that an active suppression of CEA neurons contributes to the anorectic effects of fear on feeding.

Disclosures: C.J. Reppucci: None. **G.D. Petrovich:** None.

Poster

256. Central Pathways: Anatomy and Development

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 256.11/NN1

Topic: E.07. Food Intake and Energy Balance

Support: Carleton University Faculty Research Award

Title: Mice exposed perinatally to the obesogenic endocrine disruptor bisphenol-A (BPA) show impaired central and behavioral leptin sensitivity in advance of diet-induced obesity: evidence for developmental programming of hypothalamic pro-opiomelanocortin (POMC) regulation

Authors: *H. A. MACKAY, Z. R. PATTERSON, A. ABIZAID
Carleton Univ., Ottawa, ON, Canada

Abstract: Bisphenol-A (BPA) is a component of polycarbonate plastic, and is commonly found in food and drink containers. BPA has been characterized as an endocrine disruptor capable of acting as a xenoestrogen in a variety of experimental models, and because it is capable of leaching out of food and drink containers, human exposure is nearly ubiquitous. Since BPA can cross the placenta and is also found in breast milk, adverse organizational effects due to early-life exposure are of particular concern. Recent evidence from our lab has demonstrated in mice that early-life exposure to environmentally relevant doses of BPA yields a phenotype characterized by a predisposition to adult obesity in female mice and metabolic disturbance in males. Given the sensitivity of the developing hypothalamus to the organizational effects of sex steroids, we hypothesized that early-life BPA exposure adversely affects the development of hypothalamic feeding circuitry in order to bring about this phenotype. To test this hypothesis, we used male and female CD-1 mice exposed pre- and post-natally to either a control diet, a diet containing BPA (appx. 13.5 μg/kg/day), or the estrogenic diethylstilbestrol (DES) as a positive control (appx. 3 μg/kg/day). Serum from pups was collected on PND2, 8, 10, 12, 16, and 21 for analysis of circulating leptin. Results from this study show that BPA and DES exposed pups have respectively delayed and blunted postnatal leptin surges - a state of affairs that points to a role for leptin in the organizational effects of early-life xenoestrogen exposure. Adult offspring from this experiment were resistant to leptin-induced suppression of food intake, body weight loss, and hypothalamic POMC upregulation. Taken together, these data suggest that BPA, a known obesogen, may exert its effects through developmental programming of the hypothalamic melanocortin circuitry.

Disclosures: H.A. Mackay: None. **A. Abizaid:** None. **Z.R. Patterson:** None.

Poster

256. Central Pathways: Anatomy and Development

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Program#/Poster#: 256.12/NN2

Topic: E.07. Food Intake and Energy Balance

Support: MRDC Pilot Grant (S. F.)

Diabetes Quebec (Diabete Estrie) Postdoctoral Award (S. T.)

Title: ABHD6 loss-of-function in nucleus accumbens neurons reduces food intake and prevents diet-induced obesity

Authors: *S. TOBIN[1], D. MATTHYS[2], S. ZHAO[3], S. MADIRAJU[3], T. ALQUIER[4], M. PRENTKI[3], S. FULTON[1]
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Abstract: Excessive consumption of energy-rich food largely drives the development of obesity and related metabolic disorders. The nucleus accumbens (NAc), a central component of brain reward circuitry, plays a significant role in the control of feeding and food-motivated behaviour. Alpha-beta-hydrolase domain 6 (ABHD6) is an enzyme that degrades the endocannabinoid 2-arachidonoylglycerol (2-AG) in neurons and has recently been shown to regulate de novo fat synthesis within metabolic tissues and contribute to the peripheral regulation of body weight and energy metabolism. Here, we used conditional gene targeting to determine the impact of ABHD6 in NAc neurons in feeding, energy expenditure, body weight and mood. Methods: An adeno-associated virus (AAV2/1.hSyn.eGFP-Cre.WPRE) delivering Cre recombinase ("KO") or GFP ("control") under the control of the synapsin promoter was stereotaxically injected into the NAc (500nl/side; 5x109 GC) of adult ABHD6lox/lox mice (Bl6 background). Food intake and body weight were measured in mice (n=9-10/group) consuming chow or high-fat diet for 8-10 weeks. EchoMRI (n=9-10/group) and CLAMS metabolic cages were used for measures of body composition and energy expenditure, respectively CLAMS metabolic cages were used for measures of energy expenditure. Elevated-plus maze, open-field and forced swim tests were used to assess changes in anxiety- and depressive-like behaviour (n=9-10/group). Results: AAV infection was specific to neurons as assessed by GFP + NeuN immunofluorescence, and KO mice had an average 70% reduction in NAc ABHD6 mRNA relative to controls. Male KO mice on high-fat diet consumed significantly less food, were completely protected from diet-induced weight gain and had elevated locomotor activity (dark cycle) relative to controls. KO mice did not differ from controls with respect to anxiety- and depressive-like behaviour. Conclusion: Our results show that selective deletion of ABHD6 from NAc neurons prevents diet-induced hyperphagia and obesity and increases spontaneous physical activity without inducing negative emotional states, and thus identify an important role for this enzyme in NAc controls of feeding and locomotion. These findings also support the current pursuit of ABHD6 inhibitors for the treatment of obesity and type 2 diabetes.

Disclosures: **S. Tobin:** None. **D. Matthys:** None. **S. Zhao:** None. **S. Madiraju:** None. **T. Alquier:** None. **M. Prentki:** None. **S. Fulton:** None.

Poster

256. Central Pathways: Anatomy and Development

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Program#/Poster#: 256.13/NN3

Topic: E.07. Food Intake and Energy Balance

Support: NSF IOS-1121866

Title: Ventral hippocampal neurons influence meal onset and meal frequency

Authors: ***R. C. HANNAPEL**, M. B. PARENT
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Abstract: Decades of research suggest that different mechanisms regulate meal initiation versus termination. There is extremely limited evidence regarding the mechanisms that regulate meal onset and the interval between two meals (i.e., the postprandial intermeal interval; ppIMI). The ppIMI influences meal frequency and is a major determinant of total food intake. Emerging evidence indicates that the hippocampus, which is critical for memory, also regulates energy intake. We discovered that dorsal hippocampal neurons delay meal onset during the period following a meal. Specifically, we found that temporarily inactivating dorsal hippocampal neurons after the end of a sucrose meal with unilateral infusions of the GABA-A receptor agonist muscimol decreases the ppIMI (Henderson et al, 2013; Hippocampus). Ventral hippocampal neurons may also influence meal onset because this region has been implicated in motivational, emotional and affective processes; and permanent ventral hippocampal lesions increase total intake. Additionally, ventral hippocampal neurons project to brain areas involved in eating (i.e. hypothalamus, bed nucleus of the stria terminalis, lateral septum and nucleus accumbens). To test the hypothesis that ventral hippocampal neurons also inhibit meal onset, adult male Sprague-Dawley rats (n = 8) were implanted with a unilateral cannula aimed at the left or right hippocampus (hemisphere counterbalanced). The rats were trained to consume a 32% sucrose solution at a scheduled time daily. On experimental days, the rats were given vehicle or muscimol infusions (0.5 μg/μl; 1μl; within subject design) after they had stopped consuming the sucrose solution (i.e., during the ppIMI). Latency to consume the sucrose and the number of sucrose meals were recorded for 4 hr after the infusion. The results demonstrated that ventral hippocampal muscimol infusions significantly decreased the ppIMI and increased the number of

postinfusion meals (ps < 0.05 vs. vehicle). The present findings indicate that ventral hippocampal neurons also inhibit meal onset during the ppIMI and raise the possibility that hippocampal dysfunction may contribute to the development or maintenance of diet-induced obesity. Interestingly, the effect of inactivation was significant with fewer rats in the ventral experiment than in the dorsal study, suggesting that ventral hippocampal neurons may have a stronger inhibitory influence on meal onset. To test this, we are now directly comparing the effects of dorsal versus ventral manipulations.

Disclosures: R.C. Hannapel: None. **M.B. Parent:** None.

Poster

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Topic: E.07. Food Intake and Energy Balance

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NIH Grant GM109817

UTEP Grand Challenges Award

Title: Afferent and efferent projections of the periventricular hypothalamus: A combined anterograde and retrograde tract tracing study in the adult male rat

Authors: *B. DE HARO, A. M. KHAN
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Abstract: The periventricular hypothalamus is an enigmatic area thought to be involved in neuroendocrine function. The PV is parcellated in the Swanson rat brain atlas into the preoptic (PVpo), anterior (PVa), interior (PVi), and posterior (PVp) parts. The circuitry of the PV with other regions of the brain has mostly been investigated through indirect means, with the PVp being the best characterized region. Currently, there is a lack of complete understanding of other PV regions. In order to investigate further the PV circuitry we injected a cocktail of anterograde and retrograde tracers into the PV. Specifically, we injected Cholera toxin subunit b (Ctb) and *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the PVi. The projections were then characterized with the use of a companion Nissl series, immunohistochemical staining for the tracers, and wide-field imaging of the PV and surrounding regions. We found projections to the

PVi arising mainly from several hypothalamic nuclei. First, confirming the anterograde analysis of ventral premammillary (PMv) projections provided by Canteras *et al*., 1992 (*J. Comp. Neurol*. 324:195-212) we found robustly labeled retrogradely filled neurons confined to the PMv. Second, several additional hypothalamic regions also displayed retrogradely labeled neurons, including the arcuate nucleus, the ventromedial nucleus of the hypothalamus, dorsomedial nucleus of the hypothalamus, and lateral hypothalamic area. The axonal projections of the PVi, in turn, mainly target anterior regions of the hypothalamus. Collectively, these data suggest that the PVi might have an important role in the control of multiple homeostatic functions.

Disclosures: B. De Haro: None. **A.M. Khan:** None.

Poster

256. Central Pathways: Anatomy and Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 256.15/NN5

Topic: E.07. Food Intake and Energy Balance

Title: Effects of the food form of apple products on satiety and brain responses in humans: An fMRI study

Authors: M. SOTO, *N. P. DARCEL, A. MARSSET-BAGLIERI, J. PIEDCOQ, D. TOMÉ, G. FROMENTIN, N. NADKARNI
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Abstract: Background: There is little evidence as to whether satiety induced by different food forms having the same caloric content differentially alters brain responses to food cues, and which brain regions are involved. **Objective:** We assessed the differences in brain activation in response to food cues between different states of hunger induced by ingestion of sweet foods of different forms (liquid, semi-liquid and solid). **Design:** Twenty-five normal-weight men (mean ± SEM age: 23.2 ± 0.4 yrs.; BMI: 22.4 ± 0.4 kg/m^2) participated in the study. Subjects arrived fasted in the morning and had to eat a breakfast consisting of one of three apple products (cross-over design): apple juice, puree or slices, each with the same caloric content. After breakfast, a fMRI session was conducted as follows: one 'liking' session consisting of viewing images (food and objects) whilst rating their appeal value, and one 'choice' session in which participants had to choose between two images of different categories (high-fat/low-fat sweet/savory). VAS questionnaires were completed during each session to assess perceived hunger and satiety. Statistical contrasts between the three different breakfasts were calculated, and data for regions

of interest were extracted. BOLD percent change in the regions that were differentially activated were correlated to VAS scores before the fMRI session and to TFEQ scores. **Results:** Consumption of the three apple products led to differences in short-term satiety, in the order slice > puree > juice. In the 'liking' session, three brain regions were more activated after consumption of apple juice vs. apple slices: the nucleus accumbens, the orbitofrontal cortex and the hypothalamus. Neural activity in the NAcc and OFC was positively correlated with hunger ratings after breakfast (and negatively with fullness ratings). In the 'choice' session, the OFC was more activated after the juice breakfast (vs. slices) when subjects chose sweet images (rather than savory) and high-fat images (rather than low-fat). The BOLD signal in the OFC measured when high-fat foods were chosen was positively correlated with hunger ratings and ratings of 'expected pleasure to eat' self-reported after breakfast. **Conclusions:** Eating apples in the form of a solid (slices) for breakfast elicited a greater satiety than consuming the same energy in the form of a liquid (juice). Differences in food form also led to different responses to food cues of limbic (NAcc and OFC) and homeostatic (hypothalamus) regions underlying food intake.

Disclosures: **M. Soto:** None. **N.P. Darcel:** None. **A. Marsset-Baglieri:** None. **J. Piedcoq:** None. **D. Tomé:** None. **G. Fromentin:** None. **N. Nadkarni:** None.

Poster

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Topic: E.07. Food Intake and Energy Balance

Support: NIH intramural

Title: The underlying neural network of proopiomelanocortin (POMC)-mediated regulation in food intake

Authors: *C. LI, M. KRASHES
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Abstract: Recent studies have demonstrated the functional roles of proopiomelanocortin (POMC) and agouti-related peptide (AGRP) neurons in the arcuate nucleus (ARC) of the hypothalamus. Respectively, they have been shown to promote and attenuate body weight-gain and feeding behavior. Although much attention has focused on $AGRP^{ARC}$ functions and their downstream projections, the axonal targets and mechanisms underlying $POMC^{ARC}$ stimulation-

induced satiety has yet to be elucidated. While immunohistochemistry studies show a wide range of intra- and extra-hypothalamic structures labeled by peptides released from POMC neurons, the anatomical organization, synaptic connectivity and functions of relevant downstream sites in signaling satiety remain unknown. $POMC^{ARC}$ neurons release alpha-melanocyte-releasing hormone (α-MSH), a melanocortin-4 receptor (MC4R) agonist, which acts to reduce food intake. In contrast, AGRP neurons release the neuropeptides AGRP, a MC4R inverse agonist/antagonist, neuropeptide Y (NPY) and the fast-acting neurotransmitter GABA, all of which drive feeding. These results suggest a highly orchestrated modulatory balance on appetite-controlling MC4R-expressing neurons. The MC4Rs are essential in body weight regulation as both genetic knockout mice and human mutations lead to overeating and subsequent obesity. It has been shown that the resulting drop in body weight and food intake following 24-hr optogenetic activation of $POMC^{ARC}$ soma is dependent on melanocortin signaling. Based on the above evidence, MC4R-expressing neurons are likely targets for $POMC^{ARC}$ neurons in mediating feeding, although this has never been directly tested, mainly due to the inability to reliably mark MC4R-expressing cells. In this study, using cre-dependent viral tracing, optogenetics, and chemogenetics, we determined the anatomical and cellular downstream targets of the $POMC^{ARC}$ neurons mediating satiety with a focus on those expressing MC4R. In addition, we employed electrophysiology in combination with optogenetics to investigate the functional connection between $POMC^{ARC}$ and MC4R-expressing neurons as well as measure firing activity in these downstream neurons in distinct physiological hunger states.

Disclosures: **C. Li:** None. **M. Krashes:** None.

Poster

256. Central Pathways: Anatomy and Development

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Topic: E.07. Food Intake and Energy Balance

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UTEP Grand Challenges Award

UTEP SMARTS Program

Title: Hypothalamic chemoarchitecture in the adult male rat: Creating canonical atlas maps for three co-visualized peptidergic neuronal populations and their fiber systems from a single brain

Authors: *C. E. WELLS, K. PENNINGTON, A. M. KHAN
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Abstract: While the hypothalamus is a major homeostatic control center and its functions have been extensively studied, the precise circuitry underlying many of these functions remains ambiguously delineated. Here we extend our previous studies of the rat lateral hypothalamic area (LHA) to report the distribution and interactions of α-melanocyte stimulating hormone (α-MSH), melanin-concentrating hormone (MCH), and neuronal nitric oxide synthase (nNOS) in additional locations in the adult male rat. We have identified the locations of these proteins in the rat hypothalamus using fluorescent antibody markers, and we have employed wide-field multi-fluorescent imaging to map novel immunoreactivity (-ir) patterns of their distribution onto the Swanson (2004) reference atlas of the rat brain. Our data indicate that α-MSH-ir fibers largely avoid the LHA, while nNOS-ir and MCH-ir cell bodies and fibers are dispersed throughout this region in a heterogeneous, patterned manner. In particular, there is a subpopulation of neurons expressing both nNOS-ir and MCH-ir in the dorsolateral LHA at levels 29-31 of the Swanson atlas. **Tables 1** and **2** detail additional patterns of distribution. To our knowledge, this work marks the first time that the anatomical distributions of α-MSH, MCH, and nNOS have been mapped together in the hypothalamus in detail. Further work to understand the interactions of these molecules will aid in functional studies of this region.

Table 1. Fiber Distributions

# of peptides labeled in area	Peptides	Locations (abbreviations after Swanson, 2004)
3	α-MSH, MCH, nNOS	PV, PVH, ME, ARH, PH, TU, I
2	α-MSH, MCH	TM, DMH
	MCH, nNOS	ZI, SUM
	α-MSH, nNOS	PM, SON
1	nNOS	VMH
	MCH	MM

Table 2. Potential Synaptic Interaction Zones

Interacting peptides	Locations with potential interaction zones (abbreviations after Swanson, 2004)		
α-MSH, MCH	LHA, DMH, ARH, ME, PV, PH, TU, I	PVH	TM
α-MSH, nNOS			PM, SON
MCH, nNOS		SUM	

Disclosures: **C.E. Wells:** None. **K. Pennington:** None. **A.M. Khan:** None.

Poster

256. Central Pathways: Anatomy and Development

Location: Halls A-C

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Program#/Poster#: 256.18/NN8

Topic: E.07. Food Intake and Energy Balance

Title: Glutamatergic regulation of the lateral septum by melanin-concentrating hormone neurons

Authors: *M. J. CHEE[1], E. ARRIGONI[2], E. MARATOS-FLIER[1]
[1]Endocrinol., [2]Neurol., Beth Israel Deaconess Med. Center, HMS, Boston, MA

Abstract: Melanin-concentrating hormone (MCH) is produced exclusively by neurons in the lateral hypothalamus and zona incerta. It regulates physiological processes, such as energy homeostasis and sleep, and affective behaviors, such as anxiety and aggression. However little is known about the functional connectivity of these neurons. While MCH neurons express other neurotransmitters that can contribute to MCH actions, including GABA and glutamate, it is not certain if they express the vesicular GABA (VGAT) and glutamate transporters (VGLUT) required for uptake and release. Furthermore, MCH projections were mapped by MCH-immunoreactive fibers but its functional release sites are not known. We first determined if MCH neurons express VGAT or VGLUT2 by using double immunohistochemistry to colocalize MCH with L10-green fluorescent protein in VGATcre;L10gfp and VGLUT2cre;L10gfp mice. Almost all MCH neurons (94 ± 3%) expressed VGLUT2 but none were VGAT-positive, therefore MCH neurons may release glutamate but not GABA. We then stereotaxically injected MCHcre mice with a cre-dependent channelrhodopsin (ChR2)-mCherry adeno-associated virus to identify MCH terminals. The densest mCherry-labelled fibers were found in the lateral septum (LS). To test the MCH to LS connectivity by light-evoked GABA or glutamate, we performed whole-cell LS recordings while photostimulating (5ms, 470nm) surrounding ChR2-expressing MCH terminals. LS photostimulation evoked both GABAergic inhibitory postsynaptic currents (eIPSC) that were completely blocked by a GABAA receptor antagonist (10μM bicuculline); as well as glutamatergic excitatory postsynaptic currents (eEPSC) that were blocked by a glutamate receptor antagonist (1mM kynurenic acid). eIPSC amplitude (423 ± 174pA) was greater than eEPSCs (69 ± 22pA), but the eIPSC onset delay (6.5 ± 0.3ms) was nearly twice as long as for eEPSCs (3.3 ± 0.3ms; $p < 0.0001$). Furthermore, pretreatment with kynurenic acid abolished eIPSC generation (by 98.8 ± 0.7%) to suggest that GABA release depends on glutamatergic inputs from MCH cells. Indeed, blocking activity-dependent transmission (500nM tetrodotoxin + 1μM 4-aminopyridine) extinguished GABAergic eIPSCs but not glutamatergic eEPSCs. This indicated that there is a monosynaptic glutamate release from LS MCH terminals that then triggers robust GABA release in the LS. These findings demonstrate direct glutamate but not GABA release from MCH neurons, so glutamatergic transmission may underlie the actions of MCH neurons in the LS. While the role of LS MCH efferents is not known, the LS may mediate some MCH functions, including nutrient-regulation, anxiety and aggression.

Disclosures: **M.J. Chee:** None. **E. Arrigoni:** None. **E. Maratos-Flier:** None.

Poster

256. Central Pathways: Anatomy and Development

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Topic: E.07. Food Intake and Energy Balance

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Title: Chronic energy imbalance via hyperlipidemic diet disrupts olfactory sensory systems and odor reversal learning in mice

Authors: *K. L. FERGUSON[1], G. A. BELL[2], M. C. JOHNSON[3], N. THIEBAUD[2], D. A. FADOOL[2]
[1]Biol., Florida State Univ., Tallahassee, ; [2]Biol., Florida State Univ., Tallahassee, FL; [3]Dept. of Biol., Univ. of West Georgia, Carrollton, GA

Abstract: Well-supported data show that obesity can lead to cardiovascular and cognitive declines, yet little is understood concerning obesity's impact on sensory systems. Because olfaction is linked with ingestive behavior to guide food choice, its potential dysfunction during obesity could evoke a positive feedback loop to perpetuate poor ingestive behaviors. To determine the effect of chronic energy imbalance and reveal any structural or functional changes associated with obesity, we induced long-term, diet-induced obesity by challenging mice to high-fat diets: (1) in an obesity-prone (C57BL/6J) and obesity-resistant line of mice via voltage-gated potassium channel knock out ($Kv1.3^{-/-}$), and compared this with (2) late-onset, genetic-induced obesity in mice via melanocortin receptor 4 knock out ($MC4R^{-/-}$) in which diabetes secondarily precipitates after disruption of the hypothalamic axis. Using M72*tau*LacZ and OMP*gfp* reporter backgrounds, we quantified marked loss of olfactory sensory neurons and their axonal projections after exposure to a fatty diet, with a concomitant reduction in electro-olfactogram amplitude and reduced protein expression of Golf and mouse odor receptor 28 Loss of olfactory sensory neurons and associated circuitry was linked to changes in neuronal proliferation (Ki67) and apoptotic cycles (caspase-3 and tunel labeling) as well as increased inflammatory markers,

such as Iba-1 Using a computer-controlled, liquid-based olfactometer, mice maintained on fatty diets learned reward-reinforced behaviors more slowly, had deficits in reversal learning demonstrating behavioral inflexibility, and exhibited reduced olfactory discrimination. Olfactory dysfunctions and lost circuitry persisted when obese mice were removed from their high-fat diet to regain normal body weight and fasting glucose. We conclude that a chronic energy imbalance presents long-lasting structural and functional changes in the olfactory sensory system and leads to impaired olfactory- and reward-driven behaviors.

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Poster

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Title: Efferent projections of the arcuate nucleus of the hypothalamus in the adult male rat: A dual retrograde tract tracing study

Authors: *A. MARTINEZ, B. E. PINALES, A. M. KHAN
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Abstract: By monitoring systemic signals, the arcuate nucleus of the hypothalamus (ARH) acts as an active sentinel of the metabolic needs of an animal. Such signals are additionally

transduced to several other discrete brain nuclei that function as co-regulators of energy homeostasis. Many of these nuclei send reciprocal projections back to the ARH to establish complex regulatory networks that function in concert to meet the dynamic demands of energy expenditure. While ARH function has been intensively investigated, a complete understanding of connectional patterns from the ARH to larger network targets remains poorly understood. Here we report the initial results from an ongoing study using retrograde tracers to map ARH efferents to the ventral lateral bed nuclei of the stria terminals (BSTvl), the medial preoptic nucleus (MPO) and the lateral preoptic nucleus (LPO). Using stereotaxic procedures, bilateral injections of the retrograde tracers, Fluorogold (FG) and 4',6-diamidino-2-phenylindole (DAPI), were made into the BSTvl, MPO and LPO nuclei of Sprague Dawley rats. Adjacent Nissl-stained tissue series were utilized to identify the cytoarchitecture and nuclear boundaries of the ARH for tissue series used to visualize injection sites. Retrogradely backfilled ARH neurons were mapped to the Swanson rat brain atlas (2004). All cases displayed backfilled FG and DAPI cell bodies in the ARH, along with other regions. Rostral to caudal variations in the number of positively labeled ARH neurons in the medial-lateral and dorsal-ventral orientation were present among cases with different retrograde injection target sites. In some instances, both ipsilateral and contralateral projections from the ARH were visualized. While many nuclei receive projections from the ARH, this initial study closely examines the selected target sites located in rostral regions. The data from this ongoing project will provide a better understanding of the key networks the ARH communicates with to regulate energy balance.

Disclosures: **A. Martinez:** None. **B.E. Pinales:** None. **A.M. Khan:** None.

Poster

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Topic: E.07. Food Intake and Energy Balance

Support: P01 HD044232 to VP, MNL, and LMC

Title: Insulin sensitizers block effects of prenatal testosterone excess in female sheep on expression of tyrosine hydroxylase, insulin and androgen receptors in the ventral tegmental area

Authors: ***C. J. STEADMAN**[1], M. N. LEHMAN[2], V. PADMANABHAN[4], L. M. COOLEN[3]
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MS; [4]Departments of Obstetrics and Gynecology, Pediatrics, and Reproductive Sci. Program, Univ. of Michigan, Ann Arbor, MI

Abstract: Female sheep exposed to excess testosterone (T) during prenatal life, display an array of endocrine deficits comparable to those observed in women with polycystic ovarian syndrome (PCOS), including functional hyperandrogenism, insulin resistance, and increased risk for developing diabetes. Previously, we demonstrated increased food reward-seeking behavior and reduced motivation for feminine sexual behavior in prenatal T-treated ewes. A neural substrate critical for such goal-directed behavior is the mesolimbic dopaminergic system. Indeed, we recently showed that prenatal T treatment increased expression of tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine, and androgen receptors in the ventral tegmental area (VTA) of the adult ewe. In the current study, we hypothesize that insulin receptor activation plays a critical role in mediating the effects of prenatal T excess on the VTA dopamine and androgen receptor systems. We utilized prenatal and postnatal interventions with insulin sensitizers Rosiglitazone and Metformin to test this hypothesis. Ewes were treated prenatally with T (twice weekly; 100 mg testosterone propionate; ~1.2 mg/kg) during days 30-90 of the 147 day gestation period (T; n=5) or vehicle (Control; C; n=7). In addition, prenatal T-treated and control females were co-treated prenatally with rosiglitazone (daily oral 8 mg/ewe; TR; n=7; and CR; n=6) or received postnatal treatments starting at 8 weeks of age with insulin sensitizers rosiglitazone (daily oral 0.11 mg/kg; T+R; n=8; and C+R; n=6) or metformin (daily oral 7.1 mg/kg; T+M; n=4; and C+M; n=5). At two years of age, all ewes were ovariectomized and administered progesterone and estradiol sequentially to mimic late follicular phase. Brains were harvested and immunohistochemistry for tyrosine hydroxylase (TH) was used to analyze VTA dopamine expression. In addition, co-expression of TH with insulin receptors (IR) was determined and expression of androgen receptors was analyzed. Prenatal T increased VTA androgen receptor and TH expression, but reduced IR expression in TH neurons. Moreover, both prenatal and postnatal insulin sensitizer treatment partially blocked or ameliorated these effects. These findings indicate that prenatal T organizes the VTA dopamine system partly via actions on insulin receptors. Moreover, the data indicate potential interactions between androgen and insulin receptors underlying effects of prenatal T.

Disclosures: C.J. Steadman: None. **M.N. Lehman:** None. **V. Padmanabhan:** None. **L.M. Coolen:** None.

Poster

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Topic: E.07. Food Intake and Energy Balance

Support: R01-DK078049

Title: Phenotypic characterization of Urocortin 3 in the paraventricular nucleus of the hypothalamus

Authors: *C. VAN HOVER[1], C. LI[2]
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Abstract: Urocortin 3 (Ucn 3) is a member of the corticotropin releasing factor (CRF) neuropeptide family implicated in feeding, the stress response, and sympathetic outflow. Ucn 3 nerve fibers innervate the ventromedial hypothalamic nucleus (VMH), an area known for its role in satiety and sympathetic modulation. Ucn 3 neurons in the anterior parvicellular part of the paraventricular nucleus of the hypothalamus (PVHap) project prominently to the VMH. In the present study, anatomical features of the Ucn 3 cells in the PVH area were characterized to explore their functional role. Transgenic mice with Cre recombinase (cre) expressed in Ucn 3-positive cells (Ucn 3-cre) were used to visualize the distribution of Ucn 3 in the mouse brains. The expression of Ucn 3 in these mice was first verified by crossing Ucn 3-cre mice with reporter mice with mCherry fluorescence protein driven by a ROSA promoter (Ucn3-cre-mCherry). A high degree of co-localization of Ucn 3 immunoreactivity and mCherry was observed in reported Ucn 3-enriched areas including the PVHap and medial amygdala. To further characterize the Ucn 3 cells in the PVH, brain sections of Ucn3-cre-mCherry mice were labeled for CRF, vasopressin, and oxytocin (OXY) as markers to identify subregions of the PVH. Scant colocalization among Ucn 3, indicated by mCherry fluorescence, and these neurotransmitters was seen; only a very small number of Ucn 3 cells showed colocalization with OXY in the PVHap area. Ucn 3 cells thus appear to constitute a sub-population of PVH neurons located predominately in the PVHap area. To define projections of Ucn 3 cells from the PVHap, a cre-regulated anterograde tracing viral vector that expresses farnesylated GFP (GFPf) was injected into the PVHap of Ucn 3-cre mice. GFP-positive fibers and terminals were located in the medial and anterodorsal parts of the median preoptic nucleus, the perifornical area, posterolateral portion of the bed nucleus of the stria terminalis, many parts of the PVH (the PVHap, lateral magnocellular part, ventral part, and posterior part), the anterior hypothalamic nucleus, dorsomedial part of the VMH, the arcuate nucleus, and the external zone of the median eminence. In summary, we have determined that Ucn 3 shows little overlap with other well known substrates expressed in the PVH and that these Ucn 3 cells project prominently to nuclei in the hypothalamus and the median eminence. The Ucn 3 cells in the PVH connect with a number of brain areas implicated in regulating energy balance and feeding, and the Ucn 3 PVH system appears to constitute a circuit that is separate from other well known neuropeptide regulators in energy homeostasis in the PVH.

Disclosures: **C. Van Hover:** None. **C. Li:** None.

Poster

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Title: Characterization of c-Fos activation following limited access to palatable food and subsequent binge eating

Authors: ***M. M. HURLEY**, J. M. RESCH, B. MAUNZE, D. A. BAKER, S. CHOI
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Abstract: Binge eating disorder is defined by aberrant and unhealthy feeding patterns, driven by a state of extreme motivation. To advance our ability to distinguish between homeostatic and motivated feeding states we have developed a paradigm where male Sprague Dawley rats exhibit a binge-like behavior for limited access to a "western diet" (WD). This paradigm includes daily ad Libitum access to a nutritionally complete standard chow while simultaneously providing restricted access to a high energy WD for 30 minutes. As a result, animals consumed over half of their daily caloric intake from the WD. This design replicates several fundamental characteristics observed in binge eating disorder in humans, with the most prevalent being voluntary food restriction in exchange for brief access to WD, despite having daily ad Libitum access to standard chow. In addition to selective food restriction, the escalating quantity of WD consumption in the brief 30 min period, thereby increasing the rate of feeding, further reflects characteristics of binge eating behavior. Moreover, this behavior is highly dependent on the palatability or preference for the food provided during the "binge session," as this behavior is not observed when the WD is substituted with standard chow. Taking this into consideration, the current study will identify brain regions demonstrating changes in c-Fos expression in animals that are offered either standard chow or WD for 30 minutes in addition to their ad Libitum access to standard chow for 14 days. We anticipate that regions involved with hypothalamic-reward system interactions specific to feeding behavior will be altered by the 30 minute intake of WD compared to standard chow. Additional analysis will include co-labeling c-Fos immunoreactivity

with *in situ* hybridization detection of mRNA for feeding neuropeptides to construct a neurocircuitry underlying binge feeding behavior. In addition to utilizing limited access to WD, we will modify this behavioral paradigm to delineate and separate homeostatic feeding from motivated feeding. This will include food restriction of standard chow up to a 6 hour period then followed by 15 minutes of limited access to either standard chow or WD (restricted feeding-limited access binge paradigm). This protocol ensures the animals are homeostatically sated when they are offered limited access to palatable or standard food and allows for the examination of feeding that is driven more by motivation or preference than caloric need. Similarly, we will examine co-labeling of c-Fos and mRNA expression to identify the potential circuits involved in motivated feeding.

Disclosures: M.M. Hurley: None. **J.M. Resch:** None. **B. Maunze:** None. **D.A. Baker:** None. **S. Choi:** None.

Poster

257. Sleep

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Topic: E.08. Biological Rhythms and Sleep

Support: DGAPA-UNAM Grant IN224314 to OPG

CONACyT Grant 129103 to OPG

Title: 4-aco-dmt affects sleep-waking cycle of rats

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Abstract: Hallucinogens drugs have a long history of use in healing ceremonies, exerting their effects primarily by activating serotonin (5-HT) 2A receptors. Despite renewed interest as therapeutic tool (e.g. psychotherapy, addictions, depression) there are little systematic research about its pharmacological effects on brain changes and behavioral states. In this work we analyzed the effect of 4-AcO-DMT (O-Acetylpsilocin) on the sleep-waking cycle, anxiety and

motor control of male Wistar rats. 4-AcO-DMT was ip administered at a dose of 1, 2 and 5 mg/kg right immediately before the beginning of the sleep recording. Likewise, open arms and RotaRotd test were carried out during the dark phase of the photoperiod. Results showed that all the doses tested decreased REM sleep during the first four hours of recording and the effect remained for a total of 12h with the higher dose. Additionally, 1 mg/kg decreased the time spent in open arms; whereas 2 mg/kg interferes with the motor control. We concluded that 4-AcO-DMT modify the sleep-waking cycle by decreasing REM sleep while increasing waking facilitating anxiety and reducing motor control.

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Poster

257. Sleep

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Program#/Poster#: 257.02/NN15

Topic: E.08. Biological Rhythms and Sleep

Title: Using microarray data for an improved sleep related gene ontology and identifying candidate genes for sleep QTL

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Abstract: Humans spend approximately one third of their lives sleeping, but compared with other biological processes, most of the molecular and genetic aspects of sleep have not been elucidated. A nearly random gene ontology and lack of a dedicated database containing a comprehensive list of sleep related genes and their function presents a hurdle for sleep researchers. Using a two-pronged approach to solve this problem, publicly available microarray data from NCBI GEO (National Center for Biotechnology Information - Gene Expression Omnibus) database was used to develop a list of sleep related genes for traits of interest. The data was analyzed using R Bioconductor and custom Perl scripts. The genes from this list were then matched with the genes in QTL (Quantitative Trait Loci) for the trait. The genes within the QTL chromosomal region matching any in the list of sleep-related genes were considered as potential candidates for causing variations in the Quantitative trait. Here we present the results from our preliminary study conducted for sleep deprivation (SD) using this approach. Three microarray

datasets belonging to two superseries in GEO database were analyzed. The datasets were selected on the basis of similarity of experimental design. 227 candidate sleep related genes were identified by comparing data from control and sleep deprived mice. We were able to identify 4 candidate genes in Dps1 QTL, 2 in Dps2, and 9 genes in Dps3. These Dps loci are the QTL associated with delta power in slow wave sleep. The list contains Homer1 that has already been established as a molecular correlate of sleep loss, with alleles that appear responsible for Dps1. A second highlighted gene, Asrb, has also been previously reported as a candidate gene. Analysis of additional datasets from mice and *Drosophila* is underway. The advantage of this approach is that it provides more information and cross support than a simple list of sleep related candidate genes. Experimental validation of candidate genes identified using this approach will help in establishing the validity of this method. The use of microarrays and other data for improved lists of sleep related genes is not perfect, but should represent a substantial improvement over the existing list of genes returned using the query "sleep" or other similar terms in gene ontology database, and should be useful for many sleep researchers.

Disclosures: S.S. Joshi: None. **B.F. O'Hara:** None.

Poster

257. Sleep

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Program#/Poster#: 257.03/NN16

Topic: E.08. Biological Rhythms and Sleep

Title: Melatonin effect over ERK/MAPK signaling pathway on hippocampus of adult BalbC mice, submit to 96 hours of REM sleep deprivation

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Abstract: Melatonin (MEL) is an import indole released from the pineal gland, and it possesses a wide variety of neurobiological actions because is consider a pleiotropic molecule, some roles

include control of seasonal reproduction, regulation of circadian rhythms, besides is an excellent antioxidant. By the other hand consequences of sleep deprivation (SD) at the molecular level are largely unexplored. Knowledge of such molecular events is essential to understand the restorative processes occurring during sleep as well as the cellular mechanisms of sleep regulation. Until now, is documented at the literature that in the hippocampus, ERK1/2 activation is regulated by sev¬eral neuromodulators, such as trophic factors, monoamines, and neuropeptides, and this can affect a broad array of cellular functions including proliferation, survival, apoptosis, motility, transcription, metabolism and differentiation. So in this work we investigate if MEL can activate this signaling pathway by MT1 and MT2 receptor or also by anti- apoptotic proteins like BCL2 and BCLX. We administrated oral melatonin at 10mg/kg for 14 days before to performance 96 hours of REM SD by the flower pot technique. Groups were: control, MEL+96 h PS, 96hrs PS and Luzindole (30mg/Kg) I.P+MEL+96 PS. MEL was administrated daily in water consumption and oral cannula administration at 18:00 p.m, luzindole (antagonist of MT1y MT2) once daily I.P at 17:30. After 96h of SD we sacrificed by decapitation and quickly we remove hippocampal tissue and stored -80°C until used. We quantify protein/RNAm by western blot(WB) technique and Real Time-PCR respectively. Our results suggest that mice treated with MEL were significant different compared with the other groups for both techniques WB and RT-PCR.

Disclosures: G.L. Armas: None. **S. Soto-Rodriguez:** None. **G. Yañez-Delgadillo:** None. **S. Luquin de Anda:** None. **R. Ramos-Zuñiga:** None. **M. Flores-Soto:** None. **V. Chaparro:** None. **R. Gonzalez-Castaneda:** None.

Poster

257. Sleep

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Program#/Poster#: 257.04/NN17

Topic: E.08. Biological Rhythms and Sleep

Title: The dual orexin receptor antagonist lowers rat brain histamine levels without lowering rat brain acetylcholine

Authors: *S. M. SMITH, L. YAO, A. RAMIREZ, A. GOTTER, P. TANNENBAUM, J. USLANER, C. WINROW, S. KUDUK, P. COLEMAN, J. RENGER
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Abstract: Orexin, promotes wakefulness via interaction with two G-protein coupled receptors, Orexin 1 and Orexin 2. Pharmacological antagonism of these two orexin receptors promotes sleep and has been clinically validated for the treatment of insomnia. DORA-12 and DORA-22, analogs of Suvorexant, are potent dual Orexin receptor antagonists (DORAs) that promote sleep across all species tested. Previous work has demonstrated that sleep-promoting doses of DORA-22, unlike currently-marketed sleep medications eszopiclone and zolpidem (Z-drugs), do not disrupt memory in a novel-object recognition paradigm in rodents (Uslaner et al., 2013). To understand the sleep-promoting effects of DORAs and Z-drugs, we used *in vivo* microdialysis to assess histamine or acetylcholine levels in the lateral hypothalamus (LH), prefrontal cortex (PFC) or hippocampus (HIP) following administration of DORA-12, DORA-22, or Z-drugs during the wake-phase when histamine and acetylcholine levels are elevated thus mimicking conditions associated with insomnia. At sleep promoting doses, DORA-22 and DORA-12 lowered the elevated histamine levels in the LH in a dose-dependent manner to the sleep phase levels. Eszopiclone had a weak and transient effect on the elevated histamine and zolpidem had no effect at the dose tested. Similarly, in the PFC, DORA-22 and DORA-12 lowered the increased histamine to levels observed during the sleep, whereas both Z-drugs only transiently lowered histamine. In hippocampus, both histamine and acetylcholine are increased during waking phase. DORA-22 did not significantly reduce the acetylcholine (ACh) levels at 3mg/kg, which is beyond the dose needed to introduce sleep (1mg/kg). In contrast, Z-drugs doses tested below sleep induction produced a significant decrease in ACh levels with similar doses showing a negative impact in rodent memory experiments (Uslaner et al., 2013). Interestingly, recent results indicate that DORA-22 attenuates caffeine induced elevation of histamine in the LH and PFC, but has no effect on the caffeine induced acetylcholine increase in the LH and PFC during the sleep phase. In contrast, Lunesta attenuated caffeine induced both histamine and acetylcholine increase. Collectively, our data provides added insights into the mechanism of action of DORAs and Z-drugs during the sleep-wake phase and further differentiates the effects of DORAs from current standards of care.

Disclosures: **S.M. Smith:** A. Employment/Salary (full or part-time):; Merck. **L. Yao:** A. Employment/Salary (full or part-time):; Merck. **A. Ramirez:** A. Employment/Salary (full or part-time):; Merck. **A. Gotter:** A. Employment/Salary (full or part-time):; Merck. **P. Tannenbaum:** A. Employment/Salary (full or part-time):; Merck. **J. Uslaner:** A. Employment/Salary (full or part-time):; Merck. **C. Winrow:** A. Employment/Salary (full or part-time):; Merck. **S. Kuduk:** A. Employment/Salary (full or part-time):; Merck. **P. Coleman:** A. Employment/Salary (full or part-time):; Merck. **J. Renger:** A. Employment/Salary (full or part-time):; Merck.

Poster

257. Sleep

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 257.05/NN18

Topic: E.08. Biological Rhythms and Sleep

Title: Effects of zolpidem on sleep pattern in an animal model of insomnia

Authors: *K. A. ZANIN[1,2], C. L. PATTI[1], A. ZAGER[3], L. B. LOPES-SILVA[2], C. S. BIZERRA[1], R. SANTOS-BALDAIA[1], A. W. HOLLAIS[1], M. L. ANDERSEN[2], S. TUFIK[2], R. FRUSSA-FILHO[1], D. L. R. POYARES[2]
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Abstract: Purpose: Zolpidem (Zolp) is a hypnotic drug prescribed to treat insomnia, in some conditions, as a long term treatment. Although the polysomnographic findings after Zolp administration have already been described, they remain controversial. Additionally, few studies have characterized the effects of Zolp on sleep pattern in animal models of sleep restriction. Thus, the aim of this study was to evaluate the effects of the repeated administration of Zolp on sleep parameters of mice submitted to total sleep deprivation (TSD). Methods: Three-month-old Swiss male mice were subjected to control condition (CTRL) or to TSD by gentle handling for 3 h per day during 10 days. Sleep-wake cycle was assessed via electrocorticographic and electromyographic records. Two pairs of electrodes were surgically implanted in the fronto-parietal medial derivation and a pair of nickel-chrome electrodes was implanted in the dorsal muscle of the neck of mice. All of the animals were recorded for 24 h before treatment to evaluate baseline sleep parameters. On the 2nd day mice were subjected to CTRL or to TSD. After sleep deprivation mice were treated with saline (Sal) or Zolp during 10 days forming the following groups: CTRL-Zolp (n=4), TSD-Sal (n=4) e TSD-Zolp (n=4). Sleep was recorded using the Somnologica software. The sleep pattern was visually and manually scored by an experienced single blinded researcher. The following sleep parameters were considered: total sleep time (TST), slow-wave sleep time (SWS), paradoxical sleep time (PS) and wake time (W). Results: The TSD protocol was able to enhance the W time and to decrease the SWS, PS and TST when compared to baseline. Zolp administration enhanced SWS in both CTRL and TSD groups. Concerning the CTRL group, Zolp increased SWS and decreased PS in the light period. Still, we found an increase in PS in the dark period, suggesting a compensatory effect. However, when comparing the 10th to the 1st day of treatment, there was a decrease in PS in the dark period. Finally, in the PST-Zolp group the compensatory enhancement in sleep parameters was observed in the light phase and persisted throughout the treatment. Importantly, opposite to which was observed in the other groups, in the PST-Zolp group we observed modification on sleep pattern during recovery when compared to baseline sleep. Conclusions: Zolp increased

SWS in both CTRL and TSD conditions. However, this effect did not persist in mice kept in the CTRL group. Sleep deprived animals seemed to tolerate the SWS (but not PS) enhancement. Importantly, sleep-deprived mice treated with Zolp did not completely recover the sleep parameters to baseline levels during rebound.

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Poster

257. Sleep

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Program#/Poster#: 257.06/NN19

Topic: E.08. Biological Rhythms and Sleep

Title: Dual orexin receptor antagonists demonstrate efficacy with chronic treatment and restore REM sleep following induced insomnia in healthy animals

Authors: J. I. BRUNNER[1], *A. L. GOTTER[3], J. STEVENS[2], P. L. TANNENBAUM[2], S. V. FOX[2], S. GARSON[1], J. USLANER[2], J. J. RENGER[1], C. J. WINROW[1]
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Abstract: Insomnia treatments ideally increase all sleep stages to a similar extent, and induce natural, restorative sleep. The current pharmacological standard of care which includes GABA-A modulators promote sleep by globally inhibiting CNS activity. These drugs characteristically and preferentially promote non-rapid eye movement (non-REM) sleep at the expense of reduced REM sleep. Dual orexin receptor antagonists (DORAs), representing a novel potential treatment for insomnia, block the arousal-promoting activity of orexin peptides and promote sleep proportionately in all sleep stages. Unlike that seen following GABA-A modulator administration, quantitative electroencephalography (qEEG) spectral analysis in sleep stages is not disrupted by DORAs. Insomnia treatments should also alleviate sleep debt created by deprivation according to homeostatic need for specific sleep stages of sleep. Even in the presence of increased sleep drive in REM deprived animals, treatment with Zolpidem (30 mpk, po, QD) has no immediate impact on REM sleep in comparison to control animals, and even suppressed REM at later time points. On the other hand, sleep deprived animals treated with DORA-12 (100 mpk, po, QD) exhibit a significant increase in both non-REM and REM sleep. In addition to

promoting natural sleep, sleep medication should maintain effectiveness with chronic treatment. GABA-A modulators have previously been shown to exhibit tolerance and dependence such that sleep promoting efficacy diminishes with chronic exposure. Animals treated with Zolpidem (30MPK, po, QD) exhibited desensitization over a 5 day period, with reduced or total loss of efficacy on the last day of treatment. Treatment with DORA-12 (30 mpk, po, QD) was fully efficacious throughout the same 5 day treatment window, and also had equal effectiveness in animals previously desensitized to Zolpidem (10 mpk, po, QD). Together, these results in animal models suggest that DORAs promote sleep that is similar to natural sleep, maintain effectiveness with chronic treatment, and have efficacy that is resistant to prior desensitization to GABA-A modulators.

Disclosures: **J.I. Brunner:** A. Employment/Salary (full or part-time):; Merck & Co. **A.L. Gotter:** A. Employment/Salary (full or part-time):; Merck & Company. **J. Stevens:** A. Employment/Salary (full or part-time):; Merck & Co. **P.L. Tannenbaum:** A. Employment/Salary (full or part-time):; Merck & Co. **S.V. Fox:** A. Employment/Salary (full or part-time):; Merck & Co. **S. Garson:** A. Employment/Salary (full or part-time):; Merck & Co. **J. Uslaner:** A. Employment/Salary (full or part-time):; Merck & Co. **J.J. Renger:** A. Employment/Salary (full or part-time):; Merck & Co. **C.J. Winrow:** A. Employment/Salary (full or part-time):; Merck & Co.

Poster

257. Sleep

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CONACYT 179927

Secretaría de Ciencia, Tecnología e Innovación del DF PINV11-30

Title: Non-steroidal anti-inflammatory drug alters c-fos expression during rebound after rapid eyes movement sleep deprivation

Authors: *K. GUZMAN VASQUEZ, D. MILLAN-ALDACO, M. PALOMERO-RIVERO, R. DRUCKER-COLIN
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Abstract: Several works have shown that total sleep deprivation (TSD) or Rapid eyes movement (REM) sleep deprivation is detrimental for animals and humans. Moreover, mechanisms sleep rebound after sleep deprivation are unknown. Our group showed that nabumetone reduces rebound after REM sleep deprivation, therefore, the goal is shown if non-steroidal anti-inflammatory drugs (Nabumetone) modifies c-fos expression in regions of brain related with sleep regulation. Male Wistar rats (250-280g) were maintained under a controlled light-dark cycle (12:12, lights on at 07:00 A.M.) with food and water ad libitum. Rats were divided in six groups and received a treatment: cage control (Vehicle or Nabumetone); deprivation control (Vehicle or Nabumetone) and REM sleep deprivation (Vehicle or Nabumetone). During deprivation rats received each 24 hours vehicle or nabumetone i.p. administration. At the end of deprivation, rats were anesthetized intraperitoneally with sodium pentobarbital and perfused transcardially with 200 ml PBS and 200 ml 4% paraformaldehyde. Then, the brains were prepared for cryostat sectioning; coronal slices were made at the hypothalamic and pons level. Immunofluorescence was developed Nabumetone administration modifies c-fos expression in the hypothalamus. Therefore, we think proinflammatory molecules are involucre in REM sleep rebound.

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Poster

257. Sleep

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Topic: E.08. Biological Rhythms and Sleep

Support: Department of Veterans Affairs

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Title: Optogenetic stimulation of basal forebrain cholinergic neurons promotes cortical activation both directly and indirectly

Authors: J. C. ZANT[1], T. KIM[1], A. V. KALINCHUK[1], C. YANG[1], R. E. BROWN[1], J. MCNALLY[1], S. THANKACHAN[1], J. T. MCKENNA[1], R. E. STRECKER[1], R. W. MCCARLEY[1], *R. BASHEER[2]
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Abstract: The complexity of the neuronal composition in the basal forebrain (BF) has long prevented a clear understanding of the causal role of each neuronal subtype on modulating cortical activity. Cortically-projecting cholinergic and parvalbumin expressing GABAergic neurons are suggested to modulate cortical activation. To define their specific roles, we have developed a novel opto-dialysis method combining EEG/EMG recording, *in vivo* microdialysis, and optogenetics. ChAT-ChR2-EYFP-BAC (ChAT-ChR2) mice, and AAV-ChR2 transduced Parv-cre (PV) mice (for selective cholinergic and GABAergic stimulation respectively) were outfitted with EEG/EMG electrodes, and an opto-dialysis probe, aimed at the BF area. The experiment consisted of a baseline day and an experimental day, when a 10s stimulation paradigm was repeated every min for 2h, using 10ms laser pulses (473nm) at selected frequencies (8Hz for ChAT-ChR2, 40Hz for PV). To antagonize the muscarinic receptor-mediated input on BF GABAergic neurons in ChAT-ChR2 mice, atropine was administered by reverse-microdialysis during cholinergic stimulation. The 8Hz stimulation in ChAT-ChR2 mice (n=5) resulted in a 39% reduction in EEG delta power (0.5-4Hz) when compared to the pre-stimulation period; decreased NREM to wake latency by ~15s (117 trials); and increased the total amount of wakefulness by 84% (n=5). This increased wakefulness was attenuated to 28% when atropine was simultaneously perfused (n=3). 40Hz stimulation in PV mice (n=2), to excite PV expressing GABAergic neurons, increased wakefulness by 24%. These results indicate that stimulating BF cholinergic neurons may promote cortical activation both directly and indirectly mediated by activation of neighboring cortically-projecting GABAergic and/or glutamatergic neurons.

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Poster

257. Sleep

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Topic: E.08. Biological Rhythms and Sleep

Support: Novartis Institutes for BioMedical Research

Title: Sleep-wake profile and EEG power spectrum: Distinct effects of zolpidem and dual orexin receptor antagonists in rats

Authors: *L. H. JACOBSON[1], L. PERROT[2], M. FENDT[3], D. BEHNKE[2], S. COTESTA[2], G. LAUE[2], S. OFNER[2], E. LEGANGNEUX[2], S. HINTERMANN[2], C. BETSCHART[2], C. E. GEE[4], D. HOYER[5]
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Abstract: Orexin neurons selectively clustered in the lateral hypothalamus project widely across the brain and have a distinct role in sleep-wake regulation. Orexin 1 & 2 receptors (Ox1R, Ox2R) are drug discovery targets for sleep disorders and several dual Ox1R/Ox2R antagonists (DORAs) have been clinically validated in primary insomnia. Advantages of DORAs over $GABA_A$ receptor targeted hypnotics may include reduced interactions with ethanol, absence of tolerance, withdrawal and addictive potential, yet the detailed effect of DORAs on sleep architecture remains to be investigated as only a few studies have examined the effects of DORAs on EEG power spectrum. We therefore compared the effects of the four DORAs SB-649868 (50 mg/kg), almorexant (50, 150 mg/kg), MK-6096 (filorexant; 50,100 mg/kg) and MK-4305 (suvorexant; 30 mg/kg) with those of zolpidem (10 mg/kg), on sleep-wake profile and EEG power spectrum in rats. Male Sprague-Dawley rats were implanted with 4 electrocorticogram electrodes positioned on the cerebellum, visual and frontal cortex (frontal electrode serving as an earth). Pizoelectric detectors were placed on the bottom of the cages to detect activity and aid in defining vigilance states. Each rat served as its own control in a 3 day experiment: dosing at the start of the dark phase with water on day 1, vehicle on day 2, and drug on day 3. EEG signals were acquired using Harmonie and analysed with Somnologica. Post-hoc correction of vigilance states using the piezoelectric signal was performed using an internal algorithm. Relative spectral power (Hanning window) was analysed in REM epochs and in selected, continuous wake and NREM epochs. All compounds reduced wake: the four DORAs had similar effects on sleep-wake, increasing total sleep and REM sleep as a proportion of total sleep, in contrast to Zolpidem which reduced REM sleep. SB-649868, almorexant (150 mg/kg), filorexant (50 mg/kg) and zolpidem increased NREM sleep, while filorexant (100 mg/kg) and suvorexant had no effect on NREM sleep despite potent REM-enhancing effects. Wake and NREM spectral ratios were

unaffected by DORAs, in contrast to zolpidem which increased ß / γ / 5Hz power, but decreased theta power during wake. During NREM, zolpidem enhanced delta, but reduced theta power and higher frequencies. Both filorexant and Suvorexant enhanced theta power within REM sleep. In summary, the DORAs investigated preferentially enhance REM sleep, suggestive of a class action of the DORAs. Further studies are needed to investigate the effects of DORAs on enhanced REM sleep theta power and its consequences, especially in human subjects, to assess translation from rodent to human sleep studies.

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Poster

257. Sleep

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 257.10/NN23

Topic: E.08. Biological Rhythms and Sleep

Title: Enhancing effects of Ursolic acid on pentobarbital-induced sleeping model

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Abstract: *Prunella vulgaris* (PV) is widely used as an herbal medicine for cancer, inflammatory disease, or other infection. Although its long-time usage, its effects on the function of the central nervous system have not been done so much. Here, we first observed that ethanolic extracts of *Prunella vulgaris* (EEPV) had anti-anxiety and sedative effects in stressed mice. As already

known, many active components including triterpenoid (such as ursolic acid and oleanolic acid) which possess many biological activities have been isolated. Therefore, we wanted to evaluate which component of PV induces sleep extension in pentobarbital mediated sleeping model in mice. Surprisingly, in spite of their similarity in structure and other common functions like anti-inflammation, anti-cancer, and tissue protection, only ursolic acid showed enhancement of sleep duration in pentobarbital-treated mice. These activities were abolished by bicuculline, $GABA_A$ receptor antagonist treatment. To clarify this GABA-induced effect, we measured intra brain GABA level by ELISA assay. With an effective dose of ursolic acid administration, total GABA concentration is significantly increased compared to that of vehicle treatment. Taken together, the present results suggest that ursolic acid from *Prunella vulgaris* prolongs sleep duration through $GABA_A$ receptor activation and would be useful for hypnotic agent.

Disclosures: J. Jung: None. **B. Kim:** None. **Q. Gao:** None. **S. Ko:** None. **Y. Lee:** None. **H. Lee:** None. **S. Park:** None. **S. Jeon:** None. **J. Cheong:** None. **J. Ryu:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: USAMRAA Grant DR080789P1

Title: The dual hypocretin receptor antagonist almorexant is permissive for the activation of wake-promoting systems

Authors: *G. S. PARKS[1], D. R. WARRIER[1], L. DITTRICH[1], A. J. WILK[1], M. D. SCHWARTZ[1], T. C. NEYLAN[2], S. R. MORAIRTY[1], T. S. KILDUFF[1]
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Abstract: The dual hypocretin receptor (HcrtR) antagonist almorexant (ALM) has potent hypnotic actions and is thought to promote sleep by selective disfacilitation of wake-promoting systems whereas benzodiazepine receptor agonists (BzRAs) such as zolpidem (ZOL) induce sleep through general inhibition of neural activity. Consequently, HcrtR antagonists are predicted to cause less functional impairment than BzRAs. Recent behavioral studies have supported this hypothesis as ALM causes less impairment in spatial memory tasks in rats awoken from

hypnotic-induced sleep than ZOL does. Other dual HcrtR antagonists also promote sleep at doses that do not disrupt locomotor activity or cognition. In order to gain insight into the neural mechanisms underlying the differential functional impairment of these drugs, we compared the effects of ALM and ZOL on functional activation of the currently known wake-promoting systems. Sprague Dawley rats, implanted for EEG/EMG recording, were orally administered vehicle (VEH), 100mg/kg ALM, or 100mg/kg ZOL during their active phase and were either left undisturbed or kept awake (i.e., sleep-deprived; SD) for 90 min after which their brains were collected. We measured Fos coexpression with markers for wake-promoting cell groups in the basal forebrain (BF; ChAT), tuberomammillary nuclei (ADA), lateral hypothalamus (Hcrt), and dorsal raphe (DR; 5HT). In the locus coeruleus (LC), we counted singly-labelled Fos+ cells because the density of DBH staining obscured Fos immunoreactivity in double-labeled sections. Following sustained wakefulness, Fos coexpression in histamine and Hcrt neurons was higher in VEH and ALM-treated rats than in ZOL-treated rats; moreover, the level of co-expression was indistinguishable between the VEH- and ALM-treated groups. In these neuronal populations, Fos levels were consistently elevated in ALM-treated SD rats compared to undisturbed animals whereas Fos levels were unchanged by SD in ZOL-treated animals. In contrast, no significant differences were found between groups regardless of treatment in the BF and DR. Interestingly, there were no differences in Fos expression between VEH and ALM-treated animals in the LC following SD, but ZOL-treated rats exhibited elevated Fos compared to vehicle. These results indicate that, in contrast to ZOL, ALM does not inhibit activation of the histamine and Hcrt systems and is thus unlikely to prevent activation of wake-promoting systems in response to situational demands. These results may also relate to the lower levels of cognitive impairment produced by dual HcrtR antagonists compared to ZOL.

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Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant MH59839

Title: Behaviorally activated localized expression of brain-derived neurotrophic factor (BDNF) in the brainstem is a molecular signature for homeostatic regulation of rapid eye movement (REM) sleep

Authors: *S. DATTA, R. SINGH
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Abstract: Multiple lines of evidence indicate that cholinergic neurons within the pedunculopontine tegmentum nucleus (PPT) are critically involved in the generation of REM sleep. However, the specific molecular mechanisms operating within PPT neurons that underlie the homeostatic regulation of REM sleep remain largely unknown. In the present study, we tested the hypothesis that BDNF levels in key regions of the REM sleep generating network are modulated by behaviorally induced REM sleep deprivation. Adult male SD rats (n = 102) were chronically implanted with sleep-wake (S-W) recording electrodes. After surgical recovery, habituation, and final baseline S-W recordings, rats were separated into 3 groups (34 rats/group), which were each further divided into 5 subgroups. Group 1 (baseline S-W [BLS]): These animals were recorded for undisturbed S-W activity for a length of time ranging between 2 and 6 hours (between 10 AM to 4 PM). After each hour, rats from one of the five subgroups (BLS-2h, BLS-3h, BLS-4h, BLS-5-h, and BLS-6h) were quickly euthanized with CO2. The brains were then rapidly chilled on dry ice for further molecular analyses. Group 2 (selective REM sleep deprivation [RSD]): The experimental protocol and five subgroups for these animals are almost identical to those of Group 1, except that for group 2 animals, from 10 AM to 1 PM, REM sleep episodes were selectively terminated at their beginning while the animals remained connected to the recording system. Group 3 (total sleep deprivation [TSD]): The experimental protocol and subgroups for these animals are almost identical to those of Group 1, except that from 10 AM to 1 PM, Group 3 animals were deprived of total sleep. The results demonstrated that: a) during the selective RSD period, the number of interventions required to prevent REM sleep, total % of time spent in non-REM sleep, and cortical EEG slow-wave activity (SWA) increased progressively; b) during the recovery S-W period, the total % of REM sleep and total number of pontine (P)-waves were significantly greater in the RSD group than in the BLS and TSD groups; c) during the selective RSD period, the levels of BDNF expression in the PPT and subcoeruleus nucleus (SubCD), but not in the medial pontine reticular formation, ventrolateral periaqueductal gray, and preoptic area, increased progressively in the RSD group; d) there was a strong positive relationship between: REM sleep pressure and SWA, REM sleep pressure and levels of BDNF in the PPT; BDNF in the PPT during RSD and rebound REM sleep. These results suggest that selective RSD-induced increased expression of BDNF in the PPT and SubCD are determinant factors in the development of the homeostatic drive for REM sleep.

Disclosures: **S. Datta:** None. **R. Singh:** None.

Poster

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Support: NIH Grant MH59839

DBT Research Grant, India

Title: Water extract from Withania Somnifera (Ashwagandha) leaves (ASH-WEX) decreases sleep deprivation-induced anxiety, impairment in motor coordination, and decline in cognitive function

Authors: *R. SINGH[1,2], G. KAUR[2], S. MANCHANDA[2], T. KAUR[2], R. MISHRA[2], S. DATTA[1]
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Abstract: Sleep restriction/deprivation (SD) leads to negative health and performance effects, including anxiety and impairment of motor coordination and cognitive functions. Most individuals in modern society experience frequent SD, yet there is no efficacious pharmacological remedy for the negative effects of SD. Herbal medicine research suggested that ASH may be effective in treating anxiety and improving cognitive function. Therefore, using a rat model, we examined whether anxiety, impaired motor skill, and decline in cognitive function could be attenuated in SD rats pretreated with ASH-WEX. Experiments were performed on 3 groups of adult Wistar rats (n = 6 rats/group). Group 1 (vehicle treated-unrestricted sleep-wake activity [V-US]): These animals received a single intra-gastric (IG) infusion of vehicle daily for 10 consecutive days; immediately after this infusion animals were allowed US. Group 2 (vehicle treated-sleep deprived [V-SD]): The experimental protocol for these animals is similar to that of Group 1, except that on day 10, immediately after IG infusion, animals were subjected to 12 hours of SD (between 8 am and 8 pm). Group 3 (ASH treated-SD [ASH-SD]): The experimental protocol for these animals is similar to that of Group 2, except that these animals received IG infusion of ASH-WEX (140mg/kg/infusion; in a volume of about 1 ml). On day 10, after 12 hours of SD or US, all rats were tested behaviorally for cognitive function (Novel Object Recognition [NOR] test), level of anxiety (Elevated Plus Maze [EPM]), and motor coordination (Rotarod task). Brains were then removed and analyzed to study the expression of stress response and plasticity markers, Heat Shock Protein 70 (HSP 70) and PSA-NCAM, in the dorsal

hippocampus. NOR test showed that ASH-SD rats showed a higher preference index for novel object compared to V-SD rats, but less than the V-US rats, indicating that the ASH treatment attenuated SD-induced decline in cognitive function. Relative to V-SD rats, the ASH-SD rats had more open arm crossings and spent more time in the open arm. Percentages of time spent in the closed arm were comparable between ASH-SD and V-US rats. These results suggest that ASH-WEX treatment normalized SD-induced anxiety. In the Rotarod task, V-SD rats showed maximum number of falls and spent minimum time on rotating rod compared to ASH-SD and V-US rats. Results showed a decrease in HSP 70 expression in V-SD rats compared to ASH-SD rats. PSA-NCAM expression was upregulated in the ASH-SD but not in the V-SD rats. These results suggest that ASH-WEX could be used as a supplement to control SD-induced anxiety and minimize SD-induced deficits in motor coordination and cognitive function.

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Poster

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Title: Purinergic P2 receptor-mediated excitation of basal forebrain cortically projecting cholinergic and GABAergic neurons

Authors: *C. YANG, R. W. MCCARLEY, R. BASHEER, R. E. BROWN
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Abstract: Basal forebrain (BF) cortically-projecting neurons are broadly involved in regulating cortical activation, attention and sleep. Previous studies from our group (Yang et al., 2013; Frontiers in Neurology) showed that adenosine, which accumulates in BF during prolonged wakefulness, inhibits the glutamatergic inputs onto BF cholinergic and GABAergic neurons via adenosine A1 receptors. However, the effects of its precursor, adenosine triphosphate (ATP), an important glial/neurotransmitter are unclear. *In situ*, ATP is rapidly broken down to adenosine, leading to activation of A1 receptors. However, ATP may also have fast P2-mediated effects which are obscured when applied via bath application. In order to investigate possible P2 receptor-mediated effects without contamination by adenosine effects due to ATP degradation, here we bath applied an ATP non-hydrolyzable agonist, ATP-γ-S (100 μM) and examined its effect on BF putative cortically-projecting cholinergic and GABAergic neurons with whole-cell patch clamp. ATP-γ-S strongly depolarized (by >10 mV) cholinergic and two subsets of GABAergic neurons with large and small Ih currents, in the presence of 500 nM TTX (n=5-6/group). A broad-spectrum P2X receptor antagonist, PPADS (30 μM), with relatively low affinity for P2X4 receptors, significantly decreased the responses in cholinergic neurons and small Ih GABAergic neurons to 10-20 % of control (n=5/group), but only slightly blocked the response in large Ih GABAergic neurons (by 30%, $p>0.05$, n=5). A selective P2X4 receptor antagonist, 5BDBD (30 μM), significantly decreased ~90% of the response in large Ih GABAergic neurons (n=5) and ~65% of the response in cholinergic neurons (n=5). These data suggest that ATP-γ-S excites BF cortically-projecting neurons via at least 2 subtypes of P2X receptors. Thus, ATP released from neurons and/or glia during wakefulness has both a postsynaptic excitatory effect on BF cortically-projecting neurons mediated via P2X receptors and a presynaptic inhibitory effect on glutamatergic inputs via A1 receptors. The overall effect likely depends on the kinetics of degradation/reuptake of ATP and adenosine. P2X receptors may be interesting novel targets for pharmacological agents regulating cortical activation and sleep.

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Poster

257. Sleep

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Topic: E.08. Biological Rhythms and Sleep

Title: Orexin (Hypocretin) levels in patients with Niemann-Pick type C and Miglustat treatment

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Abstract: Introduction: The symptoms of narcolepsy can occur during the course of other neurological conditions (i.e. symptomatic, narcolepsy). Inherited disorders, tumors and head trauma were the three most frequent causes for symptomatic narcolepsy. Among inherited disorders, Niemann-Pick type C (NPC), Myotonic dystrophy type 1 and Prader-Willi syndrome were mainly reported. NPC is an autosomal recessive and congenital neurological disorder characterized by the accumulation of cholesterol and glycosphingolipids in the peripheral tissues and of the glycosphingolipids in the brain. NPC is associated with mutations in NPC1 and NPC2 genes. Symptoms include hepato- splenomegaly, vertical supranuclear gaze palsy, ataxia, dystonia, and dementia. Some cases frequently display narcolepsy-like symptoms, including cataplexy. Miglustat inhibits glucosylceramide synthase, an essential enzyme for the synthesis of most glycosphingolipids. Method: The subjects were 7 patients with NPC (3 male and 4 female). Patients or families gave informed consent for the lumbar puncture. We checked clinical symptoms, PSG, MRI or CT, HLA and measured orexin levels. Result: Concerning the orexin levels, 2 cases were low level, 3 cases were intermediate, 2 cases were normal. Subjects having cataplexy had low or intermediate levels. One case (6F) treated with Miglustat (glucosylceramide synthase inhibitor) was shown to reduce cataplexy and her orexin level became normal from intermediate level. Conclusion: Moderate or severe decreases in CSF orexin levels were observed in some cases with NPC with cataplexy. However, the degree of reduction was small in contrast to idiopathic narcolepsy-cataplexy.

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Poster

257. Sleep

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Topic: E.08. Biological Rhythms and Sleep

Title: Caffeine improve memory performance and molecular plasticity during 48h of total sleep deprivation in rat

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Abstract: Normal sleep-wake cycle ensures the balance of synaptic potentiation and depression which called total synaptic strength. Sleep deprivation (SD) significantly impairs the basal synaptic strength. A large body of evidence suggests that chronic caffeine treatment significantly improves synaptic plasticity during stress, including SD. Here we investigated the impact of acute caffeine administration during 48h SD on short term / reference memory performance and molecular plasticity in the hippocampus. Experimental animals were sleep deprived by novel automated cage shaking stimulus based on animal activity (immobility and freezing). In five groups the rats were subjected to two behavioral paradigm, novel object recognition test and Morris Water Maze (MWM) spatial reference memory test. Hippocampal tissues were collected for qPCR, western blotting and immunohistochemical studies. SD induced a deficit in both MWM spatial navigation memory and familiar object retrieval performance. Caffeine administration during SD significantly improved both types of memory. SD significantly decreased expression of BDNF, Gria2 (AMPA), Grin2a (NMDA) and PSD-95 in terms of both mRNA and protein level. Expression of total Gria1 (AMPA), CaMK-II and CREB mRNA/protein did not alter after SD but phosphorylated Gria1 (S831), CaMK-II (T286) and CREB (S133) significantly decreased in dorsal hippocampus. Caffeine administration during SD significantly improved phosphorylation of these molecules compared to SD. Caffeine administration during SD also prevented down regulation of Gria2, Grin2a, BDNF, PSD-95, Synapsin 1 and Synaptophysin in mRNA or protein level or both. The overall results suggested that caffeine administration preserved basal synaptic strength and facilitated cognitive performance during 48h SD.

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Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: Research Grant Program at Escuela de Medicina, Universidad Anáhuac Mayab given to E.M.-R.

Title: Expression of MAP-K in hypothalamus and PONS on sleep deprivation and rebound in rats

Authors: *A. POOT-AKE[1], S. MIJANGOS-MORENO[1], A. MANJARREZ-MARTÍN[2], R. JIMÉNEZ-MORENO[1], E. PACHECO-PANTOJA[2], P. AQUINO-HERNÁNDEZ[2], E. MURILLO-RODRÍGUEZ[1]
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Abstract: Mitogen-activated protein kinase (MAP-K) is a protein that plays a fundamental role in the memory and sleep consolidation. For instance, there is evidence showing that expression of MAP-K is higher during sleep. However, no evidence is available regarding the homeostatic changes in MAP-K after sleep deprivation and sleep rebound process. Thus, in the present experiment, male Wistar rats were sleep deprived (total sleep deprivation [TSD]) during 6h during the lights-on period. After this, rats were sacrificed by decapitation and hypothalamus (HYP) and pons (PONS) were collected for analysis of MAP-K expression using Western blot means. A second experiment consisted in rats deprived for 6 hours, and allowed them to sleep for 4h after deprivation (sleep rebound period), and then sacrificed by decapitation. HYP and PONS were collected for further MAP-K expression analysis. In the first experiment, we found out that HYP and PONS displayed a higher expression of MAP-K after 6h of TSD compared to respective control. On the second experiment, MAP-K expression as determined by Western blot means was decreased in HYP and PONS in animals that were allowed to the sleep rebound period, compared to respective control. The current results suggest that MAP-K expression displays differences if sleep deprivation is induced or if sleep rebound process is allowed. Further experiments are required to determine the homeostatic mechanism of action of MAP-K expression during sleep.

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Poster

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Topic: E.08. Biological Rhythms and Sleep

Title: Evidence that glutamatergic neurons of the supramammillary nucleus are responsible for the activation of the dentate gyrus during paradoxical (REM) sleep in the rat

Authors: *F. BILLWILLER[1], L. RENOUARD[2], O. CLÉMENT[1], P. FORT[1], P.-H. LUPPI[1]
[1]Inserm U1028-CNRS UMR5 5292, Lyon, France; [2]Dept. of Neuroscience, Sch. of Med., University of Pennsylvania, PA

Abstract: Paradoxical sleep (PS) is characterized by muscle atonia, rapid eye movements (REM) and cortical and hippocampal activation. The pathways responsible for such activation are still a matter of debate. It has been proposed that pontine PS-on neurons activate the intralaminar thalamic nuclei and the basal forebrain cholinergic neurons which in turn activate the cortex. The supramammillary nucleus (SuM) might also play a role in such activation, in particular for the hippocampus. Indeed it projects to the medial septum and the dentate gyrus (DG) and it plays a role in the genesis of hippocampal theta rhythm (Kirk and McNaughton, 1991). It has been shown that the SuM contains a large number of glutamatergic neurons projecting to the DG (Boulland et al, 2009; Soussi et al, 2010). Besides, we recently demonstrated by means of Fos labeling that the lateral part of the SuM (SuML) contains a large number of neurons specifically activated (cFos+) during PS hypersomnia (Sapin et al, 2010). Further, we showed that a SuM lesion drastically decreases the number of cFos+ granular neurons in the dorsal DG during PS hypersomnia (Renouard et al, unpublished results). In view of these data, we hypothesized that the SuML-DG pathway activated during PS is glutamatergic. To test this hypothesis, we used a combination of Fos immunostaining and *in situ* hybridization of vesicular glutamate transporter 2 (vGLUT2) mRNA in three groups of rats: control (PSC, n=4), deprived of PS for 72h (PSD, n=4) and allowed to recover during 150 min after such deprivation (PSR, n=4). In addition, we performed vGLUT2 immunohistochemistry in PSR control animals (n=4) and PSR animals with an iontophoretic injection of ibotenic acid in the SuM (n=4. In rats subjected to PS recovery, we found that 85% of the Fos-labeled neurons located in the SuML also express the vGLUT2 mRNA. In contrast, no or only a few double-labeled neurons were observed in control and PS deprived animals. In PSR animals with a SuM lesion, we found a near disappearance of the vGLUT2 labeled fibers in particular in the dorsal DG compared with controls. These results indicate that the SuML-DG pathway selectively active

during PS is glutamatergic in nature and thus responsible for the activation of a subset of DG granular cells during PS.

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Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH awards R01 NS20246 and P20 GM103425.

Title: Modulation of gamma oscillations in the pedunculopontine nucleus (PPN)

Authors: ***S. M. DONOFRIO**[1], N. KEZUNOVIC[1], J. HYDE[1], B. LUSTER[1], S. MAHAFFEY[1], E. GARCIA-RILL[1,2], F. J. URBANO[2]
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Abstract: Reduced gamma band activity has been reported in schizophrenic and bipolar disorder patients. In the same disorders, increased neuronal calcium sensor protein 1 (NCS-1) expression was reported in a series of postmortem studies. These disorders are also characterized by sleep dysregulation, suggesting a role for the reticular activating system (RAS). Our discovery of gamma band activity in the pedunculopontine nucleus (PPN), the cholinergic arm of the RAS, revealed that such activity was mediated by high threshold calcium channels that are regulated by NCS-1. Here, we tested the hypothesis that a) NCS-1 normally regulates gamma band oscillations subserved by these calcium channels, and b) excessive level of NCS-1, such as would be expected with over expression, would decrease gamma band activity. Whole-cell patch-clamp responses were recorded on 9-13 day old adult timed-pregnant Sprague-Dawley rat brainstem slices. Slices were recorded at 37^0C perfused with oxygenated aCSF in an immersion chamber containing the synaptic blockers gabazine ($GABA_A$ antagonist), strychnine (glycine antagonist), 6-cyano-7-nitroquinoxaline-2,3-dione (AMPA/kainate receptor antagonist), APV (NMDA receptor antagonist), mecamylamine (nicotinic receptor blocker), and tetrodotoxin (sodium channels blocker). We found, using depolarizing 2 sec current ramps, that after 20-30 min of recording, NCS-1 at 1 uM significantly increased the frequency (percent increase 641%,

ANOVA, $p<0.03$) and amplitude (percent increase 149%, ANOVA, $p<0.02$) of gamma oscillations. Mean gamma band oscillation amplitude in PPN neurons significantly increased compared to other concentrations, including 0.5, 5 and 10 uM (Kruskal-Wallis ANOVA, $p<0.01$). Furthermore, we found that very high concentrations of NCS-1 (10 uM) reduced or blocked gamma band oscillations in these cells. NCS-1 at 10 uM concentration first remained the same or increased slightly, but decreased oscillations in PPN neurons induced by 2 sec ramps as it diffused into the cell ~15 min. These findings suggest the modulation by NCS-1 of calcium channels presumably located in the dendrites of PPN neurons that mediate gamma band oscillations. Based on these results, 1 uM NCS-1 seems to be the most critical concentration for gamma oscillation modulation, whereas 10 uM would be an excessive level in keeping with significant over expression. These results suggest that NCS-1 over expression may be responsible for the decrease in gamma band activity present in schizophrenia and bipolar disorder patients.

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Poster

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Support: NIH awards R01 NS20246 and P20 GM103425

Title: Mechanisms behind gamma band activity in the pedunculopontine nucleus (PPN)

Authors: ***B. R. LUSTER**[1], J. HYDE[1], S. DONOFRIO[1], F. J. URBANO[2], E. GARCIA-RILL[1]
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Abstract: The pedunculopontine nucleus (PPN) is a component of the brainstem Reticular Activating System (RAS), and is active during waking and paradoxical sleep. High frequency, especially beta/gamma band activity drives our cognitive function during waking and REM sleep, two markedly different states of awareness. Previous results show that every cell in the PPN plateaus at gamma band frequencies and this high frequency activity is mediated by high threshold, voltage-dependent N- and P/Q-type Ca^{2+} channels. However, these results do not

indicate whether some PPN cells manifest this high frequency activity through only P/Q-type Ca^{2+} channels or only N-type Ca^{2+} channels. The proposed studies were designed to determine whether some PPN cells have only N-, only P/Q-, or both N- and P/Q-type Ca^{2+} channels, and determine how many have neither one of these. Sagittal slices containing the PPN were cut at 400 μm from 9-17 day rat pups, an age at which developmental changes have shifted and plateaued. Slices were recorded and perfused with oxygenated aCSF in a perfusion chamber containing the synaptic blockers (SB): gabazine ($GABA_A$ antagonist), strychnine (glycine antagonist), 6-cyano-7-nitroquinoxaline-2,3-dione (AMPA/kainate receptor antagonist), and APV (NMDA receptor antagonist), and also Tetrodotoxin (TTX) to block sodium channels. Whole cell patch-clamp experiments (current and voltage clamp modes) were performed at body temperature (~36.5°C) using a Multiclamp 700B amplifier. Capacity transients were cancelled using computer-controlled circuitry and series resistance was compensated in all experiments (>35%, ranging from 5-16). PPN neurons were identified as to cell type (type I has LTS, type II has I_A, type III has LTS+I_A) using voltage and current clamp I-V curves. We found that all rat PPN cell types (n=50) showed gamma oscillations in the presence of SB+TTX when membrane potential was depolarized using current ramps. PPN neurons showed gamma oscillations when voltage-clamped at holding potentials above -30 mV. The N-type calcium channel blocker CgTx partially reduced gamma oscillations (decrease of 34% compared to control, $p>0.05$), while the P/Q-type blocker AgA abolished the remainder. Both ω-CgTx and ω-Aga blocked voltage-dependent calcium currents. These results suggest that all cells in the PPN have both N- and P/Q-type Ca^{2+} channels, and that voltage-dependent P/Q- and, to a lesser extent, N-type calcium channels mediate gamma oscillations in the PPN.

Disclosures: B.R. Luster: None. **J. Hyde:** None. **S. Donofrio:** None. **F.J. Urbano:** None. **E. Garcia-Rill:** None.

Poster

257. Sleep

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 257.21/NN34

Topic: E.08. Biological Rhythms and Sleep

Support: NIMH Grant R01MH099231

Title: Sleep repairs DNA double-strand breaks that normally occur during wake

Authors: *M. BELLESI, G. TONONI, C. CIRELLI
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Abstract: Sleep deprivation leads to cognitive impairments in humans and, if sustained for 2-3 weeks in rodents, it is invariably fatal. It has been suggested that neural activity associated with wake, if it is not interrupted by periods of sleep, may damage brain cells through excitotoxic or oxidative mechanisms and eventually lead to cell death, but so far direct evidence for this hypothesis has been lacking. Recently, it has been shown that physiological brain activity associated with exploration of a novel environment induces DNA double-strand breaks (DSBs) in neurons of young adult wild-type mice. Of note, the number of neurons displaying DBS foci returned to baseline levels after a 24-hour recovery period, in which mice were allowed to recover in their home cages. Here we investigated whether sleep plays an active role in repairing DNA DSBs in cortical cells of mice. One well-recognized marker of DSBs is the phosphorylation of the histone protein H2A variant X at serine 139, which can be reliably identified by using antibodies against γH2A.X. Brains of adult (10 weeks old) C57Bl/6J mice was collected after 6-8h of sleep during the day (S, n=6 mice), 6h of sleep deprivation (SD, n=6 mice) by exposure to novel objects starting at light onset, 6h of recovery sleep after 6h of SD (SDRS, n=5 mice), and 6h of enforced locomotion (without exploration of novel objects) after 6h of SD (SDSD, n=4 mice). Three coronal sections of frontal cortex for each animal were stained with antibodies against γH2A.X and counter-stained with propidium iodine to identify cell nuclei. Cells containing at least one γH2A.X-positive focus in the nucleus were scored as positive. Quantitative analysis showed that SD mice displayed a higher density of DSB foci in brain cells relative to S mice (SD: 258±40.7 cells with foci/mm3, S: 190±15.4 cells with foci /mm3, p=0.003). SDRS mice showed decreased DSB foci relative to both SD and SDSD mice (SDRS: 180±43.9 cells with foci /mm3, SDSD: 280.9±63.5 cells with foci /mm3; SDRS vs SD, p=0.01; SDRS vs SDSD, p=0.026), whereas differences between S and SDRS or SD and SDSD were not significant. These results confirm that in the cerebral cortex a period of exploration during wake induces DSBs. Moreover, they demonstrate that after induction by wake exploration, the subsequent decline in DSBs requires sleep, and not simply the passage of time.

Disclosures: **M. Bellesi:** None. **G. Tononi:** None. **C. Cirelli:** None.

Poster

257. Sleep

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 257.22/NN35

Topic: E.08. Biological Rhythms and Sleep

Support: Wellcome Trust

MRC G0800399 and G0901892

BBSRC BB/K018159/1

Title: Histaminergic neurons at the intersection of circadian rhythms and the sleep wake cycle

Authors: *X. YU[1], A. ZECHARIA[1], Z. ZHANG[1], Q. YANG[1], R. YUSTOS[1], P. JAGER[2], A. L. VYSSOTSKI[3], E. S. MAYWOOD[4], J. E. CHESHAM[4], S. G. BRICKLEY[1], M. H. HASTINGS[4], N. P. FRANKS[1], W. WISDEN[1]
[1]Imperial Col. London, London, United Kingdom; [2]Univ. Col. London, London, United Kingdom; [3]Univ. of Zurich/ETH Zurich, Zurich, Switzerland; [4]Cambridge Biomed. Campus, Cambridge, Cambridge, United Kingdom

Abstract: Circadian clocks are widely expressed in the brain, yet their functions are unknown. Here we show that one local clock regulates the expression of histidine decarboxylase (HDC), the enzyme producing the "arousal transmitter" histamine in hypothalamic neurons. The level of this enzyme varies with time of day, being highest at the start of lights off (ZT12), and is up-regulated by sleep deprivation. We disrupted this local clock by using *HDC-Cre* recombinase to delete BMAL1, the transciption factor central to circadian rhythms, selectively in histaminergic neurons, generating *HDC-Bmal1* KO mice. *Hdc* gene expression in *HDC-Bmal1* KO mice showed a disrupted 24-hour rhythm: with reduced amplitude and advanced phase peaking at ZT08, and with *hdc* transcript levels overall higher. This greatly affected natural sleep, with changes in the distribution of wakefulness and sleep during the light-dark cycle, as well as changes in the transitions between the different sleep states. In addition to this change in sleep architecture, recovery sleep following sleep deprivation was also substantially changed, with *HDC-Bmal1* KO mice having a much reduced sleep rebound. This correlated with reduced memory: after sleep deprivation *HDC-Bmal1* KO mice performed less well than controls in object recognition. In summary, we have identified a local "histaminergic clock" that regulates HDC levels, and is necessary for maintaining appropriate sleep-wake cycle architecture.

Disclosures: **X. Yu:** None. **A. Zecharia:** None. **Z. Zhang:** None. **Q. Yang:** None. **R. Yustos:** None. **P. Jager:** None. **A.L. Vyssotski:** None. **E.S. Maywood:** None. **J.E. Chesham:** None. **S.G. Brickley:** None. **M.H. Hastings:** None. **N.P. Franks:** None. **W. Wisden:** None.

Poster

257. Sleep

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Program#/Poster#: 257.23/NN36

Topic: E.08. Biological Rhythms and Sleep

Support: BBSRC

Title: Genetically mapping the sites of zolpidem-induced sleep reveals that histaminergic neurons are key targets

Authors: *D. S. UYGUN[1], A. ZECHARIA[1], Z. YE[1], X. YU[1], R. YUSTOS[1], A. ZYSSOTSKI[2], S. BRICKLEY[1], N. FRANKS[1], W. WISDEN[1]
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Abstract: Zolpidem, a positive allosteric modulator of the GABAA receptor, is a popular drug for treating insomnia, and works at α1βγ2, α2βγ2 and α3βγ2 type receptors. It is not known, however, if zolpidem enhances IPSCs in selective neuronal types to induce sleep, or if it just induces a general inhibition throughout the CNS, which then non-specifically produces sleep. A phenylalanine at position 77 of the mature γ2 subunit forms part of the zolpidem binding site. Mutation of F77 to isoleucine (γ2I77) abolishes zolpidem's actions. We are interested in which brain regions confer zolpidem's ability to sedate. To investigate this, we introduced γ2F77 subunits to specific brain regions of γ2I77 mice. First, a Cre-dependent γ2F77 transgene was expressed in the histaminergic hypothalamic area of γ2I77lox mice, enabling γ2F77 subunit expression exclusively in histaminergic neurons. Second, we genetically swapped γ2F77 subunits in the frontal neocortex of γ2I77lox mice. We assayed zolpidem-mediated sleep in both groups using behavioural criteria and EEG analysis. In C57bl6 wild-type mice, harbouring the wild-type γ2F77 subunit, zolpidem (5 mg/kg) injection produced sleep as assessed from the EEG, with a latency of approx. 2 minutes. In γ2F77I mice, however, zolpidem (5 mg/kg) produced no effect within the first half hour: the mice were zolpidem-insensitive. In mice with zolpidem sensitivity selectively placed into histaminergic neurons, zolpidem caused sleep onset at approx. 10 minutes after injection. We found that this is approximately the same sleep latency produced with an H1 histamine receptor antagonist, pyrilamine (approx. 12 minutes). This suggests that a considerable amount of zolpidem's sedative effects arise from blocking histaminergic neurons. Similarly, for frontal cortex "γ2 swap" mice, zolpidem also caused sleep onset after approximately 15 minutes. Our results demonstrate that both neurons in the frontal cortex and histaminergic neurons in the hypothalamus and are key targets of zolpidem-mediated sleep induction.

Disclosures: **D.S. Uygun:** None. **A. Zecharia:** None. **Z. Ye:** None. **X. Yu:** None. **R. Yustos:** None. **A. Zyssotski:** None. **S. Brickley:** None. **N. Franks:** None. **W. Wisden:** None.

Poster

257. Sleep

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 257.24/OO1

Topic: E.08. Biological Rhythms and Sleep

Support: NIMH grant R01MH099231

Title: Ultrastructural signatures of sleep and wake in neuronal cell bodies of mouse frontal cortex

Authors: *L. DE VIVO, G. TONONI, C. CIRELLI
Dept. of Psychiatry, Univ. of Wisconsin, Madison, WI

Abstract: Cortical activity and brain metabolism are higher during wake than during NREM sleep, and whole cortex transcriptomics studies show that sleep and wake are associated with the upregulation of genes belonging to different, often complementary functional categories. Yet, it remains unknown whether there are ultrastructural signatures that can distinguish sleep and wake at the single neuron level. Here we searched for those signatures by using electron microscopy to measure number and size of mitochondria, resting and active lysosomes, lipofuscins and multivesicular bodies (MVBs) in the cell body of layer II pyramidal neurons. Organelles were examined in frontal cortex (20 cell bodies/mouse) of YFP-H adolescent mice (1 month old) after: i) 6-8 hrs of sleep (S, n=4 mice); ii) 6-8 hrs of acute sleep deprivation (SD, n=4 mice); iii) 5 days of chronic sleep restriction (sleep reduced to ~30% of baseline values) enforced using forced locomotion and exposure to novel objects (CSR, n=5 mice); iv) more than 32 hrs of recovery sleep after chronic sleep restriction (RS, n=4 mice). Mitochondria. In CSR a shift of the entire mitochondrial population toward bigger sizes was observed relative to S ($p=0.0022$), together with the presence of extra large mitochondria. Some of these changes persisted even after several hours of RS. Lysosomes. After acute SD, fewer but bigger resting lysosomes were observed relative to S (decreased density $p=0.0024$, increased size $p<0.0001$;), while after CSR resting lysosomes did not differ from S. By contrast, active lysosomes were smaller but more numerous after CSR than after S ($p<0.0001$), and they were bigger after SD than after S ($p<0.0001$). Lipofuscins. Lipofuscins granules were rare in adolescent mice (33 granules in total), but half of them were found in the cells of CSR mice (16 granules in 5 mice), while only 6 granules were identified in S (2 mice) and RS (2 mice), and 5 in acute SD (4 mice). MVBs. The density of all MVBs was lower in CSR relative to SR ($p=0.0062$). In particular, endosomal compartments with

less than 2 intra-luminal vesicles were significantly less numerous in CSR relative to S (p=0.0116) and RS (p=0.0001), while the density of endosomal compartments with 2 or more vesicles was lower after acute SD relative to S (p=0.032) and RS (p=0.025). In S and RS, 10% of all MVBs underwent an invagination and a similar percentage underwent back-fusion. After both acute SD and CSR, an increase in the percentage of back-fusing MVBs was observed. These data show that frontal cortex pyramidal neurons respond to acute and chronic sleep loss by changing the size and/or the density of organelles implicated in energy production and in the turnover of cellular components.

Disclosures: L. de Vivo: None. **G. Tononi:** None. **C. Cirelli:** None.

Poster
[Unable to Attend]

257. Sleep

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 257.25/OO2

Topic: E.08. Biological Rhythms and Sleep

Support: VA Grant BX000729

Title: Projections from dorsal deep mescencephalic nucleus (dDpMe) to nucleus pontis oralis (PnO) utilize inhibitory and excitatory neurotransmitters

Authors: *G. A. MARKS[1,2], C.-L. LIANG[1,2]
[1]Res., VA North Texas Hlth. Care Syst., DALLAS, TX; [2]Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Both lesion and pharmacological inhibition of neurons in dDpMe resulted in an induction of REM sleep. The sublaterodorsal nucleus (SLD) also has been identified in the rat as a REM sleep induction zone partly due to the action of locally applied GABAa receptor antagonists inducing a short latency onset of a REM sleep-like state. It has been hypothesized that a critical source of GABA to SLD underlying the negative control of REM sleep is from the dDpMe. A long-lasting increase in REM sleep also resulted from application of GABAa antagonists to PnO. This GABAa receptor mediated REM sleep increase is blocked by the cholinergic receptor antagonist atropine. We previously published a study of the dDpMe projection to SLD utilizing orthograde tract-tracing, immunohistochemistry and confocal microscopy. Here utilizing the same techniques and tracer ejections, we examined dDpMe

innervation of PnO. Male, Long-Evans Hooded rats (N=4) underwent stereotaxic surgery in which the orthograde tracer biotinylated dextran amine (BDA, 10k) was iontophoretically ejected into the dDpMe to visualize varicose axon fibers in ipsilateral PnO. Six days later, rats were intracardialy perfused with fixative under pentobarbital anesthesia and coronal sections (20 um) obtained through the PnO. Tissue was prepared for fluorescence immunohistochemistry and visualized with laser scanning, confocal microscopy (LSM 510). Antibodies against the vesicular GABA transporter (VGAT) and vesicular glutamate transporter-2 (VGLUT2) were used to visualize glycine/GABA and glutamate presynaptic varicosities, respectively. These preliminary data yielded a sampled mean of 215.4 BDA labeled varicosities per section of PnO (12 fields/section). Significantly greater VGLUT2 double labeled varicosities were observed (36.5±11.1) than double-labeled VGAT (27.5±9.8)(t-test for dependent means; r=0.996). The four ejection sites in dDpMe resulted in approximately 50% more BDA labeled varicosities in SLD than in PnO, and 200% more for both VGLUT2 and VGAT double labeled varicosities in SLD than in PnO. At least some VGAT double labeled varicosities were observed apposed to the membrane of presynaptic cholinergic terminals in PnO. We hypothesize interactions among dDpMe, SLD and PnO constitute an important part of the brainstem REM sleep control network. GABAergic neurons in dDpMe may act to modulate acetylcholine release in PnO to promote and inhibit REM sleep. Glutamatergic dDpMe neurons may act in PnO to promote wakefulness.

Disclosures: G.A. Marks: None. **C. Liang:** None.

Poster

257. Sleep

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 257.26/OO3

Topic: E.08. Biological Rhythms and Sleep

Support: VA Medical Research

MH55772

NS79940

NS84477

Title: Optogenetic activation of melanin-concentrating hormone (MCH) containing neurons powerfully increases sleep in rats

Authors: *C. A. BLANCO-CENTURION[1], D. PELLURU[1], R. KONADHODE[1], M. LIU[1], A. VAN DEN POL[2], P. SHIROMANI[3]
[1]Psychiatry and Behavioral Sci., Med. Univ. of South Carolina, Charleston, SC; [2]Yale University. Sch. of Med., New Haven, CT; [3]Ralph Johnson VAMC, Charleston, SC

Abstract: MCH neurons are the only phenotype of neurons that induce both NREM and REM sleep in response to optogenetic stimulation (Jego, Glasgow et al. 2013, Konadhode, Pelluru et al. 2013). Indeed, sleep is induced against a strong circadian waking drive suggesting that this is a principal group responsible for sleep (Konadhode, Pelluru et al. 2013). The initial studies were performed in mice, and for the results to be valid the sleep induction must be replicated in other species. Therefore, the present study used a proven MCH promoter (Van den Pol, Acuna-Goycolea et al. 2004) to insert the gene for channelrhodopsin-2 in MCH neurons in rats. CSF levels of MCH and orexin were also measured at 6h intervals to determine oscillations of these peptides in conjunction with sleep-wake. Ten Long-Evans rats were administered rAAV-MCHp-ChR2-EYFP bilaterally in the lateral hypothalamus (LH), and implanted with sleep-wake electrodes and bilateral fiber optic probes (400 μm) in the LH. Subsequently, at the start of the lights-off (night) period the rats were stimulated with 5, 10 or 30 Hz (random order) of blue light pulses (10 msec duration). The light pulses were delivered for 1 minute every 5 minutes for 24h, and 48h elapsed between the three stimulation rates. Throughout the experiment the rats were maintained on a 12-12h light-dark cycle with food and water available ad libitum. We found that at night optogenetic activation of MCH neurons (10 Hz) significantly increased both NREMS ($P=0.002$) and REMS ($P = 0.014$). Sleep was increased because the wake bouts were shortened (-50%) and the number of both NREMS (>150%) and REMS (>300%) bouts increased. During the day the optogenetic activation of MCH neurons at 5 Hz significantly increased REM sleep ($P<0.02$) During the day when the stimulation train occurred during NREMS, REMS was promptly induced (>200%) although the length of the REMS bout was not increased. In a separate group of rats the activity of single neurons was recorded in freely behaving rats in conjunction with optogenetic stimulation. Light directly activated some neurons and decreased the activity of wake-active neurons. CSF levels of orexin and MCH were opposite each other and correlated with the sleep-wake cycle. Our studies have shown that all of the MCH neurons need not be activated to induce both NREM and REMS. This indicates the potency of MCH neurons in suppressing the action of all arousal neurons. By driving sleep in another specie that sleeps, these results cement the status of MCH neurons as sleep neurons in network models of sleep-wake regulation. Pharmacological activation of MCH neurons could potentially counteract insomnia.

Disclosures: C.A. Blanco-Centurion: None. **D. Pelluru:** None. **R. Konadhode:** None. **M. Liu:** None. **A. Van den Pol:** None. **P. Shiromani:** None.

Poster

257. Sleep

Location: Halls A-C

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Program#/Poster#: 257.27/OO4

Topic: E.08. Biological Rhythms and Sleep

Support: VA Medical Research

MH55772

NS79940

NS84477

Title: Optogenetic activation of MCH neurons simultaneously with DREDD-induced inhibition of LC neurons has an additive effect in enhancing both NREM and REM sleep

Authors: *P. J. SHIROMANI[1], E. VAZEY[2], D. PELLURU[2], R. KONADHODE[2], M. LIU[2], G. ASTON-JONES[2], C. BLANCO CENTURION[2]
[1]Psychiatry, VA - Med. Univ. of South Carolina, Charleston, SC; [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: New genetically engineered tools, such as optogenetics and DREDD, permit specific phenotypes of neurons to be manipulated. We optogenetically activated neurons containing melanin concentrating hormone (MCH) and found a robust increase in both NREM and REM sleep (Konadhode et al. 2013). In particular, the increase in sleep was induced at night when the animals were awake indicating that MCH neuron activation can be driven to suppress the activity of all of the arousal neurons. There are a number of phenotypes of arousal neurons and to identify where MCH neurons are most effective we combine optogenetics and DREDD. We start with the LC where the noradrenergic neurons are active during waking and virtually silent during sleep, especially REM sleep. We hypothesize that MCH neuron activation combined with LC inhibition would have an additive effect on sleep. In tyrosine hydroxylase-Cre Long-Evans rats (gift from Diesseroth) the LC neurons were targeted by directly injecting rAAV-hSyn-DIO-hM4D(Gi)-mCherry in the LC. This delivered the inhibitory DREDD receptor to TH-Cre neurons in the LC. In the same rats, rAAV-MCH-ChR2-EYFP was injected bilaterally into the lateral hypothalamus. This delivered the excitatory light-activated ChR2 into MCH neurons (see Konadhode et al., 2013). The experiments were started three weeks later at lights-off. Initially, baseline sleep recordings were made (12:12 light on cycle). Then light stimulation (473 nm light; 10ms duration pulse; 1 minute on every 5 minutes for 5h; 5Hz), vehicle (IP injection), CNO

(3mg/kg;IP), or CNO+light was administered. Preliminary data are in 3 rats employing a repeated measures design. Sleep during the first 6h was analyzed based on the time-course of CNO's effect. During the first two hours inhibition of LC (DREDD) by itself did not induce NREM or REMS. However, during this same time period MCH neuron activation (optogenetics)+LC inhibition (DREDD) increased NREM and a four-fold increase in REMS indicating an additive effect. In the 3rd hour LC inhibition by itself did not induce REMS whereas MCH neuron activation+LC inhibition increased REMS two-fold. It was not until the 4th hour that LC inhibition by itself increased REMS. These results demonstrate that MCH neuron activation coupled with inhibition of target arousal neurons can be used to ferret out the target site where MCH neurons most potently act to initiate NREM and/or REMS. The time-course of increase in NREM and REMS can serve to identify the relative contribution of specific arousal neurons in the sleep process. This has advantage over method referred to as ChR2 circuit mapping (CRACM) which involves stimulating ChR2 containing terminals at target sites.

Disclosures: P.J. Shiromani: None. **E. Vazey:** None. **D. Pelluru:** None. **R. Konadhode:** None. **M. Liu:** None. **G. Aston-Jones:** None. **C. Blanco Centurion:** None.

Poster

257. Sleep

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 257.28/OO5

Topic: E.08. Biological Rhythms and Sleep

Support: VA Medical Research

MH55772

NS79940

NS84477

Title: Optogenetic activation versus inhibition of melanin-concentrating hormone (MCH) containing neurons in MCH-Cre mice

Authors: *R. KONADHODE[1], D. PELLURU[2], C. BLANCO-CENTURION[2], M. LIU[2], A. VAN DEN POL[3], P. SHIROMANI[4]
[2]Dept. of Psychiatry, [1]Med. Univ. of South Carolina, Charleston, SC; [3]Dept. of Neurosurg., Yale Univ. Sch. of Med., New Haven, CT; [4]Ralph H. Johnson VA Med. Ctr., Charleston, SC

Abstract: In current models of sleep-wake regulation only one group - the VLPO, is listed as driving sleep. However, their status in the sleep process is unknown since these neurons have never been selectively manipulated. There are other neurons that become active during sleep (Hassani et al. 2009).One such phenotype contains MCH and it is intermingled with orexin neurons in the lateral hypothalamus. Last year we discovered that optogenetic activation of MCH neurons in WT mice robustly increased both NREM and REM sleep (Konadhode et al. 2013). The effects were evident during the animal's normal waking cycle indicating that the MCH neurons can effectively silence all of the arousal neurons. In our published report we used an MCH promoter to insert channelrhodopsin 2 (ChR2) into the MCH neurons. The advantage of this approach is that it opens explorations into other animals besides mice (see adjacent poster), but not all MCH neurons may contain the light-sensitive opsin. To optogenetically manipulate a larger percentage of MCH neurons we test our hypothesis in MCH-Cre mice. Independent groups of MCH-Cre mice were administered either excitatory (rAAV-EF1a-DIO-hChR2(H134R)-EYFP) or inhibitory (rAAV-EF1a-DIO-eArch3.0-EYFP) opsins into the LH and implanted with sleep recording electrodes. Three weeks later a 48 h baseline sleep recording was obtained (0 Hz). At the start of the lights-off (night) period the mice were stimulated with 5, 10 or 30 Hz (random order) of blue or yellow light pulses (10 msec duration). The light pulses were delivered for 1 minute every 5 minutes for 24h, and 36h elapsed between the three stimulation rates. Activation of MCH neurons (10Hz) for the 12h night period significantly decreased waking ($P<0.009$; n=6), increased NREM ($P<0.014$) and REMS ($P<0.049$). Activation during the second half of day cycle had no effect on NREM or REMS. Both these results in MCH-Cre mice are consistent with and support our report in WT mice. Preliminary data analysis indicates a trend to sleep less and more waking in response to inhibition of MCH neurons. Histology is pending and will identify the number of MCH neurons containing the reporter gene, a proxy marker of the light-sensitive opsins. Our combined studies in WT rats (Blanco-Centurion et al. sfn abstract 2014) and mice (Konadhode et al., 2013), and in MCH-Cre mice (this study) demonstrate conclusively that activation of MCH neurons at night shortens wake bouts, hastens sleep onset and increases the percent of both NREM and REMS. Pharmacological activation of MCH neurons could potentially treat insomnia.

Disclosures: R. Konadhode: None. **D. Pelluru:** None. **C. Blanco-Centurion:** None. **M. Liu:** None. **A. Van den Pol:** None. **P. Shiromani:** None.

Poster

258. Sleep: Systems and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 258.01/OO6

Topic: E.08. Biological Rhythms and Sleep

Support: Icelandic Technological Devlopment Fund 131828-0611

Title: High throughput behavioral sleep assay for larval zebrafish

Authors: *K. KARLSSON, Dr.[1], A. ÁGUSTSDÓTTIR[2], H. THORSTEINSSON[2]
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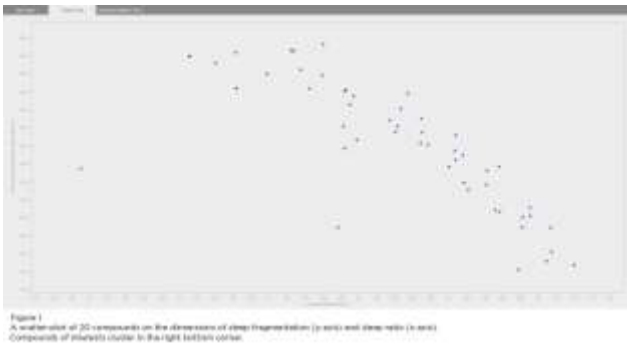
Abstract: Currently we describe a set-up for automated parallel 2304 larval zebrafish sleep-wake recording and sleep-wake modulating drug screening. Fertilized embryos are collected using a custom built embryo collector that yields up to 5000 embryos a day. The embryos are incubated at 28.5° C for 120 hrs and then placed in 96 well-plates by a custom built dispenser which utilizes a cost-effective break beam detection system. Next, twenty-four 96 well-plates are placed in light and temperature controlled recording apparatus which is fitted with 24 separate cameras for behavioral data collection and a microfluidics dispenser. After a 24 hr acclimation period, drugs are dispensed to the wells, and the larvae are recorded for 24 hrs. In a typical run each group comprises 16 larvae. Each drug is applied in three dose sizes, in addition to 3 control groups (47 different drugs in a single run). Behavioral data is collected using the Noldus Ethovision image analysis module but analyzed for sleep percentage, sleep fragmentation, average sleep and wake bout length, and sleep latency, using custom written software. Following automated analysis a report is generated highlighting drugs of interest. Future developments include adaptation of the assay to other CNS disorders with a behavioral phenotype such as ALS and epilepsy.



Disclosures: **K. Karlsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 3Z Pharmaceuticals. **A. Águstsdóttir:** None. **H. Thorsteinsson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 3Z Pharmaceuticals.

Poster

258. Sleep: Systems and Behavior

Location: Halls A-C

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Program#/Poster#: 258.02/OO7

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant OD011185

NIH Grant HG006332

Title: Analysis of sleep and wake in the KOMP2 mouse population using an improved non-invasive, automated sleep phenotyping system

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Abstract: We developed a noninvasive, high-throughput piezoelectric system that distinguishes sleep and wake in mice with high accuracy. A piezoelectric sensor placed at the bottom of the mouse cage records gross body movements, while an automatic classifier analyzes signal features and differentiates sleep from wake. Sleep signals are characterized by specific features of the signal waveform when the dominant motion is the mouse breathing rhythm. The classifier correlates approximately 95% with EEG and 90-95% with human observation. This system has been used in a variety of studies, including quantitative trait loci mapping of sleep-related traits, as well as characterizing sleep in mouse models of Alzheimer's disease, traumatic brain injury, and other disorders. We are expanding the system to identify other behaviors such as grooming and locomotion, as well as being able to differentiate REM from NREM sleep. Quality control is an important factor in high-throughput systems. In the piezoelectric system the signal may be erroneous for a variety of reasons, such as faulty wire connections, electronic interference, and mice with weak or unusual breathing patterns. In the past, assessments of signal quality were subjective. Recently we developed a more robust quantitative measure of signal quality, which takes into account the distribution and response of decision statistics over multi-day recordings and assigns a score from 0 to 1. A higher score indicates that the mouse sleep and wake behaviors generated more distinct decision statistics. Then we use a threshold for rejecting poor quality signals. The data confidence metric was developed to provide a well-defined signal quality measure for the Knockout Mouse Phenotyping Program (KOMP2), which aims to characterize a variety of physiological and behavioral phenotypes in over 5000 single-gene

knock-out mouse lines during this phase of the project. The piezoelectric sleep phenotyping system is part of The Jackson Laboratory pipeline, which characterizes over 200 phenotypes. The data from this pipeline can be used to elucidate the physiological and behavioral effects of single genes, as well as find correlations and connections between different genes and phenotypes. We present the results of the sleep phenotyping performed thus far in this project.

Disclosures: **M. Striz:** F. Consulting Fees (e.g., advisory boards); Signal Solutions, LLC. **N. Cole:** None. **J. Ashley:** F. Consulting Fees (e.g., advisory boards); Signal Solutions, LLC. **J.M. Denegre:** None. **K.D. Donohue:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **E.J. Chesler:** None. **K.L. Svenson:** None. **B.F. O'Hara:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC.

Poster

258. Sleep: Systems and Behavior

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 258.03/OO8

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant 1R43MH098595

Title: A system for applying sleep deprivation to a single animal in a group-housed environment

Authors: *D. A. JOHNSON, H. P. HARMON, S. GABBERT, E. L. AKERS, D. V. AILLON, D. A. JOHNSON, P. A. PETILLO, E. NAYLOR
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Abstract: Social isolation of rodents results in physiological deficiencies including altered sleep patterns. Characterization of the beneficial effects of social housing compared to social isolation for sleep and sleep-related disorders is difficult. For example, sleep deprivation of a single animal within a group housed setting requires tracking and identification of multiple animals, and the selective deprivation of the target animal with minimal interference to others. We designed an automated grouped-housing system that facilitates a variety of interventions to a single animal within a social setting, including the ability to selectively sleep deprive individual members of an experimental cohort. Animal movement was tracked using high-definition video cameras. RFID tags were used to differentiate animals when video tracking was lost, usually

when animals slept in piles. Sleep deprivation was achieved using a robotic arm that simulated gentle handling via a 0.75" diameter spring-loaded cylinder at the end of the arm. Evaluation of the total amount of sleep was based on inactivity automatically measured from the video stream. In real time, when the software indicated that the target animal was inactive (no motion greater than a 5 pixel change over 40 seconds), the animal was gently nudged by the robot arm until movement was resumed. Two independent trials successfully tracked a single rat within its cohort over a three day period and selectively sleep deprived this target animal. All animal studies were performed at the University of Kansas Animal Care Facility and approved by the University of Kansas IACUC. Two cohorts of four rats were implanted with RFID tags and socially housed in a 2' x 2 'x 1.5' cage with food and water available ad lib. After recording 24 hours of baseline activity, six hours of sleep deprivation was applied ending at lights off, which was followed by 24 hours of recovery. The amount of sleep following the end of the deprivation period was compared to the sleep measured from the same animal during the equivalent time period of the baseline day. In cohort 1, the target animal increased sleep time during recovery by 65%, while the target animal in cohort 2 increased sleep time by 22%. In both cohorts, the number of sleep bouts increased by about 40%. The grouped-housing system proved successful in depriving a specific animal within a cohort of four, and demonstrates the utility of the design to effectively apply selective interventions within a social environment. **SUPPORT:** This research was supported by NIH grant #1R43MH098595.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH DP2-OD006454

Title: Characterizing the continuous behavioral and physiological dynamics of the sleep onset: A novel behavioral and computational approach

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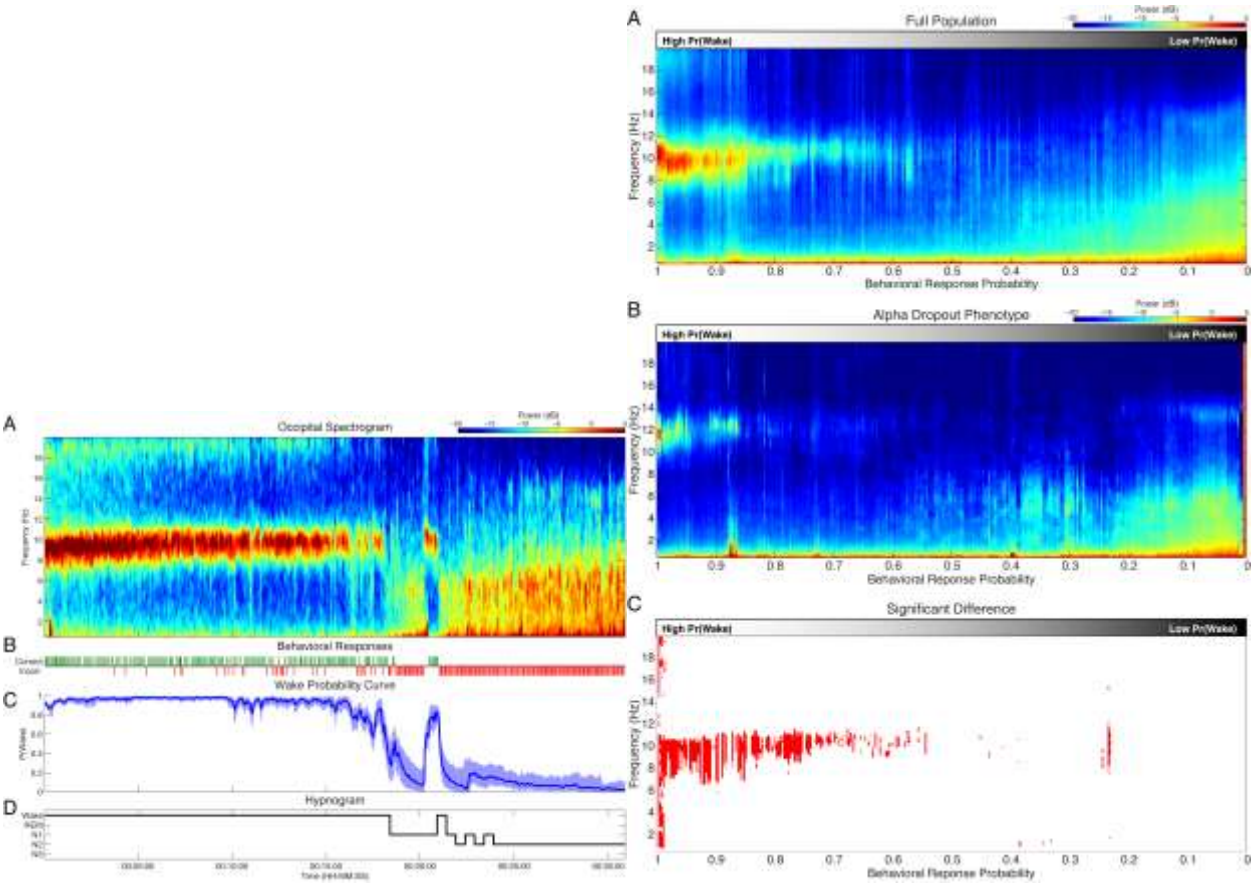
Abstract: The act of falling asleep is a continuous, dynamic process involving multiple behavioral and physiological systems. Standard methods for characterizing the sleep onset process (SOP) do not incorporate current knowledge of the neurophysiological dynamics governing the wake/sleep transition, and fail to integrate information from both behavioral and physiological data. To keep pace with our scientific understanding, we developed a physiologically-principled model of the SOP within a statistically-principled Bayesian framework. Our empirical model of the SOP integrates information from both behavioral and physiological observations, combining simultaneously recorded EEG, EMG, and behavioral task data to estimate the instantaneous probability that a subject is awake. We fit the model to the EEG, EMG, and respiratory data from 10 healthy subjects. As subjects fell asleep, they performed a novel task, which uses breathing as a prompt for behavioral response and thus removes the need for any arousing external sensory stimuli. The model output successfully captured the continuous dynamics of the SOP, significantly outperforming standard clinical metrics in predicting behavior. By performing a cross-subject alignment of EEG activity with respect to response probability, we revealed a subset of subjects with significantly lower alpha power compared to the other subjects at identical levels of wakefulness.



Disclosures: **M.J. Prerau:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Prerau has patents pending on sleep data analysis. **K. Hartnack:** None. **G. Obergon:** None. **A. Sampson:** None. **M. Bianchi:** None. **J. Ellenbogen:** None. **P. Purdon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Purdon has patents pending on sleep analysis.

Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant HD062618

NIH Grant HD062864

Title: Validation of an assessment strategy to accurately measure sleep in non-human primates from actigraphy data

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Abstract: Omnidirectional accelerometers (i.e. actigraphy) are widely used to measure activity levels in humans and animals. Because activity levels are highly correlated with sleep-wake patterns, actigraphy has also proved a useful noninvasive means of characterizing sleep. However, established algorithms and scoring criteria for assessing sleep from actigraphy have only been developed and validated for humans. The goal of this study was to identify and validate an algorithm to identify sleep in non-human primates from actigraphic measurement. In our study, adult female cynomolgus monkeys (Macaca fascicularis) wore collars housing accelerometers (Actical®, Philips Respironics Inc., Pittsburgh PA). Activity counts were recorded in 60 second epochs and downloaded with Actiware® software. Behavioral sleep was monitored continuously for 18 hours from late afternoon until mid-morning in each animal using infrared videography. Videos were scored frame-by-frame as either sleep or wake by three scorers (inter-rater reliability was 0.94). For sleep in monkeys, we tested a range of scaling multipliers for the Actiware algorithm, seeking out the highest ratio of sensitivity to specificity in the monkey data. The sensitivity of the original, unaltered Actiware algorithm to detect video-defined monkey sleep was 95.8%, but the specificity was only 46.3%. Kappa was k=0.49, but the low specificity means that there was a tendency to misidentify wake, further evidenced by a 35.9% specificity during the night that only rose to 48.8% during the day. After optimizing the equation for cynomolgus monkeys using a series of different scaling multipliers, the best multiplier for the night (lights off) period was M = 1.4, that resulted in a sensitivity of 74.4% and a specificity of 74.3%. Kappa for this form of the equation was k=0.5. The best multiplier for the day (lights on) period was M=0.08, which resulted in a sensitivity of 72.1% and a specificity of 70.5%, with an overall kappa of k=0.57. Thus, different parameters appear to be necessary to accurately determine sleep-wake states in the monkey in the night vs. day from actigraphy, reflecting differences in activity in these two time periods. By using monkey-optimized algorithms, specificity of sleep determined from actigraphic measures is considerably improved compared to the algorithm optimized for humans.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: TrygFonden Charitable Foundation, Denmark

BIAL Foundation 220/12

Title: Using whole-brain computational modelling for identifying hubs necessary for transitioning between sleep stages measured with MEG

Authors: *A. B. STEVNER[1,3], G. PIANTONI[4], G. COLCLOUGH[2], M. WOOLRICH[2], C. PARSONS[1,5], J. CABRAL[1,5,6], E. VAN SOMEREN[7], Y. VAN DER WERF[7], G. DECO[6], M. L. KRINGELBACH[1,5]
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Abstract: Sleep in normal adults is characterised by highly consistent state-transitions in the brain over time. Compared to the descent to sleep, which is, at least partly, a voluntary act, the switching between sleep stages appears almost mechanistic. The temporal order and relationship between the brain states of various sleep stages are remarkably constant. Describing the whole-brain activity of individual sleep stages was one of the first merits of electroencephalography (EEG), and more advanced forms of neuroimaging have expanded our understanding of the spatiotemporal unfolding of sleep. Yet, the mechanisms underlying, and brain regions orchestrating the transitions between wakefulness and the various sleep states remain unresolved. Understanding this may lead to important insights into not only the fundamental principles of the human brain function but also the causes of sleep disorders. Viewing the brain as an intricately connected network, in which activity occurs as a result of communication between parts of this network has helped the investigation of spontaneous brain activity. By combining analysis of structural imaging data, such as diffusion tensor imaging (DTI), and functional imaging data, such as functional Magnetic Resonance Imaging and magnetoencephalography (MEG), computational modelling has successfully been applied to describe how spontaneous dynamics

can arise from the structural properties of the network. Modelling of whole-brain activity can assist in elucidating the causal links facilitating the transitions between brain states of sleep. In computational terms the aim is to understand the interplay between integration and segregation in the brain and to find the important binding regions that are necessary and sufficient for network transitions between states. In the current study we used MEG to measure whole-brain activity of 11 healthy adults that went through the different phases of sleep. We obtained the spatiotemporal dynamics of brain activity by extracting the slow fluctuating changes in the Hilbert power envelope of frequency filtered and beamformed time-series. A Hidden Markov Model (HMM) makes it possible to resolve non-stationarity of functional networks. Thus each sleep stage was tested as individual transient states of the network. Finally, we applied a whole-brain computational model that allowed us to identify the necessary and sufficient brain regions binding information across the brain and facilitating the transitions between brain states during sleep.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: Medical Research Council (MR/J004448/1)

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Deafness Research UK (552:STR:SS)

Title: Two different mechanisms alternate during the slow oscillation

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Abstract: A large amplitude slow oscillation (<2 Hz) is seen during slow-wave sleep and under anesthesia throughout the cortex. Within the slow oscillation UP phases are characterized by

global spiking while DOWN phases are characterized by global silence. It is currently unclear whether this slow oscillation is generated by the cortex alone or through interactions between the thalamus and cortex. In our study, applying independent component analysis (ICA) to recordings from urethane-anesthetized rat neocortex reveals two different mechanisms for slow oscillations. The mechanisms are distinguished by two key neural sources identified by ICA: a strong broad source centered in layer 5 (BL5) and an apparent sub-cortical source producing a clock-like 3.5 Hz oscillation which resembles hippocampal theta oscillations (SUB). While these two sources are independent on a timescale of milliseconds, they are anti-correlated and alternate on a timescale of minutes. The BL5-dominated slow oscillation often resembles cortically generated oscillations: UP phases are initiated in the infragranular layers akin to traveling neocortical waves, and the oscillation is relatively slow. The SUB-dominated slow oscillation can resemble thalamo-cortically generated oscillations: UP phases are initiated in granular as well as infragranular layers, and the oscillation is faster and relatively regular in some experiments. These findings suggest that both hypothesized mechanisms for the slow oscillation are at work in the cortex - in alternation.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: Funding support from Department of Anesthesiology

Title: Comparison of corticocortical coherence between spontaneous and recovery sleep-wake states in rat

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Abstract: Background: Sleep deprivation induces functional deficits at multiple organizational levels ranging from molecules to behavior. Some of these deficits, especially neurobehavioral and cognitive, have been documented to persist even after the recovery of sleep architecture. Electroencephalogram (EEG) based brain connectivity measures can provide insights into the interaction and causal relationships between different brain regions and may reveal differences in

functional states that remain undetected by coarse EEG analysis. To the best of our knowledge, there has been no comprehensive study to characterize brain connectivity during spontaneous sleep-wake (S-W) states and the recovery period after total sleep deprivation (TSD). Therefore, we characterized and compared the corticocortical connectivity between spontaneous S-W states and the S-W states during recovery from 9 h of TSD. Methods: Male Sprague Dawley rats (n=7) were instrumented to record EEG from frontal, parietal, occipital cortices, and electromyogram (EMG) from nuchal muscles. After 7-10 days of post-surgical recovery, monopolar EEG (0.1-300 Hz), bipolar EEG (0.1-100 Hz), and EMG (1-100 Hz) were recorded across 24 h light-dark period (lights on at 6 am). Thereafter, the rats had 9 h of TSD through gentle handling between 9 am - 6 pm. After TSD, the rats were allowed ad libitum sleep and electrophysiological recordings were conducted for 24 h. Bipolar EEG and EMG were used for classifying the S-W records into wake (W), slow wave sleep (SWS), rapid eye movement sleep (REMS), and intermediate state (IS). Monopolar EEG was used for measuring changes in corticocortical coherence, a measure of non-directional brain connectivity. Paired t test was used for statistical comparisons. Results: As expected, after TSD there was a rebound increase in SWS, REMS, and IS ($p<0.05$). The differences were significant only for the 12 h recovery period (dark phase) immediately after the completion of TSD. Broadband (0.5-115 Hz) coherence analysis for the recovery period showed a significant ($p<0.05$) suppression of coherence during SWS and REMS as compared to spontaneous sleep. Percent S-W states in the post-TSD light phase were not statistically different from the pre-TSD light phase, indicating behavioral recovery from the effects of TSD. However, coherence during SWS and REMS in the recovery light phase remained significantly suppressed ($p<0.05$). Conclusions: Despite the recovery of sleep macroarchitecture after TSD, changes in corticocortical coherence persist. Such changes may underlie persistent cognitive deficits and motivate further study of corticocortical connectivity during recovery sleep.

Disclosures: **D. Pal:** None. **B. Silverstein:** None. **A. Lee:** None. **U. Lee:** None. **G. Mashour:** None.

Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: Health and Medical Research Fund, Food and Health Bureau, Hong Kong

Title: REM-sleep latency predicts napping improvement on planning ability

Authors: *M. WONG[1], E. LAU[2]
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Abstract: E.08. Biological Rhythms and Sleep E.08.f. Sleep: Behavior REM-sleep latency predicts napping improvement on planning ability Objective Basic cognitive functions, including memory and attention, were found to improve after sleep when compared to wakefulness. We investigated whether sleep also facilitated higher-order cognitive function such as planning ability, which is subserved by the prefrontal cortex. Methods Fifty healthy adults (aged 17-25, 60.7% female) completed two different sets of the Tower of London task(ToL) separated randomly by either a 90-minute nap (Nap, n=25) or wakefulness (Wake, n=25). The number of steps and RTs in completing the tasks were used as outcome measures of their planning ability. Results The two groups were matched on age, sex, BMI and average 5-day actigraphy-measured sleep duration (ps>.05). The groups did not differ in the change in RT, t(48)=.599, p=.551, but in steps, t(48)=2.011, p=.048, with the nap group having decreased steps and the wake group having increased steps post-condition, indicating improvements and deteriorations, respectively. Reduction in steps was associated with shorter REM-sleep latency in the nap group, r(23)=.546, p=.016. Conclusion This was the first study demonstrating the benefits of daytime napping, particularly REM-sleep on planning ability. While previous studies showed that executive function deficits in sleep-restricted and clinical populations (e.g. Attention Deficit Hyperactivity Disorder) were correlated with REM-sleep latency, our data shed light on the potential use of a daytime nap to reverse executive function deficits through its impact on REM-sleep.

Disclosures: M. Wong: None. **E. Lau:** None.

Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: MICINN (SAF2009-10560)

JJAA (P09-CVI-4712)

FEDER

Title: Cortical dynamic of an EEG high frequency band during rapid eye movement (REM) sleep

Authors: *A. SÁNCHEZ LÓPEZ, L. C. CERVANTES, M. ESCUDERO
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Abstract: Electroencephalographic activity (EEG) remains one of the main physiological activities used in the identification of neurofunctional states. Here we present a detailed study of the high frequency EEG along different vigilance states in the rat. Five animals were implanted with electrodes for the recording of the EEG, neck muscle activity and search coils for the recording of eye movements by mean of the scleral search coil technique. A spectral analysis of the EEG signals was performed revealing the existence of a frequency band peaking between 120 and 140 Hz which characterized REM sleep. In order to study the cortical dynamics of this frequency band, another nine animals were implanted with 18 EEG epidural electrodes. High frequency oscillations showed the highest amplitude in the centro-parietal cortex, just previous to lambda. This high frequency oscillations occurred in bursts in synchrony with oscillations in the theta band. A high coherence among locations in a same hemisphere but not between hemispheres characterized high frequency oscillation cortical activity. Looking for the origin of the high frequency oscillations, another four animals were implanted with electrodes in the cortex and hippocampus to study this frequency band and its relation to theta in these two structures. The power of the high frequency was higher in the cortex than in the hippocampus and a high coherence was found between every hippocampus and the ipsilateral cortex. Nevertheless, electrolytic lesions in the hippocampus produced no changes in cortical high frequency, suggesting that this frequency band in the cortex must have a generator independent from hippocampus. Finally, two additional animals were implanted with 4 electrodes in each thalamus. No high frequency was recorded by any of these electrodes during REM sleep. In conclusion, the high frequency band recorded in the EEG during REM sleep seems to have a cortical origin.

Disclosures: A. Sánchez López: None. **L.C. Cervantes:** None. **M. Escudero:** None.

Poster

258. Sleep: Systems and Behavior

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Support: BIAL Foundation 220/12

ONR N00014-13-1-0672

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NIH R01 MH099645

Title: Insights into local properties and synchrony of sleep spindles from ECoG in humans

Authors: *G. PIANTONI[1], E. HALGREN[2], S. S. CASH[1,3]
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Abstract: During non-REM sleep, neuronal firing is grouped by the slow oscillations at around 1 Hz, and sleep spindles, 0.5-2s long bursts of oscillatory activity between 11 and 18 Hz. Spindles are thought to arise from the interplay between thalamic and cortical neurons and are hypothesized to be involved in a wide variety of fundamental processes including the solidification of learning during sleep. However many of the physiological mechanisms and characteristics of spindles remain poorly understood. The spatial extent of spindle activity, as one key feature, has been the object of substantial debate, with conflicting evidence pointing both to an increase in synchronization, assessed with scalp EEG, and to strong regional localization, as measured by MEG and intraparenchymal depth electrode recordings (iEEG). Here, we examine spindle localization and synchrony across broad areas of neocortex using recordings from, on average, 80 electrodes (ECoG) placed directly on the pial surface in six patients undergoing evaluation for intractable epilepsy. The research was approved by the local institutional review board and electrode placement was determined solely by clinical criteria. At least one hour of visually scored sleep was included in the analysis. Spindles were automatically detected on each electrode independently, based on custom code. Spindles were considered synchronous when two spindles on separate channels overlapped in time. We observed that spindles were more likely than expected by chance to appear at the same time over multiple cortical locations. However, when we estimated the distribution of spindles in relation to the number of active electrodes, we observed that there was a strong positive skew. This indicates that most spindles were synchronous only in one or a few channels at the same time but some spindles were widely distributed. This distinction between focal spindles and distributed spindles was further investigated by measuring the spreading over cortical regions with a 3D reconstruction of the electrode locations and by comparing it with the underlying anatomical structures. These results indicate a dynamic interplay between local properties of focal spindles and widespread synchrony of global spindles. We suggest that the multiple spatial scales at which sleep oscillations operate might provide a flexible framework to support the many

functions ascribed to sleep and to spindles in particular, such as sensory gating, memory consolidation, and synaptic homeostasis.

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Poster

258. Sleep: Systems and Behavior

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Title: Differences between transcranial magnetic stimulation-evoked brain activity during N2 and N3 sleep measured by electroencephalography

Authors: *J. O. NIEMINEN[1,4], O. GOSSERIES[1,2], F. SICLARI[1], M. BOLY[3], M. MASSIMINI[5], G. TONONI[1]
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Abstract: Combined transcranial magnetic stimulation and high-density electroencephalography (TMS-EEG) is a non-invasive technique that allows assessing changes in brain activity that occur in response to the stimulation of the cortex. In earlier studies, TMS-EEG has been used to reveal differences between wakefulness, rapid-eye-movement (REM) sleep, and non-REM (NREM) sleep. TMS-evoked brain activity in NREM sleep has been described to be less complex than the response evoked during REM sleep or wakefulness, paralleling the reduced level of consciousness. However, previous studies did not differentiate between light (stage 2) and deeper (stage 3) slow wave sleep. In this study, we investigated differences between TMS-

evoked activity in NREM stage 2 (N2) and NREM stage 3 (N3) sleep on healthy adults. TMS was targeted on the precuneus using navigated TMS and the magnetic resonance images of the subjects. Brain activity was recorded using a 60-channel TMS-compatible EEG. The online EEG recording was used to detect sleep-stage transitions; after the subjects entered N2 or N3 sleep, the brain state was let to stabilize for at least 5 minutes before TMS was applied. A single TMS session consisted of about 150 biphasic pulses with an inter-stimulus interval of 2 - 2.3 seconds. In a typical night, 5 to 10 TMS sessions were recorded. After each TMS session, the subject was awakened to ask for a dream report in order to correlate the TMS-EEG responses with the level of consciousness. After removing bad EEG channels and trials contaminated by artefacts, the data were filtered and baseline-corrected. The TMS-evoked responses were studied at the sensor level using the global-mean-field-amplitude (GMFA) measure. Our preliminary results suggest that the TMS-evoked EEG response during N3 sleep appears larger and slower than during N2 sleep. The GMFA is higher in N3 than in N2 sleep, and the peak latencies vary between the sleep stages. Further analyses are currently undergoing to quantify these brain activity changes and to correlate them with the level of consciousness and the absence or presence of dream reports. TMS-EEG might provide valuable information for characterizing underlying neurophysiological differences during sleep.

Disclosures: **J.O. Nieminen:** None. **O. Gosseries:** None. **F. Siclari:** None. **M. Boly:** None. **M. Massimini:** None. **G. Tononi:** None.

Poster

258. Sleep: Systems and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 258.13/OO18

Topic: E.08. Biological Rhythms and Sleep

Support: NSF Grant No. 0903622

Title: Steady-state visually evoked potentials as a method for studying information processing during sleep

Authors: ***J. NORTON**, S. UMUNNA, T. BRETL
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Abstract: Sleep is a rapidly reversible period of altered consciousness during which awareness of external stimuli is greatly reduced. This reduction in awareness is caused by an inhibition of

information processing during sleep. Event-related potentials (ERPs) have provided a key research tool in the investigation of the extent to which information processing is inhibited during sleep. The majority of research in this area has concentrated on the auditory and somatosensory modalities; the elicitation and detection of visual ERPs during sleep has been unreliable. To improve the reliability of recording visual ERPs during sleep we have developed a head mounted stimulation system for the elicitation of steady-state visually evoked potentials (SSVEPs), expanded the number of occipital electroencephalograph (EEG) channels recorded during polysomnography, and implemented a detection system based on canonical correlation. To validate our system we collected experimental data from eight individuals during non-paradoxical sleep. Results demonstrate a significant and frequency specific increase in the correlation between participants' recorded EEG data and the visual steady-state stimuli during sleep. Our system provides a promising platform for the further study of visual information processing during sleep.

Disclosures: J. Norton: None. **S. Umunna:** None. **T. Bretl:** None.

Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: Defense Science and Technology Agency Singapore (POD0713897)

National Medical Research Council Singapore (StaR/0004/2008)

Title: Co-activated yet disconnected: Neural correlates of eye closure during sleep deprivation

Authors: J. ONG[1], D. KONG[1], T. T. Y. CHIA[1], J. TANDI[1], B. T. T. YEO[1,2], *M. W. CHEE[1]
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Abstract: When an individual is deprived of sleep, the duration spent during eye closure increases. More pronounced eye closures are linked to periods of diminished responsiveness even to auditory stimuli (Ong, et al., 2013). Here, we studied BOLD signal changes accompanying eye closures in sleep deprived healthy young adults seeking to understand these might be related to diminished responsiveness. 18 undergraduates underwent two 6-minute

resting-state scans in darkness after being kept awake for ~24h. Although instructed to keep their eyes open, multiple epochs of spontaneous eye closure were observed. A trained observer scored 4-second epochs of eye video recordings as 'Eyes Closed' (EC) or 'Eyes Open' (EO). To model BOLD activity during EC periods, a random-effects GLM-based analysis was employed using a boxcar regressor corresponding to the length of each EC epoch convolved with a hemodynamic response function. To investigate the effect of eye closure duration on BOLD signal changes, event-related activity time-locked to the start of each 0.5-4s, 4-8s and 8-12s EC epoch was determined by signal averaging. In addition, EO and EC epochs were extracted for computation of functional connectivity maps within nodes of the 'default-mode' (DMN) and dorsal attention network (DAN). BOLD signal during EC contrasted to EO epochs revealed extensive activation and deactivation in multiple brain regions. There was pronounced deactivation of the thalamus, which could reflect the transition between wakefulness to sleep and closure of sensory gates. Paradoxically however, there was increased activity in hippocampal and parahippocampal areas, and in the sensorimotor, somatosensory, visual extrastriate and auditory cortices, which could suggest a switch to a dissociated interoceptive state characterized by multisensory activity (Marx et al., 2003). Further evidence of dissolution of cognate processing lay in the increased co-activation of both DMN and DAN regions, which could represent 'wake-state instability' (Doran, et al., 2001). In addition, longer duration eye closures were associated with increased BOLD peak amplitude and latency. Finally, eye closure was also associated with reduced functional connectivity within both the DMN and DAN. Together, these findings show that during drowsiness, dramatic local changes in BOLD signal and global reduction of coupling within attentional networks can be observed during periods of eye closure. These changes likely represent a shift to interoceptive processing as well as state-instability in drowsy sleep deprived persons.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: NSERC DG 386522-2010

AHFMR Polaris Award

Title: The effects of non-oscillating transcranial direct current stimulation on sleep in rats

Authors: L. KALVI, M. ECKERT, K. ALI, *M. TATSUNO
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Abstract: Accumulating evidence supports the idea that sleep plays an active role in memory formation, suggesting that external manipulations, such as transcranial direct current stimulation (tDCS), applied during sleep may alter memory consolidation. Indeed, studies in humans and rodents have shown that transcranial application of oscillating potentials during sleep can alter endogenous brain oscillations and enhance memory retention. At the same time, tDCS applied during sleep may disrupt sleep architecture and have a negative effect on memory performance. As the effect of tDCS during sleep has not yet been investigated systematically, the aim of this study was to determine the effects of non-oscillating tDCS on sleep properties in rats. We hypothesized that non-oscillating tDCS during NREM or REM sleep would also affect fundamental brain oscillations and modify sleep architecture. Local field potentials were recorded from the cortex and hippocampus of rats during daily 3 hour sleep sessions. After habituation to the recording room, rats underwent a period of baseline sleep recordings followed by two weeks of stimulation sessions in which 5 minutes of mild non-oscillating tDCS was administered during REM or NREM sleep. Using a within-subjects design we found that many measures of sleep architecture, such as total sleep time, percentage of REM and NREM sleep, duration of REM and NREM episodes, and REM and NREM density, were not affected by tDCS either during REM or NREM. To determine the effects of stimulation on oscillatory activity, we compared power spectra following stimulation episodes with corresponding periods of sham "stimulation" during baseline days. Unlike oscillatory stimulation, tDCS during NREM sleep had no effect on delta power but decreased gamma power. On the other hand, tDCS during REM sleep did not affect power in the wide range of frequencies (delta to gamma). To investigate if stimulation affected interactions between the hippocampus and cortex, we computed the cross-correlation of hippocampal sharp wave-ripples (SPWRs) and cortical K-complexes. We found that SPWRs tended to occur around K-complexes but tDCS did not affect this timing. Contrary to previous studies in which rats engaged in learning tasks exhibited SPWRs that tend to precede K-complexes, our data from rats not engaged in any learning showed that SPWR's were equally likely to occur just prior to or after a K-complex. Taken together, these results suggest that sleep architecture is very robust to mild non-oscillating tDCS. It is possible that oscillating current is necessary to modify sleep.

Disclosures: L. Kalvi: None. **M. Tatsuno:** None. **M. Eckert:** None. **K. Ali:** None.

Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant MH087934

Title: The effects of sleep deprivation on pre and post-training in a one-trail discriminative learning task

Authors: J. J. IZYGON, D. M. NGHIEM, J. SHAHIN, D. MCGHIEY, C. MADISON, A. MASOOD, M. HENCEROTH-CHOMIAK, G. SRINIVASARAO, D. H. MALIN, *C. P. WARD
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Abstract: Experimental models of sleep deprivation have been shown to adversely affect memory consolidation in the brain. The present study explored the effects of a pre/post-training sleep deprivation period on a one-trial discriminative learning task. 27 male Sprague-Dawley rats, 4 months old, were randomly assigned to three groups: a control group, a pre-training sleep deprived group, and a post-training sleep deprived group. The behavioral task involved a training trial where rats were able to explore a five arm radial maze in order to discover the location of a food reward. Rats were either sleep deprived 24 hours prior to training or 24 hours after their training. Regardless of their condition, each group performed a retention trial 24 hours after the training trial where rats had to find the food reward in the same arm as before. Latency to find the food reward and number of incorrect arms entered were recorded. Rats were housed in a 12:12 light/dark cycle and trials occurred at the end of the dark cycle and sleep deprivation began at the start of light cycle. All groups showed a decrease in latency and errors from training to retention ($F(1,22)=20.288$, $p<.001$; $F(1,20)=16.028$, $p<.001$ respectively) indicating that the rodents learned the location of the food reward with one training trial. There was no significant main effect among the three groups in latency or errors ($F(2,22)=1.341$, $p=.282$; $F(2,20)=0.160$, $p=.489$ respectively). There was also not a significant group X trial interaction in latency or errors ($F(2,22)=3.21$, $p=.055$; $F(2,20)=0.742$, $p=.489$ respectively). Previous research has shown that spatial memory in a task such as the water maze is impaired by sleep deprivation. However, sleep deprivation did not impair memory consolidation in the present one-trial spatial task. This task is different than other tests of spatial memory (e.g., water maze) in that it can be learned in a single trial. This task also differs from other one-trial tests (e.g., contextual fear) in that it relies on positive reinforcement. While sleep deprivation often interferes with performance on tasks

that rely on the hippocampus (water maze, contextual fear, social transmission of food preference), sleep deprivation did not interfere with performance on the one-trial task. Likely, this is due to the multi-modal aspect of the one trial task and while sleep deprivation may cause impairment in hippocampal function, rodents were able to recruit motor and visual discrimination memory systems for task performance.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: PROMEP-SEP Grant 14412118

Title: Effect of immune challenge and/or REM-sleep restriction on memory, anxiety and helplessness

Authors: *S. A. CASTILLO-GARCIA[1], E. BECERRIL[2], J. VELAZQUEZ-MOCTEZUMA[3], L. PAVON[2], E. DOMINGUEZ-SALAZAR[3]
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Abstract: One-third or more of the global population suffer from significant sleep loss by vocational or lifestyle reasons. Rapid eye movement (REM) sleep loss is associated with memory deficits and behavioral changes related to alteration in the brain neurochemistry. REM sleep loss also has been highly associated with the induction of immune changes including cytokine release and leukocytosis. Here short term memory, spatial memory, anxiety and helplessness (depression) were evaluated in Wistar Kyoto male rats under three experimental conditions for periods of 15 or 21 days of REMr: 1) REM sleep restriction (REMr), performed by the island technique, in a daily schedule of 20 hours of REMr and 4 hours of rest during 15 or 21 days; 2) Ovalbumin (OVA) administration at days 1 and 8 and at days 1, 8 and 15; and 3)

REMr with ovalbumin (REMr/OVA) at the same times than the other groups. A control group without manipulation was included. During the last seven days of sleep restriction and/or treatment with ovalbumin, short term memory was evaluated by the object recognition task; anxiety with the elevated plus maze; spatial memory with the Morris water maze, and helplessness (or depression) with the Porsolt test. Our results showed that by 15 days REMr impaired spatial learning and decreased anxiety, apparently helplessness (depression) also diminished; OVA attenuated these effects observed in REMr rats, but OVA by itself did not show any significant effect. However, the group treated with OVA by 21 days showed memory/learning deficits, these effects were eliminated with REMr. Also, REMr/OVA group by 21 days showed low levels of anxiety. REMr group by 21 days did not show any behavioral changes as compared to intact controls. In conclusion, the memory/learning changes induced by REMr can be attenuated or eliminated by the immune challenge at 15 days, and the effect of OVA administration was mitigated by REMr at 21 days. The effects on anxiety or depression depend of the duration of REMr or OVA treatment.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: CIHR Grant MOP259183

Title: Effects of chronic sleep restriction on cardiac and thermoregulatory functions in rats

Authors: *T. BAH, S. DEURVEILHER, E. E. EGOM, B. RUSAK, R. A. ROSE, K. SEMBA
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Abstract: Chronic sleep restriction (CSR) has been linked to cardiovascular morbidities (tachycardia, cardiac arrhythmias, and heart failure) and other physiological abnormalities. To study the autonomic impacts of CSR and underlying mechanisms, we continuously monitored electrocardiogram (ECG) and body temperature (BT) in rats before, during, and after a "3/1" protocol of CSR (continuous cycles of 3 h of sleep deprivation [SD] using slowly rotating wheels, followed by 1 h of sleep opportunity) for 4 days. We previously showed that this

protocol initiated both homeostatic and allostatic changes in sleep parameters and psychomotor vigilance task performance. Adult male Wistar rats were implanted with transmitters to monitor ECG, BT, and gross motor activity under a 12 h light/12 h dark cycle. A CSR group (n = 9) was recorded during 2 baseline days, followed by 4 days of the 3/1 protocol, and during 7 subsequent recovery days. A wheel-running control (WRC) group (n = 5) underwent the same experimental procedures except that they could turn the wheels freely for the first 6 days of recording and during a preceding 5 day period of habituation. Transthoracic echocardiography was conducted immediately before transmitter implantation and again at the end of the experiment to assess cardiac morphology and performance. As expected, the WRC rats showed increased heart rate (HR) and BT in the dark phase, when they were active, compared to the light phase. In the CSR rats, HR and BT increased when they were forced to walk during SD periods, in both the light and dark phases. When the CSR rats had the opportunity to sleep between SD periods, their HR decreased in both the light and dark phases but, unexpectedly, their BT remained as high as during SD periods, regardless of the time of day. This elevated BT in the CSR rats continued for several days after CSR, more noticeably in the light phase, despite the lack of forced activity. Thus, BT returned to baseline levels by the 5th day of recovery, whereas HR returned to baseline levels by the 4th day. Echocardiography one week after CSR showed signs of cardiac hypertrophy compared to baseline levels (increases in left ventricular wall thickness, ejection fraction, and fractional shortening). These data indicate that the 3/1 CSR protocol for 4 days resulted in alterations in both cardiac and thermoregulatory regulation that lasted 4-7 days after CSR. These alterations in autonomic functions may be part of the mechanisms by which CSR can promote cardiovascular disease in people.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NS072942

Title: Phenotypic characterization of sleep, circadian rhythm, and drug response in MitoPark mouse, a model of Parkinson’s disease

Authors: *N. SAKAI, N. CHAN, S. NISHINO
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Abstract: Hypersomnia can affect 20-50% of patients with Parkinson's disease (PD) and in 1 to 4% of the patients, culminates in sleep attacks. However, the pathological mechanism involved in hypersomnia and drug-induced sleep attack is unknown. A MitoPark mouse has been validated to show the progressive development of key PD features due to the selective loss of midbrain DA neurons. We therefore evaluated age-dependent changes in sleep, circadian rhythm, and drug response in MitoPark mice. Adult male mice (n=8 each for control and MitoPark mice) underwent surgery for EEG and EMG electrodes and E-mitters. Baseline sleep was recorded at 10 (absent), 15 (mild), 20 (moderate), and 25 (severe) weeks of age. Sleep deprivation was performed by gentle handling for 6 hours after one full day of baseline. Another set of mice was kept under constant dark conditions for 3 weeks from 11 and 21 weeks of age. Three doses of ropinirole, a non-ergot dopamine D2-like receptor agonist, were administered before the light off at 13, 17, 21, and 25 week olds. MitoPark mice showed an age-dependent decline of up to 60% in locomotor activity following a new cage environment, while there was no difference between control and MitoPark mice in the spontaneous locomotor activity through 24 hours on the day of baseline in all ages examined. MitoPark mice had normal amounts, and natural diurnal distributions, of wakefulness and sleep in the 12:12 LD condition at 10, 15, and 20 weeks of age. There was also no difference in the responses to 6-hour sleep deprivation. Once the movement abnormalities severely exacerbated at 25 weeks, the sleep fragmentation and a decrease of amount spent in NREM sleep occurred during light periods in the MitoPark mice. There was no abnormality in the circadian rhythm through the course of motor symptoms. Ropinirole induced the opposite effect on locomotion between genotypes, and these changes were age-dependent. In control mice, the middle and high doses (2 and 20 mg/kg) of ropinirole significantly decreased locomotion at all ages, while MitoPark mice showed a significant increase of locomotion by the high dose of ropinirole at 21 and 25 weeks of age. The vigilance changes after ropinirole injections are currently under analysis. Sleep abnormalities were observed in the MitoPark mice only at 25 week old, and it is not certain if this correlates to sleepiness in human subjects. The sleepiness could not be caused solely by the loss of midbrain DA neurons. Since the sleepiness observed in PD could be triggered by both a function of disease as well as its treatment, age-dependent changes in response to ropinirole may explain the mechanism of drug-induced sleep attacks in PD.

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Poster

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Title: Chronic sleep deprivation alters theta and gamma powers during REM sleep in mice

Authors: B. KIM[1,2], Y. KIM[3], *E. HWANG[1], R. STRECKER[3], R. MCCARLEY[3], J. CHOI[1,4]
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Abstract: Chronic sleep restriction (CSR) has been shown to impair people's health and cognitive functions, as well as change sleep homeostasis. Previous studies have shown that CSR may impair non-rapid eye movement (NREM) sleep generation in addition to reducing NREM delta power. However, little is known about how CSR alters rapid eye movement (REM) sleep or EEG power spectra during REM. Here, we used a high-density EEG method in freely behaving mice to assess the REM sleep response to CSR. Mice (N=9) were sleep deprived daily for 18-h using periodically rotating wheels, followed by 6-h of sleep opportunity that started at the beginning of each light period. This sleep restriction (SR) protocol was repeated for 5 consecutive days. High-density EEG was analyzed for only 2-h of each 6-h sleep opportunity (1-3h after the initiation of sleep opportunity), whereas conventional EEG data using skull screw electrodes was analyzed for the full 24-h experimental days. The REM sleep time during the daily 6-h sleep opportunities increased as the SR days progressed, compared to the corresponding baseline levels. A power spectral analysis of the high-density EEG revealed that low theta power (5-7 Hz) increased significantly in the frontal cortex on SR day 1, then continuously decreased on SR3 and SR5; high theta power (7-10 Hz) was persistently elevated throughout all SR days, especially in the centro-parietal cortex. A close examination of theta oscillation revealed a transition from unimodal to bimodal oscillation showing that a peak frequency at 7 Hz during baseline was split into two peak frequencies at 7 and 9 Hz. Regarding REM gamma power, a gradual but significant increase in low gamma power (30-50 Hz) was

observed near the prefrontal cortex especially on SR3 and SR5, while robust increases in high gamma power (70-100 Hz) were observed most significantly in the centro-parietal cortex on SR3. Additionally, the analysis of the cross-frequency coupling between theta phase and gamma power showed that modulation of theta on gamma oscillation was not altered during CSR. Thus, CSR produces opposite effects on the low and high theta power of REM sleep in mice. In addition, this study indicates that CSR significantly increases REM gamma power while 18-h of acute sleep deprivation does not. Further studies are needed to determine if REM high theta power and gamma power actually correlates with the behavioral sleepiness and cognitive impairments observed in CSR.

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Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Title: The effects of sleep deprivation on spatial representations in young and aged adult mice during an object-place recognition task

Authors: *R. K. YUAN, I. A. MUZZIO
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Abstract: Sleep is thought to play a key role in memory consolidation. Research has shown that neuronal ensembles activated during navigation are reactivated during sleep, and that sleep deprivation causes deficits in several hippocampus-dependent tasks. However, the direct effects of sleep deprivation on cellular activity are still unclear. Our preliminary findings indicate that sleep deprivation affects place cell stability, a correlate of spatial memory that affects the reliability of a cell firing. Additionally, in agreement with the existing literature showing that older subjects tolerate sleep deprivation better than younger subjects, we also found that aged mice show more resistance to sleep deprivation than younger animals. We are currently exploring the effects of sleep deprivation on learning by testing how the cellular alterations produced by sleep deprivation correlate to behavioral performance on an object recognition task. On day 1, C57BL/6 male mice were habituated to a novel environment, in which they subsequently explored 3 objects over the course of 3 consecutive sessions. Immediately after the

last object session, mice were sleep deprived for 5 hours, then returned to their home cages. On day 2, mice were reintroduced to the environment for a single test session in which one object was displaced. Sleep deprivation was induced using an automated system consisting of a cylindrical enclosure with a continuously rotating panel that spanned the enclosure, preventing the animal from maintaining a sleeping posture. Control mice were tested at the same time intervals and spent the same amount of time in the context and deprivation chamber but were not sleep deprived. We anticipate that the results of this study will provide important information about the role of sleep on cognitive performance in young and old animals.

Disclosures: R.K. Yuan: None. **I.A. Muzzio:** None.

Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: Dept Veterans Affairs

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Stonehill College

Title: Environmental enrichment reduces sleep deprivation induced impairments of attention and recall in the novel object recognition task

Authors: J. W. CORDEIRA[1], J. M. MCNALLY[1], J. T. MCKENNA[1], C. V. BAGLINI[2], *R. E. STRECKER[1], R. W. MCCARLEY[1,2]
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Abstract: The novel object recognition task (NOR) has been widely used to test recognition and working memory in rodent models of CNS disorders. In phase 1 of the NOR, two identical objects are presented simultaneously to allow object familiarization. Subsequently, when both a

familiar and a novel object are presented in phase 2, time spent investigating the novel object typically increases. Sleep deprivation (SD) following the initial associative learning in phase 1 has been shown to impair object recall in phase 2, an effect thought to reflect interference with memory consolidation. Attention in humans and rodents is also impaired by sleep deprivation as assessed using operant tasks (e.g., the psychomotor vigilance task); however, the NOR task is preferable to use in rodents as it does not require any training or reinforcement. Here we assessed the impact of 24h SD placed either before, or after, NOR phase 1 in mice ($N \geq 8$/group). Mice were sleep deprived by the movement of an activity wheel in which they were housed using a schedule of 3s on and 12s off for 24h. The frequency and time mice spent investigating each object was accurately determined using infrared beam breaks. Mice in the environmental enrichment condition were group housed with several toys (e.g., nesting material, running wheel, etc.) that were alternated weekly for 8 weeks prior to the NOR experiment. As previously reported, SD placed after phase 1 significantly impaired object recall in phase 2. SD prior to NOR phase 1 significantly decreased the amount of time spent per object investigation in phase 1. We interpret this finding to indicate that sleep deprived mice had an impaired ability to attend to the objects. Consistent with the observation that 24h SD prior to phase 1 impaired attention (and presumably learning), we observed that object recognition (memory) 1h later in phase 2 was also impaired. SD mice that received environmental enrichment, however, displayed object recognition in phase 2 that resembled that of untreated control mice. Specifically, ~66% of the object investigation time was spent on the novel object compared to 50% (chance level) in the sleep deprived mice. Preliminary data also indicate that high frequency cortical EEG activity evoked by novel object investigations is altered by SD. In summary, the findings suggest that: 1. the NOR task can be used as a high throughput assay of attention in mice and it is sensitive to impairments produced by SD, and 2. environmental enrichment can reverse the cognitive impairments produced by 24h SD in the NOR task.

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Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: AA020334

AA0174720

Title: Mice exposed to predator threat display protracted sleep disruptions

Authors: A. PATEL, R. SHARMA, P. SAHOTA, *M. M. THAKKAR
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Abstract: BACKGROUND: Post-traumatic stress disorder (PTSD) is a severe debilitating disorder which develops after an individual experiences a life threatening event. PTSD is characterized by hyperarousal, sleep disruptions, flashbacks (re-experiencing trauma) and avoidance to trauma associated stimuli. Although several animal models have been developed and used, most models involve physical pain and discomfort. In contrast, the predator threat (predator odor) model is considered highly relevant since it does not involve pain or discomfort rather; exposure to predator threat (cat, fox or coyote odor) is innately life-threatening, fearful and ecologically relevant. Rodents exposed to predator odor display several symptoms of PTSD including fear, exaggerated startle response (hyperarousal) and anxiety. Although sleep disturbances are considered to be hallmarks of PTSD, sleep has never been examined in the predator threat model. This study was designed to examine the effects of a single exposure to predator threat on sleep-wakefulness in C57BL/6J mice. METHODS: Using standard surgical procedure, mice were instrumented to record hippocampal EEG and EMG to examine sleep-wakefulness. Mice were exposed to contextual conditioning by replacing their recording cages with contextual cages (recording cages wrapped with aluminum foil on the bottom and outside half). After allowing 30 min of exploration, PTSD mice were exposed to predator threat by spreading soiled cat litter in their (contextual) cages. Controls were exposed to clean/unused cat litter. After 90 minutes, contextual cages were replaced with original recording cages and mice were left undisturbed for 4 days. Fear learning was verified on day 5 by housing the animals in contextual cages. Hippocampal EEG and EMG were continuously recorded for 5 days. RESULTS: As compared to controls (N=4), PTSD mice (N=5) exposed to predator threat displayed a significant ($p<0.05$) and a protracted increase in the wakefulness along with a concomitant reduction in sleep, on all 5 days during the light period including during conditioning and testing. These results support the relevancy of predator threat model in mimicking human PTSD. CONCLUSIONS: A single exposure to predator threat results in severe and protracted sleep disruptions.

Disclosures: A. Patel: None. **R. Sharma:** None. **M.M. Thakkar:** None. **P. Sahota:** None.

Poster

258. Sleep: Systems and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 258.24/OO29

Topic: E.08. Biological Rhythms and Sleep

Support: T32 Grant HL007953

Title: Acute dietary manipulations alter sleep/wake architecture in mice

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Abstract: There is a strong association between sleep and metabolism, though most studies have focused on how sleep disruption impacts metabolic function. Exploring the reciprocal relationship--how acute dietary changes influence sleep architecture--will complete our understanding of the sleep and metabolism interaction and may provide novel therapies for patients with sleep disorders and metabolic syndromes, including obesity. In this study, we used a within-animal approach to investigate how acute dietary manipulations affect sleep/wake behavior. Briefly, adult mice were implanted with EEG/EMG electrodes to monitor sleep/wake states. Following baseline sleep recording, mice were randomized to either a 25-hour food removal (FR) or one week of high fat diet (HFD). Mice were then returned to regular chow for one week before being switched to the other dietary manipulation. Diet had differential and significant effects on total wake time in the dark phase ($p < 0.01$, Friedman), with FR trending towards increased wake ($p < 0.08$, Wilcoxon Rank). HFD mice displayed increased sleep fragmentation in the dark phase, evidenced by increased wake bout number and decreased wake bout length ($p < 0.02$ and $p < 0.04$, respectively). Additionally, the multiple sleep latency test showed a significant diet interaction for sleep propensity at the beginning of the dark phase ($p < 0.05$). Diet did not affect total wake and sleep time during the light phase. Taken together, this study provides a foundation for exploring molecular mechanisms and brain regions that influence diet-induced changes in sleep/wake architecture.

Disclosures: I.J. Perron: None. **S. Veasey:** None. **A. Pack:** None. **P. Fenik:** None.

Poster

258. Sleep: Systems and Behavior

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Program#/Poster#: 258.25/OO30

Topic: E.08. Biological Rhythms and Sleep

Support: R01MH084980

P51OD011106

Title: The long term effects of early rearing environment on daytime and nighttime behavioral activity of adult male rhesus monkeys (Macaca mulatta) across the lifespan

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Abstract: In humans, early life adversity is associated with deleterious health outcomes across the lifespan. Young nursery-reared animals are reported to be more reactive than their mother-reared counterparts, but show less gross motor activity. Previous studies have evaluated the effects of differential early rearing experience on infant and juvenile behavior in monkeys, relatively few have examined their persistence through adulthood. Thus, little is known about differences in physical health, activity, and morbidity in later life. In this study, activity levels of adult male rhesus macaques with different early infant experiences, either mother-reared (n=6, MR) or nursery-reared (n=6, NR), were measured yearly in their home cage environments over the span of the 6 years from 10 to 16 years of age. The age range is comparable to young adulthood through middle-age in humans. Behavioral activity was recorded at 2-min intervals with a Actiwatch™ actimeter for 30 consecutive 24hr periods. The 24hr activity count averages were calculated for the measures: total activity, daylight and night-time activity. As expected, all monkeys were more active during the light portion of the light:dark cycle. Daytime activity decreased significantly with age ($F(5,50)=3.51$, $p=.008$). Analysis of the dark phase of the light cycle revealed no differences in behavioral activity between the two early rearing groups. Few differences between the two rearing groups in activity levels were observed, suggesting that early rearing does not produce long-lasting effects on baseline home cage activity levels in adulthood. These data provide evidence that monkeys with differential early rearing experiences exhibit similar activity patterns in adulthood and into the middle-age period of declining activity. Taken together with previous reports, our data suggest that rearing group differences in motor activity observed in the infant and juvenile period are not maintained as animals progress to adulthood and that activity differences later in life may be "reactive," requiring a salient environmental challenge to be observed.

Disclosures: P.J. Pierre: None. **A.J. Bennett:** None.

Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

Support: STW Grant OnTime12195

ZonMw AGIKO Grant 92003592

Title: Dim light at night reduces the amplitude of the sleep wake cycle in rats

Authors: D. STENVERS[1], R. VAN DORP[2], A. L. OPPERHUIZEN[3], E. FLIERS[1], P. H. BISSCHOP[1], J. H. MEIJER[2], *A. KALSBEEK[4,3], T. DEBOER[2]
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Abstract: Millions of people around the world are exposed to dim light at night (dLAN). Light is the major synchronizer of the central circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) that regulates sleep-wake behavior, but also hormone rhythms and daily energy metabolism. In principle light at night might adversely affect many of these rhythms. In the current study we investigated the effects of dLAN in a rat model. We exposed male Wistar rats to either a regular 12:12 light (200 lux):dark (0 lux) cycle (LD) or a 12:12 light (200 lux):dim light (5 lux) cycle (LDim). In a first group, electrodes were implanted for 24-h EEG and EMG sleep-wake recordings at baseline, and at day 1, 7 and 14 in LDim. In a second group, circadian rhythmicity was assessed by passive infrared monitoring (PIR) of locomotor activity for 2 months (14 days LD, then 36 days LDim, then 12 days constant darkness [DD]). In a third group, the effects on food intake and energy expenditure were monitored for 72 h in calorimetric cages at baseline, and after 1, 7, and 14 days in LDim, with animals on either a regular chow or a high fat diet. At the end of the experiments, animals were sacrificed: trunk blood was collected and white adipose tissue was weighed. The EEG recordings showed that LDim exposure immediately reduces the amplitude of the daily rhythm of REM sleep on day 1, and gradually reduces the amplitude of the daily rhythms of NREM sleep and wake in the course of 14 days in LDim. Fourier analysis showed that within NREM sleep EEG the rhythm amplitude of 0.75-4.0Hz and 16-19 Hz power density was reduced, the latter indicating reduced

internal circadian rhythm strength. Indeed, PIR recordings of the LDim animals showed a reduced amplitude of the daily rhythm in activity with an underlying free running rhythm of ~26 h, next to the 24 h rhythm imposed by the LDim. After transfer to DD some animals were arrhythmic, some showed a sudden phase shift and some showed a normal transition to a free running rhythm. In the calorimetric cages, animals showed a reduced amplitude of the daily rhythms in food and water intake and energy expenditure. However, this did not affect body weight or body fat distribution. Our results show that in rats dLAN dramatically reduces the amplitude of the daily rhythm of sleep-wake behavior, food intake and energy expenditure, probably due to a reduced output strength of the central clock rhythm. The dual rhythms observed in the current study are very similar to the LD-dissociated rhythms of forced desynchronized rats, in which rhythm separation is due to a desynchronization of the ventrolateral and dorsomedial subpopulation of SCN neurons, suggesting that a similar desynchronisation may occur due to dLAN.

Disclosures: D. Stenvers: None. **R. van Dorp:** None. **A.L. Opperhuizen:** None. **E. Fliers:** None. **P.H. Bisschop:** None. **J.H. Meijer:** None. **A. Kalsbeek:** None. **T. Deboer:** None.

Poster

258. Sleep: Systems and Behavior

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Topic: E.08. Biological Rhythms and Sleep

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Title: Gender effects in the decline of slow-wave sleep during adolescence

Authors: *J. GAINES, J. FERNANDEZ-MENDOZA, A. N. VGONTZAS, D. LIAO, E. O. BIXLER
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Abstract: The relatively higher proportion of slow-wave sleep (SWS) in adult women compared to men has been well-documented. The time at which this dimorphism occurs, however, is not clear. While previous studies in adolescents have addressed this question, they are limited by selective cohorts and small sample size. The aim of this study was to characterize the decline of SWS with age in a large cross-sectional population of adolescents. A sample of 421 adolescents (ages 12-23y, mean 17.0 ± 2.3y; 53.9% male) from the Penn State Child Cohort, a representative general population sample, underwent a single 9-hour polysomnography (PSG) recording. Multiple linear regression models for percentage of SWS versus age were generated separately for males and females, adjusting for race. Linear regression models for SWS across age suggest that while males at age 12 have more SWS than females, males undergo a more rapid reduction cross-sectionally across adolescence (-2.64% per year versus -0.96% per year in females). The interaction between age and gender on SWS was significant ($p < 0.001$), and remained when controlling for Tanner (pubertal) stage. According to the models, it is at age 17.1y that the genders diverge, with males henceforth having a lower proportion of SWS. Importantly, this loss across age was not accompanied by a change in total sleep or wake time during the night. This gender divergence may signal the initiation of dimorphic sleep patterns seen in adulthood and may also shed light on underlying mechanisms of human brain maturation, especially those related to development of mental and physical health problems.

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Poster

258. Sleep: Systems and Behavior

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 258.28/PP1

Topic: E.08. Biological Rhythms and Sleep

Title: Sleep quality and alcohol use in older adults of Mexico City

Authors: *M. M. MELENDEZ[1,2], A. GALLEGOS-CARI[1], N. HERNANDEZ-LLANES[1], R. CAMACHO-SOLIS[1], J. VELAZQUEZ-MOCTEZUMA[4], A. JIMENEZ-ANGUIANO[4], J. SANCHEZ-SOSA[3], G. BORGES[5], M. MEDINA-MORA ICAZA[5]
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Abstract: Elderly population represents a challenge for health systems, because aging is associated with significant changes in health, including changes in sleep architecture and patterns. In addition, quality of life and sleep can also be altered by the consumption of alcohol and other drugs. In Mexico City lives around 500 thousand people over 65 years old, but there is a lack of information about alcohol use and misuse in Mexico City. The aim of the present study was to assess the association between sleep quality and alcohol use, abuse and dependence among older people of Mexico City. Method: Cross-sectional probabilistic house survey with a simple random representative sample of 2501 older adults residents of Mexico City. 2,098 agreed to participate with a response rate of 83.9%. Only those who accepted the informed consent were included in the study. Sociodemographic data were collected, sleep quality was assessed with the Pittsburgh Sleep Quality Index (PSQI) and a cut point of 5 or more were considered to indicate bad sleep quality. A survey form was designed to collect information about current (last 12 months) and lifetime (anytime in life) alcohol drinking. Association analysis between sleep quality and alcohol drinking patterns was made using the Odd Ratios (OR). Results: Descriptive statistics of sociodemographic variables were determined and the prevalence of alcohol and subjective sleep quality were obtained. OR between substance use and subjective sleep quality index was applied. The prevalence in the past year for alcohol consumption was 32.3%. Alcohol consumption variables like use lifetime (OR = 1.608, 95% CI = 1250-2068), hazardous drinking lifetime (OR = 1.748, 95% CI = 1345-2270) and alcohol dependence (OR = 1.684, 95% CI = 1055-2688) lifetime were significantly associated with poor subjective quality of sleep. Risky alcohol consumption in the past 12 months was also associated (OR = 1.609, 95% CI = 1051-2462) with poor subjective quality of sleep. Conclusion: The results showed an association between poor subjective quality of sleep and alcohol consumption in older adults. The results of this study provide information on possible areas of intervention that need to be taken into account by public policy makers to plan programs of care and treatment of older adults. It is also necessary to understand the underlying physiological causes between substance use and sleep disorders in older adults and its relationship to other diseases.

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Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.01/PP2

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Nordea Foundation

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Center for Healthy Aging

Title: Spontaneous glial calcium waves in cerebellar cortex *in vivo* reduce blood vessel diameter possible via constriction of pericytes

Authors: *C. MATHIESEN[1], B. L. LIND[2], A. BRAZHE[3], B. GESSLEIN[2], M. LAURITZEN[2,4]
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Abstract: Intercellular glial Ca2+ waves (GWs) constitute a signaling pathway between glial cells, and between glia, neurons and blood vessels. We report here that GWs which occur spontaneously in cerebellar cortex of aged mice *in vivo* reduce arteriole and capillary diameters through activation of pericytes. The activation of GWs are known to be associated with reduced oxygen tension. We tested two hypotheses: 1) GWs decrease vessel diameter via activation of pericytes, and 2) GWs reduce oxygen tension directly (re-establish ion gradient) and indirectly (reduce oxygen supply). We used two-photon imaging of cerebellar cortex of aged anaesthetized NMRI and NG2-DsRed (red fluorescent pericytes) mice. Bolus injection of Ca2+ indicator (Oregon Green BAPTA) was used to identify GWs as propagating Ca2+ signals in Bergmann glia. Line analysis was used to measure vessel diameter and oxygen tension was monitored using a Clark-type electrode. Calcium activity in pericytes were measured pixel-by-pixel in Dsred-pericyte-positive cells. In wild-type NMRI mice 3-5% of spontaneous GWs induced local constriction of capillaries and arterioles in a restricted part of the vessels, indicating that contractile elements were activated. ATP-evoked GWs were associated with a decrease in oxygen tension, indicating an increase of CMRO2 and a reduced perfusion. GWs - blood vessel interaction was then further evaluated using NG2-DsRed mice with red fluorescent pericytes, showing that the constriction associated with pericyte location. Conclusion: 1) GWs reduce arteriolar and capillary diameters by activation of pericytes. 2) GWs were associated with prolonged reduction in tissue oxygen tension. The increased GW activity during aging, lowered blood oxygen saturation, and self-amplification by GW-induced constriction suggests a relation to brain pathology.

Disclosures: **C. Mathiesen:** None. **B.L. Lind:** A. Employment/Salary (full or part-time):; University of Copenhagen, Denmark. **A. Brazhe:** None. **B. Gesslein:** None. **M. Lauritzen:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

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Program#/Poster#: 259.02/PP3

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Grant-in-Aid for Scientific Research 25460280

Title: Development of newly optical imaging systems of simultaneous measurement of changes in cerebral oxygen metabolism and hemodynamics by awake-mice

Authors: ***T. MATSUURA**[1,2], H. TAKUWA[2], A. NISHINO[1,2], Y. TAJIMA[2], H. ITO[2]
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Abstract: Hemodynamic response associated with neural activity has been investigated using positron-emission tomography (PET) and functional magnetic resonance imaging (MRI). Especially, PET allows the measurement of cerebral blood flow (CBF), cerebral blood volume (CBV) and cerebral metabolic rate of oxygen (CMRO2) in human and plays an important role to understand a mechanism of neurovascular coupling in clinical research. On the other hand, in animal studies, there is no optical imaging system, which evaluates changes in CBF and CBV, and oxygen metabolism, from the same brain area under awake condition, although they can be useful for investigating the mechanism of neurovascular coupling. In the present study, we tried to develop the simultaneous measurement method of laser speckle imaging (LSI) and intrinsic optical signal imaging (IOSI), which was verified by laser-Doppler flowmetry (LDF), during cerebral activation induced by whisker stimulation using awake-mice. Moreover, to measure oxygen metabolism, flavoprotein autofluorescence imaging (FAI) was performed from the same brain area as LSI and IOSI measurements. The increase in CBF according to LSI was correlated with that by LDF. Similarly, the increase in CBV obtained by IOSI was also correlated with RBC concentration change measured by LDF. The change in oxygen metabolism by FAI was correlated with that in CBF obtained by LSI, whereas the change in CBF was greater than that in oxygen metabolism. We revealed that the relationship between oxygen metabolism evaluated by FAI and CBF as measured by our system was in good agreement with the relationship between CMRO2 and CBF in human PET studies. In conclusion, our measurement system of CBF, CBV

and oxygen metabolism is not only useful for studying neurovascular coupling, but also easily corroborates human PET studies. All experiments were performed in accordance with the institutional guidelines on humane care and use of laboratory animals and were approved by the Institutional Committee for Animal Experimentation.

Disclosures: T. Matsuura: None. **H. Takuwa:** None. **A. Nishino:** None. **Y. Tajima:** None. **H. Ito:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

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Title: Coupling of spontaneous and sensory evoked hemodynamic signals to neural activity in the barrel cortex of the awake mouse

Authors: *A. WINDER[1], P. J. DREW[1,2]
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Abstract: Increases in cerebral blood flow, volume and oxygenation follow sensory evoked neural activity, but it is unknown whether this neurovascular coupling holds for spontaneous neural fluctuations. Understanding whether the neurovascular relationship remains unchanged during spontaneous fluctuations in neural activity is important for the interpretation of resting state fMRI signals. We sought to determine if spontaneous fluctuations in cerebral blood volume (CBV) were driven by neural activity by simultaneously measuring CBV, using intrinsic optical signals, and neural activity in the barrel cortex of alert, head-fixed mice. Gamma-band power of the local field potential (LFP) and CBV were positively correlated in the absence of sensory stimulation or volitional behavior. We calculated the hemodynamic response functions (HRF), which relate the increase in blood volume to neural activity, using data from three different

behavioral states (sensory stimulation, volitional whisking, and resting behavior). All three behaviors yielded the same HRF, indicating that neurovascular coupling is the same for spontaneous and sensory evoked behavior. In order to determine how well CBV fluctuations represent neural activity, we predicted the measured hemodynamics by applying the HRF to neural activity for each behavior type. The trial-average sensory evoked hemodynamics were well fit by the trial-average neural activity (R^2=0.7±0.20), however the correlation was much lower on a trail-by-trial basis (R^2=0.30±0.57). Spontaneous hemodynamic signals were not well predicted from spontaneous neural activity (mean R^2=0.02±0.11) during individual trials. These findings are consistent with the presence of a separate, additive, uncorrelated hemodynamic signal which is of similar magnitude as the neurally evoked changes in blood volume. Thus, hemodynamic signals are coupled to neural activity on average, but isolated changes in hemodynamic signals cannot be taken as reliable indicators of changes in neural activity.

Disclosures: A. Winder: None. **P.J. Drew:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant T32-NS07391

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Title: fMRI measurements of spiking vs. synaptic activity in the rat olfactory bulb

Authors: *A. J. POPLAWSKY[1], H. FUKUDA[1], S.-G. KIM[1,2]
[1]Radiology, Univ. of Pittsburgh, Pittsburgh, PA; [2]Biol. Sciences, Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci. (IBS), SKKU, Suwon, Korea, Republic of

Abstract: Introduction Functional magnetic resonance imaging (fMRI) measures the hemodynamic response to neural activity; but more evidence is needed to understand the relative contributions of spiking and synaptic activities to neurovascular coupling. The olfactory bulb is

an ideal model system to study this issue because spiking activity from mitral cells (output neurons) can be selectively decreased while preserving synaptic activity. In particular, odors excite olfactory receptor neurons that project to the olfactory bulb through the olfactory nerve layer and form excitatory synapses with the apical dendrites of mitral cells in the glomerular layer, which descend through the external plexiform layer and initiate action potentials to cortical targets at the mitral cell layer. In addition, inhibitory granule cells are excited by anterior commissure (AC) stimulation, which form dendro-dendritic synapses with mitral cells in the external plexiform layer and inhibit mitral cell spiking. **Methods** In anesthetized Sprague-Dawley rats, excitatory and inhibitory pathways were preferentially activated with individual and simultaneous stimulations of odor (5% amyl acetate in mineral oil) and electrical stimulation of AC, respectively; and the cerebral blood volume (CBV)-weighted fMRI responses were compared. This contrast-enhanced fMRI technique was previously shown to have increased sensitivity in capillaries near the site of neural activity. High-resolution fMRI experiments (110x110x500 μm^3) were performed at 9.4 T. **Results** fMRI signals increased for the individual stimulations of odor and AC in layers specific to neuro-modulation. Particularly, activation peaked in olfactory nerve and glomerular layers for odor, and in granule cell layer for AC. To assess the relative contributions of spiking activity to the hemodynamic response, differential analysis was performed between the sum of the individual stimulations and the simultaneous stimulation ($[Odor_{alone} + AC_{alone}] - Odor+AC_{together}$), where odor-evoked mitral cell spiking activity should be suppressed in the $Odor+AC_{together}$ condition, but remain intact in the $[Odor_{alone} + AC_{alone}]$ condition. We observed significant fMRI responses in glomerular, external plexiform, mitral and granule cell layers (one-sample t-test, $p<0.05$), with the most significant difference occurring at the site of output spiking activity (mitral cell layer: $p = 0.007$, $0.28 \pm 0.08\%$ fMRI signal change). At this layer, $3.71 \pm 0.37\%$ signal change is attributed to synaptic activity ($Odor+AC_{together}$). **Conclusions** Our preliminary results indicate that synaptic activity is the dominant source of the hemodynamic response.

Disclosures: **A.J. Poplawsky:** None. **H. Fukuda:** None. **S. Kim:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

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Program#/Poster#: 259.05/PP6

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: CIHR

Title: Neural correlates of spontaneous BOLD fluctuations: A simultaneous LFP-fMRI investigation in the nonhuman primate

Authors: *N. HASHEMI[1,3], R. M. HUTCHISON[4,3], J. S. GATI[3], R. S. MENON[3], S. EVERLING[1,2,3]
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Abstract: Resting-state fMRI (rs-fMRI) is widely used to explore functional connectivity (FC) between brain regions and is being applied to characterize alterations in the coupling patterns across neurological and psychiatric diseases. However, the neural basis of spontaneous low frequency blood-oxygen level dependent (BOLD) rs-fMRI fluctuations is poorly understood, limiting the interpretation that can be made from the findings. Here, we acquired rs-fMRI data in macaque monkeys together with simultaneous recordings of local field potential recordings. Multiple scans (40, 150 vol, TR = 2 s) were acquired from two macaque monkeys (M. fascicularis) anesthetized at 1% isoflurane in a 7T Agilent scanner. Local field potentials were recorded in prefrontal area 8d using bilaterally implanted 16-channel multi-electrode laminar arrays. BOLD fMRI data were preprocessed using standard resting-state analysis techniques without global mean signal regression and normalized to a standardized atlas. After removal of gradient artifacts, band-limited power was computed by band-pass filtering the signal in different frequency bands (delta: <4 Hz, theta: 4-7 Hz, alpha: 8-16 Hz, beta: 24-36 Hz, gamma: 44-56 Hz), rectifying the signal, and resampling it every 2 s. We then convolved the band-limited power LFPs with a standard double-gamma hemodynamic response function (HRF) to account for the hemodyamic lag and used these timecourses together with the timecourse of the BOLD signals in the vicinity of the electrodes as predictors in a regression model for FC analysis. For the BOLD signal in area 8d, we found strong, distributed FC with the medial parietal lobe, the inferior temporal lobe, and areas in the superior temporal sulcus, a pattern consistent with our early work in a larger sample of monkeys. Critically, we observed connectivity maps with the same spatial topology when using the power envelops of LFPs in the alpha and beta frequency bands. Our results suggest that intrinsic connectivity networks may be specifically driven by unique LFP profiles that are dominated by alpha and beta frequency bands unique to different networks.

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Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NSF Grant BCS1063774

Title: Comparison of high-resolution functional magnetic resonance imaging performance among spiral trajectory variants and echo-planar imaging

Authors: *V. SINGH[1], D. RESS[2]
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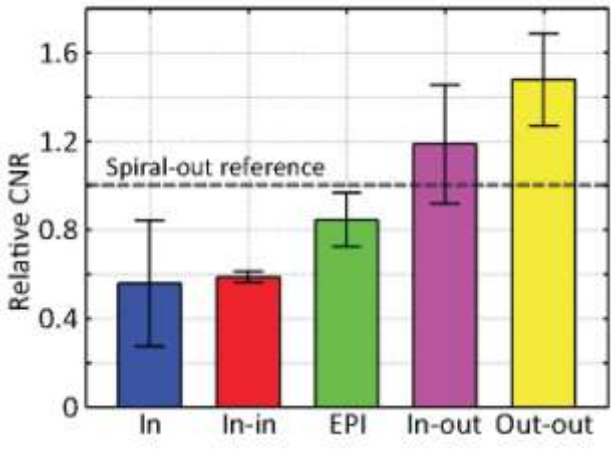
Abstract: Introduction: High-resolution functional magnetic resonance imaging (fMRI) operates in a regime with low signal levels and requires long readouts that lead to T2* signal decays that can reduce performance. Accordingly, there is a need to compare the performance of the most popular acquisition schemes for fMRI: echo-planar imaging (EPI) and spiral at high spatial resolution. Methods: We compared the performance of these trajectories for 1.2-mm sampling fMRI using a moving-dot stimulus presented alternately to left-and-right visual fields with a 24-s period. This stimulus evokes a strong response in visual areas that was quantified by calculating a functional contrast-to-noise ratio (CNR). Measurements were performed in early visual cortex and superior colliculus (SC) using 8-12 quasi-axial slices. CNR data was obtained for EPI as well as both single- and dual-echo variants of spiral trajectories. Echo time was first adjusted for each trajectory to achieve optimal CNR. Comparisons were then obtained over many scanning sessions in which each trajectory variant was compared (at its optimal echo time) against a single-echo spiral-out used as a reference. This data was obtained for three subjects using a Siemens Skyra 3T scanner. Results: Figure below shows ratio of CNR obtained for each sequence variant to the reference CNR. The preliminary data show that dual-echo spiral out offers the best performance in SC, significantly outperforming both EPI and single-echo spiral trajectories. Pure spiral-in trajectories perform comparatively poorly. Analysis of data in visual cortex is underway, and further sessions on additional subjects are planned.



Disclosures: **V. Singh:** A. Employment/Salary (full or part-time):; The University of Texas at Austin. **D. Ress:** A. Employment/Salary (full or part-time):; The University of Texas at Austin, Baylor College of Medicine.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.07/PP8

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Intramural Research Program of NINDS

Title: Investigating the spatiotemporal characteristics of the bold and the non-bold response across cortical layers in awake marmosets

Authors: ***C. C.-C. YEN**, D. PAPOTI, A. C. SILVA
NINDS/LFMI/CMS, Natl. Institues of Hlth., Bethesda, MD

Abstract: Blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) has a strong prognostic potential in ischemic stroke due to its non-invasive nature, high spatial resolution in differentiating cortical layers, high sensitivity to changes in hemodynamics, and unlimited penetration depth. However, reports of the spatiotemporal characteristics of the cortical fMRI response are sparse and non-conclusive. For instance, there is no consensus about the onset time (OT) of the BOLD fMRI response across each cortical layer. One source of contamination to BOLD fMRI is the inflow effect, which may contribute differently at the pial

surface versus parenchyma due to the different orientation of tangential pial vessels and penetrating cortical vessels. To address these concerns, we adopted a dual-echo sequence to separate the pure-BOLD component from non-BOLD components, including the inflow effect, and used it to map laminar BOLD responses with high spatiotemporal resolution in conscious, awake marmosets, a small New World non-human primate model of increasing value to neuroscience. Four adult male marmosets were acclimated to head restraint by custom-built helmets in the sphinx position inside a horizontal 7T/30cm MRI spectrometer. High-resolution BOLD functional images were obtained every 200ms using a dual-echo gradient-recalled EPI sequence from a single coronal slice. The median and ulnar nerves were noninvasively stimulated by pairs of electrode pads placed across both wrists. The bilateral stimulus paradigm consisted of 4s off, 4s on and 24s off epochs. AFNI and Matlab were used for processing the data, calculating the pure-BOLD (T2*) and non-BOLD (S0) components, laminar segmentation and statistical analysis. Robust BOLD responses were bilaterally detected in primary and secondary somatosensory areas. Laminar OT values were determined to be 1.4s in layers 1 to 3, 1.2s in layer 4 and 1.6s in layers 5 to 6, which was consistent with the order previously reported in rodents by our group. Robust pure-BOLD responses were observed across all layers except layers 1 and 2, which showed an insignificant pure-BOLD response but a strong and delayed non-BOLD response. We have demonstrated the feasibility of measuring the dynamics of pure-BOLD and non-BOLD changes across the cortical layers of conscious, awake marmosets. A significant contribution of the non-BOLD component was found on the superficial layers, which may be attributed to the inflow effect from pial vessels running tangentially to the cortex and perpendicular to the imaging plane. Therefore, caution should be exercised when measuring the laminar onset time of BOLD fMRI.

Disclosures: **C.C. Yen:** None. **D. Papoti:** None. **A.C. Silva:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.08/PP9

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH NINDS F31NS084538

NIH NINDS R01NS063226

NIH NINDS R01NS076628

Human Frontier Science Program (HFSP)

NSF 0954796

Title: Mechanisms of neurovascular maturation in the developing brain

Authors: *M. G. KOZBERG[1], Y. MA[1], M. A. SHAIK[1], C. N. LUNARDI[2], A. J. GOMES[2], E. TFOUNI[3], E. M. C. HILLMAN[1]
[1]Dept. of Biomed. Engin., Columbia Univ., New York, NY; [2]Univ. of Brasilia, Brasilia, Brazil; [3]Univ. of San Paulo, Ribeirao Preto, Brazil

Abstract: Localized changes in brain blood flow are the basis of signals measured using functional magnetic resonance imaging (fMRI). In adults, this 'hemodynamic response' consists of an increase in blood delivery in regions of increased neural activity occurring in response to somatosensory stimulation. However, prior work from our laboratory in a rodent model has demonstrated that neonatal neurovascular coupling is still maturing post-natally, progressing from an entirely inverted response at postnatal day 7 in mice (P7) and P12 in rats to an adult response by approximately P23. We hypothesize that these differences in neurovascular coupling are due to underlying differences in neural activity, communication between neurons and vasculature, and/or the vasculature itself. We are studying developing neural activity in parallel to hemodynamic activity using exposed-cortex wide-field multi-spectral optical intrinsic signal imaging of hemoglobin absorption with interleaved fluorescence imaging in Thy1-GCaMP3 mice. These mice express a genetically encoded calcium sensor in cortical pyramidal neurons. We observed localized neural activity from P7 to adulthood, while hemodynamic responses were initially absent and increased with age. Although spatiotemporal differences in the developing neural response were observed, these changes cannot fully account for the changing hemodynamic response leading to our continued study of the vasculature. As the hemodynamic response matures, we found that functional hyperemia is initially localized to the capillary bed and/or diving arterioles and there is a marked lack of dilation in pial arteries. Based on these findings, we hypothesize that the mechanism of increasing functional hyperemia throughout development may be related to the development of cortical arteries both morphologically and functionally. Using in-vivo two-photon microscopy, we quantified changes in pial vascular architecture and found that despite large changes in pial venous and capillary architecture, the distribution of pial arteries in rats is consistent from postnatal day 12 to adulthood. We are now examining whether these arteries are physically able to dilate. Arterial function is being assessed in-vivo through the application of both endothelium-dependent and endothelium-independent vasodilators to the cortex and the quantification of resulting arterial diameter changes. We are also exploring potential changes in vascular function using a novel light-activated nitrosyl nitric oxide donor. We will present the results of our analysis aiming to understand the relationship between developing neural activity and vascular function.

Disclosures: **M.G. Kozberg:** None. **Y. Ma:** None. **M.A. Shaik:** None. **C.N. Lunardi:** None. **A.J. Gomes:** None. **E. Tfouni:** None. **E.M.C. Hillman:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.09/PP10

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

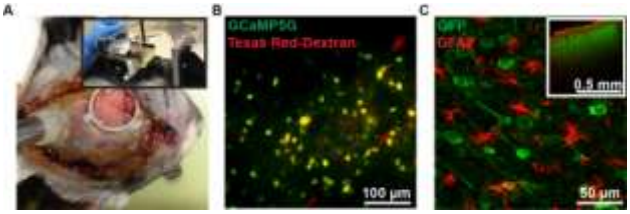
Support: National Institute of Neurological Disorders Intramural Research Program

Title: Development of long-term two-photon awake imaging in marmosets: A novel optical imaging model for studies of cerebral microcirculation and neuroglial activation in primates

Authors: ***T. P. SANTISAKULTARM**, J. L. CIUCHTA, J. PARK, S.-H. CHOI, A. C. SILVA
Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD

Abstract: Two-photon Excited Fluorescence (2PEF) microscopy is a nonlinear optical imaging technique that allows visualization of cellular structure and function located deep within the cortex, and followed longitudinally over days to months. Recently, transgenic marmoset models of Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and schizophrenia have been successfully produced, providing a research biological model that closely mirrors the human. In the present work, we aim to develop a longitudinal approach to quantify cerebral hemodynamics and neuronal activity in awake marmosets. An adult marmoset was gradually acclimated to a custom-designed, body and head restraint in the sphinx position for up to two hours, over a period of three weeks. Following behavioral training, AAV2/1-GCaMP5G virus was injected into the somatosensory cortex, and a 12-mm diameter polyetheretherketone cranial chamber was implanted over Broadman Area 3b, along with a headpost at the medioposterior to stabilize imaging (Figure 1A). After 10 days of recovery, 2PEF of intravenously labeled blood plasma revealed vascular topology and enabled linescan measurement of red blood cell (RBC) motion in microvessels 500 μm below the cortical surface in awake marmosets. 99.5% of capillaries had RBC flow during the awake imaging, indicating a robust blood flow distribution. Capillary density was 5,601 capillaries/mm3. Neurons exhibited fluorescence changes, both spontaneously and in response to electrical stimulation to fore paw (Figure 1B). Fraction of responsive neuronal dendrites was 0.45. The volume of GCaMP5G expression was estimated to be 3 mm3 using post-mortem immunohistochemistry (Figure 1C). These results demonstrate the capability to perform long-term 2PEF imaging of cerebral microcirculation and neuronal activity

in awake marmosets. This work provides a novel and insightful imaging technique to assess neurovascular coupling at the spatial resolution of the neurovascular unit, and allows for investigation of critical mechanisms in many neurological disorders.



Disclosures: **T.P. Santisakultarm:** None. **J.L. Ciuchta:** None. **J. Park:** None. **S. Choi:** None. **A.C. Silva:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.10/PP11

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Scholar Award from the McKnight Endowment Fund for Neuroscience

National Scientist Development grant from the AHA

NIH Grant R01NS078168

NIH Grant R01NS079737

Title: Neurovascular coupling and decoupling in the cortex during voluntary locomotion

Authors: *B.-X. HUO[1], J. B. SMITH[1], P. J. DREW[1,2]
[1]Ctr. for Neural Engin., [2]Neurosurg., Pennsylvania State Univ., University Park, PA

Abstract: Functional brain imaging techniques, such as fMRI, rely on localized hemodynamic responses to infer neural activity in the brain. The relationship between hemodynamics and neural activity, known as neurovascular coupling, has been widely assumed to be a one-to-one mapping, with increases in neural activity driving increases in blood flow and volume. Here, we tested whether neurovascular coupling holds true in frontal and sensorimotor cortex during natural behavior, using head-fixed mice engaging in voluntary locomotion. We recorded the

cerebral blood flow and volume changes in both frontal and parietal cortices in animals installed with bilateral, thinned-skull windows using intrinsic optical imaging; and local field potential (LFP) and multi-unit activity (MUA) in either frontal or parietal cortex in animals implanted with chronic stereotrodes. We found that locomotion induced increases of gamma-band power of the LFP and firing rate in both frontal and parietal cortices. Increased neural activity was paired with a subsequent hemodynamic response in the parietal cortex, particularly in the forelimb and hindlimb representations in the sensorimotor cortex. However, in the frontal cortex, the hemodynamic response is largely absent, regardless of the duration of locomotion. Our observation challenges the consensus that increases in neural activity are always accompanied by increases in blood flow and volume in the cortex. It also calls for caution when interpreting the functional brain imaging results.

Disclosures: B. Huo: None. **J.B. Smith:** None. **P.J. Drew:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.11/PP12

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R21 26167539

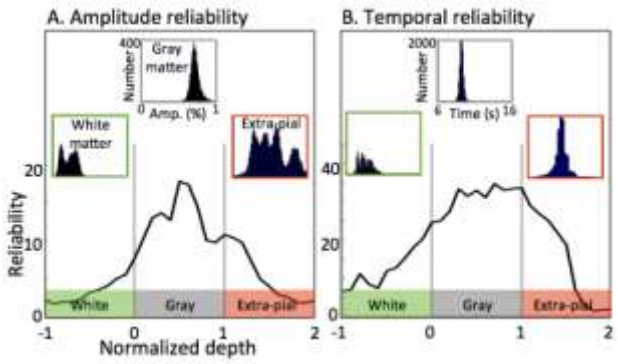
NSF BCS 1063774

Title: Depth and tissue variations of BOLD signal reliability in human visual cortex

Authors: *J. KIM, D. RESS
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Motivation: At conventional spatial sampling (~3 mm), the BOLD response includes contributions from superficial vascular sources (extra-pial tissue), white matter, as well as the desired gray matter. It is therefore of strong interest to quantify the contributions of these various tissues to the BOLD response and its signal reliability. We measured the BOLD hemodynamic response function (HRF) in visual cortex using high-resolution fMRI, then performed non-parametric statistical analyses to characterize the reliability of the HRF amplitudes and timing as a function of tissue type and gray-matter depth. Methods: Stimulus is a series of 1.7-s pulses of 4-Hz flickering dots followed by 26-s blank periods. High-resolution (0.8-mm voxels) fMRI data

is obtained using a 3T Siemens scanner and an interleaved spiral acquisition to produce ~100 HRFs/session. A self-reciprocal depth coordinate is calculated from a high-resolution (0.7 mm) volume anatomy using a weighted signed-distance approach. HRFs are averaged together as a function of tissue depth in visual areas V1-3, allowing us to distinguish between tissue types. Amplitude and timing parameters are extracted from the HRF. We use bootstrapping to estimate the distribution for each HRF parameter. The variability of each parameter is quantified by its 68% confidence intervals. Reliability is defined as the ratio of the average measured value of a parameter to its variability Results: HRF amplitude reliability showed a sharp maximum in the center of the gray matter (Fig. A). The reliability of the HRF timing showed a much broader maximum extending through the superficial and intermediate gray matter (Fig. B). Distributions of both parameters (insets) were markedly non-Gaussian in extra-pial tissue (orange) and white matter (green). Conclusion: Amplitude reliability may correlate to the vascular density that is highest in the intermediate gray matter, while temporal reliability is high throughout the gray matter. Noise distributions are complex and multi-modal outside the gray matter.



Disclosures: **J. Kim:** None. **D. Ress:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.12/PP13

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: The Finnish Funding Agency for Technology and Innovation – Tekes

UEF-Brain Strategic funding by University of Eastern Finland

Title: Comparison of five different anesthetics for phMRI using nicotine challenge

Authors: *J. K. HUTTUNEN[1], J. PAASONEN[1], R. SALO[1], K. LEHTIMÄKI[3], E. JOHANSSON[4], M. M. FORSBERG[2], O. GRÖHN[1]
[1]A. I. Virtanen Inst., [2]Sch. of Pharm., Univ. of Eastern Finland, Kuopio, Finland; [3]Charles River Discovery Res. Services Finland, Kuopio, Finland; [4]AstraZeneca R&D Mölndal, Mölndal, Sweden

Abstract: In animal functional imaging studies the choice of anesthetic is perhaps the single most important decision in the experimental design, since the exact effects of anesthetics to metabolism and neurotransmitter systems and therefore to the expected results are often unknown. The aim of this study was to investigate the blood oxygenation level dependent (BOLD) signal changes under different anesthetics with a nicotine challenge in order to find optimal experimental conditions for a pharmacological magnetic resonance imaging (phMRI) study. Altogether 79 male Wistar rats (353 ± 32 g) were used with different anesthesia protocols. After surgery isoflurane was continued for one group (ISO, n= 7) and for the rest anesthesia was switched to either α-chloralose (AC, n = 7), medetomidine (MED, n = 7), thiobutabarbital (TBB, n = 17) or urethane (URE, n = 41). All animals were ventilated except two groups under URE and TBB. MRI measurements were performed with 7 T Bruker PharmaScan. Functional data were acquired using BOLD, cerebral blood flow (CBF) and cerebral blood volume (CBV) methods. A bolus of nicotine (hydrogen tartrate salt 0.25 mg/kg) was administered intravenously. Positive BOLD response was observed in all animals except in a subgroup of TBB animals where the response showing both positive and negative signal changes were seen. Maximum BOLD changes for the groups are as follows: MED 4.5 ± 1.1 %, TBB first group 6.6 ± 2.5 %, TBB second group -1.5 ± 0.6 %, TBB (not ventilated) 4.9 ± 0.7 %, URE (not ventilated) 9.3 ± 2.3 %, URE 9.0 ± 1.9 %, and ISO 2.2 ± 1.0 %. Maximum positive BOLD response was measured 58 ± 13 s after the nicotine injection. Saline control injections did not cause detectable signal changes. In addition, local field potential (LFP) from somatosensory cortex was measured simultaneously with BOLD (URE, n = 6). Nicotine decreased the spectral power of slow potentials. Additionally, nicotine increased the spectral power at range of 13-70 Hz and this effect lasts over the recording period. With pretreatment of mecamylamine no clear decrease in slow potentials can be seen. Mecamylamine itself decreased the spectral power of 13-70 Hz dramatically and diminished the nicotine response in this range. The BOLD responses in every rat highly resemble the measured spectral power at range of 13-70 Hz. The results revealed significant differences in the responses of acute nicotine challenge with different anesthesia protocols. Despite the fact that the exact mechanisms of action of anesthetics are still mostly unknown, the phMRI method with careful study design has great potential in brain mapping and characterization of drug effects.

Disclosures: **J.K. Huttunen:** None. **J. Paasonen:** None. **R. Salo:** None. **K. Lehtimäki:** A. Employment/Salary (full or part-time):; Charles River Discovery Research Services Finland. **E.**

Johansson: A. Employment/Salary (full or part-time):; AstraZeneca R&D Mölndal. **M.M. Forsberg:** None. **O. Gröhn:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.13/PP14

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: BHF grant RG/12/6/29670

Title: Imaging brain microvascular dynamics using CellVizio confocal endomicroscopy in hypertensive rats *in vivo*

Authors: *D. N. MAYOROV, J. F. R. PATON, S. KASPAROV
Physiol. & Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstract: We first evaluated the feasibility of morphometric analysis of brain microvasculature in normotensive and spontaneously hypertensive (SH) rats in cortical and brainstem slices from P21-P45 pups using confocal microscopy (Leica SP5). The changes in vascular diameter in response to application of vasodilator agents, adenosine and sodium nitroprusside, were assessed after vascular pre-constriction with a thromboxane agonist U46619. Treatment with U46619 (100 nM) constricted arterioles to 90 ± 4% and 92 ± 3% of the resting diameter in normotensive and SH rats, respectively. Adenosine (1 μM) reversed the U46619-induced vasoconstriction by 55% and 25% in normotensive and SH rats, respectively. Sodium nitroprusside (1 μM) produced little effect on the U46619-induced vasoconstriction in both groups. Next, brain vasculature of anaesthetized rats was visualized using intravenous FITC-dextran and imaged using a fiber probe-based confocal laser endomicroscopy (CellVizio, Mauna Kea Technologies). Pial microvessels of the ventral or dorsal medulla oblongata, and the cortex were imaged in normotensive and SH rat pups (P21-P45) using S-1500, Mini-Z and Z reusable fiberoptic probes (Lateral resolution: 3.5 μm; field of view: 325 to 650 μm) coupled to a 488 nm laser scanning unit (CellVizio). In addition, beveled probes S-300/B and S-650/B (300 and 650 μm tip diameter, respectively) are being used to visualize the vascular tree in the Z-dimension. Vascular contractility is now being assessed using application of U46619 (100-500 nM), adenosine (1-10 μM) and sodium nitroprusside (1-10 μM) and data compared with that from brainstem slice preparations. Currently, we are working to adapt this method for morphometric analysis of the brainstem capillary bed. CellVizio confocal endomicroscopy provides a promising approach for

imaging and morphometric analysis of brain microvasculature *in vivo*. This approach will be useful to investigate changes in vascular reactivity of the cerebral microcirculation in hypertension and other vascular pathologies *in vivo*.

Disclosures: D.N. Mayorov: None. **J.F.R. Paton:** None. **S. Kasparov:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.14/PP15

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH NS088827

NIH EY017925

Title: Neural correlates of single-vessel hemodynamic responses *in vivo*

Authors: P. O'HERRON, P. CHHATBAR, M. LEVY, A. SCHRAMM, *P. KARA
Neurosci., MUSC, CHARLESTON, SC

Abstract: The activation of neurons in the cerebral cortex creates a demand for energy that is met by a local increase in blood flow. Hemodynamic imaging techniques, e.g., fMRI, use these vascular signals to infer the location and strength of neural activity. However the precise spatial scale and types of neural activity that drive vascular responses are still largely unknown. The relative roles of synaptic activity and action potential (spikes) activity in generating hemodynamic signals has been examined by combining hemodynamic measures with electrode recordings of spikes and local field potentials (LFPs). But due to the low spatial resolution of fMRI and intrinsic signal optical imaging methods, the relatively sparse sampling of neurons with electrodes, and the uncertainty about what LFPs represent, these studies have generated considerable controversy. Additionally, although the response selectivity profiles of individual neurons have been studied extensively, they have never been characterized for vascular signals at the resolution of individual blood vessels. Using *in vivo* two-photon microscopy, we measured blood flow changes in individual vessels and correlated them with the synaptic (using the fluorescent glutamate sensor iGluSnFR) and spiking activity (using fluorescent calcium sensors) in the surrounding neural tissue. We measured sensory-evoked vascular, spiking and synaptic activity in the cat primary visual cortex, where neurons are clustered by their preference for

stimulus orientation. We show that blood flow changes in parenchymal vessels (located within neocortical layer 2/3) have orientation preferences that match those of the surrounding neural tissue. Pial surface vessels were highly responsive to sensory stimuli but unlike parenchymal vessels, surface vessels had no orientation selectivity. Importantly, our preliminary data indicate that the orientation selectivity of parenchymal vessels matches the selectivity of glutamate release integrated over a region of a few hundred microns surrounding the vessel, whereas spiking responses were always more selective, regardless of the spatial scale over which they were integrated. These results indicate that hemodynamic signals from individual parenchymal vessels in the neocortex closely follow local excitatory synaptic activity rather than spikes. Unraveling the spatial range over which individual blood vessels may pool specific types of neural signals will allow for more accurate decoding of synaptic and spiking activity from hemodynamic signals.

Disclosures: **P. O'Herron:** None. **P. Chhatbar:** None. **M. Levy:** None. **A. Schramm:** None. **P. Kara:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.15/PP16

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: R01 NS076628 (NINDS)

R01 NS063226 (NINDS)

NSF 0954796

UL1 RR024156 (NCATS)

Title: A new non-linear model of the fmri bold response

Authors: ***J. PORTES**[1], C. B. AMOOZEGAR[2], B. R. CHEN[2], M. G. KOZBERG[3], M. S. SHAIK[2], E. M. C. HILLMAN[4]
[1]Biomed. Engineering, Columbia Univ., New York, NY; [2]Biomed. Engin., [3]Neurosci., [4]Biomed. Engin. and Radiology, Columbia Univ., New York, NY

Abstract: Blood flow in the brain changes when the brain exhibits neuronal activity. Stimulus-evoked fMRI measures these hemodynamic changes as a proxy for neuronal activity. Better understanding the cellular and vascular mechanisms that drive blood flow changes in the working brain will enable improved interpretation of fMRI data. While many have assumed that the fMRI response can be modeled as a linear convolution of a hemodynamic response function (HRF) and a neuronal input function, numerous studies have noted both spatial and temporal non-linearities in the response. For example, short duration visual stimuli lasting a few milliseconds can evoke an almost identical fMRI BOLD response to stimuli lasting 1-2 seconds, while longer duration stimuli yield responses with increasing peak amplitudes. In recent work from our lab, we have proposed a new cellular mechanism for neurovascular coupling that involves signal propagation within the vascular endothelium. This mechanism introduces a new basis for the spatiotemporal evolution of the hemodynamic response to stimulation in the brain, as well as a biological basis for the non-linear properties of the BOLD signal. To test our hypothesis, we have developed a new non-linear model of the BOLD response using high spatiotemporal resolution exposed cortex optical imaging data in rodents. We have found that our model can accurately predict many of the complex features of the hemodynamic response to stimuli of varying durations, and is wholly consistent with the involvement of the vascular endothelium in neurovascular coupling. Results and validation of this model will be presented.

Disclosures: J. Portes: None. **C.B. Amoozegar:** None. **B.R. Chen:** None. **M.G. Kozberg:** None. **M.S. Shaik:** None. **E.M.C. Hillman:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.16/PP17

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: A rubber hand illusion: Lateralization of brain area that is associated with the body image

Authors: *Y. KODAN[1], A. MITANI[1], R. NAKAI[2], T. INO[3]
[2]Kokoro Res. Ctr., [1]Kyoto Univ., Kyoto City, Japan; [3]Neurol., Rakuwakai-Otowa Hosp., Kyoto, Japan

Abstract: The feeling of bodily self-attribution is critical for our daily interaction with the outside world. In experimental studies, subjects have perceived touch sensations as arising from a rubber hand when the rubber hand and their own real hand are repeatedly brushed in synchrony

with the real hand hidden from view. This rubber-hand illusion provides a cue for studying the bodily self-attribution. In the present study, using functional magnetic resonance imaging, we investigated whether there were any brain areas related with producing the bodily self-attribution. Eleven right-handed healthy subjects participated in the imaging experiments. The study was performed in conformity with the Declaration of Helsinki, and approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine (E1287-1). All subjects gave informed written consent prior to participation. We performed both visual and somatic rubber-hand illusion tests (Ehrsson et al., 2005): there were four experimental conditions. In visual rubber-hand illusion tests, (1) the right rubber hand was aligned to the subject's own right hand and the experimenter synchronously brushed the rubber hand and the subject's hidden right hand, and (2) the left rubber hand was aligned to the subject's own left hand and the experimenter synchronously brushed the rubber hand and the subject's hidden left hand. In somatic rubber-hand illusion tests, the subjects were blindfolded and (3) the experimenter moved the subject's right hand holding a brush so that it brushed the left rubber hand and synchronously brushed the subject's left hand with another brush, and (4) the experimenter moved the subject's left hand holding a brush so that it brushed the right rubber hand and synchronously brushed the subject's right hand with another brush. Each experiment was conducted as a block design. We hypothesized that brain areas related with producing the bodily self-attribution would be activated during four illusion conditions. Such activation was detected in the right medial prefrontal cortex. The right medial prefrontal cortex may play a key role in the mechanism for bodily self-attribution.

Disclosures: Y. Kodan: None. **A. Mitani:** None. **R. Nakai:** None. **T. Ino:** None.

Poster

259. Functional Neuroimaging: Neurovascular Mechanisms

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 259.17/PP18

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: K08 MH080329

Title: Parametric application of transcranial alternating current stimulation leads to selective increases in BOLD signaling

Authors: *C. WALKER[1], N. POLIZZOTTO[2], V. SHARMA[2], E. SANTARNECCHI[4], A. ROSSI[4], S.-G. KIM[3], R. CHO[2]

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Abstract: Non-invasive neural stimulation techniques have received renewed interest as causal probes of brain function as well as being studied as a clinical therapeutic approach. Transcranial alternating current stimulation (tACS) offers the promise of a frequency-specific approach targeting specific networks underlying cortical oscillations. However, the effects of frequency specific stimulation on broader cortical network function are unknown. To investigate this question, we collected functional magnetic resonance imaging (fMRI) data from 10 participants while applying 1,500 μA currents with sinusoidal waveforms with frequencies of 2, 6, 10, 20, and 40 Hz. tACS electrodes were placed over the inion and at the base of the neck in an effort to confine stimulation to occipital visual areas. Participants completed a backward-masked, visual discrimination task, with stimulation on vs. off blocks counterbalanced across subjects. Across all participants, response times and accuracy were relatively unchanged. However, fMRI data revealed significant tACS by visual stimulus interactions to 2 and 40 Hz stimulation across a broad set of cortical regions. For both frequencies, blood-oxygen level dependent (BOLD) signaling increased in anterior cingulate cortex. During periods of 2 Hz stimulation, BOLD signaling also increased in the right superior and inferior middle temporal, fusiform, and lingual gyri as well as the right claustrum and cuneus. For 40 Hz stimulation, BOLD signals were increased along several midline regions including anterior cingulate, precuneus, and the right parahippocampal gyrus, thalamus, and caudate. No frequency was found to enhance BOLD activity in primary visual areas. Taken together, these findings suggest that, while tACS may affect neural function on a local circuit level, such changes are not detectable with fMRI. Rather, the local influence of tACS is more likely manifest in downstream enhancement of cortical function resulting in increased BOLD signaling in higher level regions along the ventral visual processing pathway. That these effects are frequency specific increases our understanding of how tACS may be used to stimulate both local and network functioning in the brain, and highlights the need for a more thorough understanding of how such stimulation techniques affect neural processes.

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Poster

260. Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant RO1AG036863

Title: Old habits die hard: what happens when predictive relationships change in a probabilistic sequence learning task?

Authors: *S. A. KISER[1], K. M. O'NEIL[1], R. L. M. FULLER[1], D. V. HOWARD[2,3], J. H. HOWARD, Jr.[1,2,3,4]
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Abstract: Implicit probabilistic sequence learning (IPSL) is a specific type of implicit learning that involves extracting statistical regularities from sequences of events and is important for a variety of life skills. We are able to learn statistical regularities amidst distraction and in a continuously changing environment. The present study investigates how an altered probabilistic learning environment affects IPSL. To accomplish this, we modified the Triplets Learning Task (TLT) to incorporate changing stochastic relationships among events across sessions. Fifteen college-aged participants completed three, 500-trial sessions of the modified TLT. On each trial three consecutive "lights" called triplets appeared on a computer screen. Participants observed the 1st two "cues" and responded to the 3rd, "target" by pressing a corresponding key. Unbeknownst to them, the 1st light predicted the target location on 80% of the trials and any other location on 20% of the trials. In order to introduce changes to the predictive relationships in the task, high and low triplet type (TT) frequencies from the 1st session were reversed in the 2nd session and then restored in the final session. Therefore, in the 2nd session all formerly high frequency triplets became low frequency and some formerly low frequency triplets became high frequency. In the 3rd session, TTs reverted back to their initial frequencies from session one. Reaction time (RT) data were analyzed using a 2 (TT: high vs low) X 6 (Epoch: 2 epochs per session) repeated measures Analysis of Variance. Results showed that participants became faster with practice as evidenced by a significant main effect of Epoch ($p<0.001$). A marginally significant interaction between TT and Epoch suggests that participants learned to differentiate high from low frequency events ($p=0.076$). However, the overall main effect of TT was not significant ($p=0.836$). Close inspection of the mean RT to different TTs across Epochs revealed learning occurred in the 1st session. However, during the 2nd session participants did not adapt fully to the reversed predictive relationships, still responding more rapidly to triplets that had originally been high frequency, even though they were now occurring with low frequency. Finally during the 3rd session, when the original frequencies were restored, the reaction time difference between high and low frequency was smaller than at the end of the 1st session. We interpret these results as showing that, on average, participants are slow to adapt to changes in

stochastic relationships within the learning environment. Implications related to the unlearning of procedural skills are discussed.

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Poster

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NIA Grant F31AG047037

Title: Adult age differences in white matter integrity and implicit probabilistic sequence learning

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Abstract: Many everyday tasks, including language acquisition and acquiring new skills, involve learning predictive relationships in the environment, often without explicit awareness. Although often characterized as being preserved with age, consistent age-related deficits in implicit sequence learning have been found (e.g. Howard & Howard, 1997) and have been associated with decreased white matter integrity (Bennett, Madden, Vaidya, Howard, & Howard 2011). While implicit sequence learning is traditionally measured using variants of the serial reaction time task, one critique of this work has been the strong motor component involved in these tasks. Recently, a new paradigm has been developed to ameliorate this called the Triplets Learning Task (TLT; Howard, Howard, Dennis, & Kelly, 2008). In the TLT, participants see two successive visual cues and then respond to a third, target event. Unbeknownst to participants, there is a statistical relationship between one of the cues and the target event, such that each of the four cue positions predicts one of four target positions 80 percent of the time. Furthermore, this task has recently been adapted for use in a scanning environment. Using this scanner-adapted version of the TLT, we investigated the effect of aging on white matter integrity and implicit sequence learning in a sample of healthy adults. First, as reported in Stillman et al.,

2014, we found significant age differences in implicit sequence learning, with young outperforming older adults by the end of training. Second, using Tract-Based Spatial Statistics (TBSS) on diffusion tensor images, we found significant age differences in the structural integrity of white matter as measured by fractional anisotropy (FA), with young having higher FA values than older adults throughout the brain. Finally, we found significant relationships between implicit sequence learning and white matter integrity, with greater learning scores being associated with higher FA. Collectively, these results are consistent with prior studies of white matter integrity and implicit sequence learning (Bennett et al., 2011), and demonstrate that these relationships still exist in a task without a strong motor sequencing component.

Disclosures: K.L. Seaman: None. **C.M. Stillman:** None. **D.V. Howard:** None. **J.H. Howard:** None.

Poster

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Army Research Office Grant W911NF-09-0001

Title: Explicit, but not implicit motor sequence training contributes to choking under pressure due to large rewards

Authors: T. G. LEE[1], A. S. PANESCU[2], *S. T. GRAFTON[1]
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Abstract: Although increased motivation usually enhances performance, impaired performance can sometimes be seen *in situ*ations involving large rewards and high motivational states. Over thirty years of psychological research has provided scientific evidence of the anecdotally salient phenomenon of this "choking under pressure". However, the cognitive mechanisms underlying choking remain unclear. One proposed theory of choking asserts that reduced performance is due to an increase in explicit monitoring of a procedural skill. Here we tested whether explicit skill knowledge is a necessary prerequisite for choking under pressure. Two groups of participants

were trained across eight different blocks on three separate 8-item motor sequences in the discrete sequence production (DSP) task. Crucially, one group of participants (explicit training) was cued (using colored shapes) to the identity of the upcoming sequence just prior to each trial and they were told to attempt to learn the three sequences. The other group (implicit training) was given no cueing as to the identity of the sequences and was told the sequences were randomized. Following training, participants were asked to perform the same task for reward bonuses with time limits set on an individual-by-individual basis based on movement time at the end of training. Each trial was associated with a $5, $10, or $20 reward by a reward cue displayed just prior to the start of the trial. Explicitly trained subjects were able to modulate their performance (as measured by accuracy) based on the reward values displayed but displayed stereotypical choking behavior as indexed by reduced performance at the highest incentive level. However, implicitly trained subjects did not show any performance decrement for large rewards.]. These results suggest that skill metacognition can both positively and negatively impact performance in a feed-forward manner and that knowledge of expected ability in a motor skill can contribute to choking under pressure.

Disclosures: T.G. Lee: None. **A.S. Panescu:** None. **S.T. Grafton:** None.

Poster

260. Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Title: Cortical representations of motor sequence features

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Abstract: Performing movements in a sequential order involves numerous cortical and sub-cortical areas. However, the exact type of information that is represented in these areas is not fully understood. Neuroimaging studies comparing trained and untrained sequences are partly inconclusive since both regional increases, decreases or no change of activity were reported. The interpretation of these findings is further complicated by difficulties in separating sequence specific activity from other, non-sequence specific processes such as general motor output, attention, sequence difficulty or salience. In primary and supplementary motor cortex (M1 and SMA), sequence training leads to a stabilization of the spatial response pattern, even in the

absence of mean activity changes. This suggests that these areas represent learned motor sequences via distributed and robust patterns of activity. Yet, it remains unclear how these representations relate to sequence structure and how they are influenced by motor performance. The aim of this study is to 1) Investigate how different features of motor sequences such as familiarity (trained/ novel) and similarity (similar/ different) are represented in cortical activity patterns and 2) Characterize how these representations change with improvements in performance over the course of a session. Healthy participants were trained explicitly on three 8-element sequences (one reference, one similar and one different) of key presses using a serial reaction time task. After two days of training they underwent fMRI scanning while performing the trained as well as three (untrained) control sequences which were constructed according to the same criteria. The sequence sets used for training and control were counterbalanced across participants. We used multi-voxel pattern analysis to compare the cortical activity patterns for all sequences with each other. In M1, SMA and parietal cortex, this revealed that 1) Representations of trained and untrained sequences can be clearly distinguished 2) Trained but not untrained sequences correlate more strongly with themselves than with other sequences (they are more discriminable) 3) Changes in the performance of untrained sequences (faster RTs) are accompanied by changes in cortical representations 4) Similar sequence content is not reliably translated into similar cortical activation patterns. This suggests that cortical representations of motor sequences relate more strongly to sequence performance than to its structural content.

Disclosures: D.M. Mussgens: None. **F. Ullén:** None. **C.I. Baker:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS-1125719

Title: Motor sequencing in the human cerebellum: How movement predictability and effector influence activity

Authors: *C. J. LOPRESTI[1], T. ALVAREZ[2], J. FIEZ[3]
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Abstract: Previous research has shown there are somatotopic maps within the human cerebellum and that neural activity within the cerebellum is sensitive to whether a movement sequence is predictable or unpredictable. It is unclear, however, whether the same regions that exhibit effector-specific activity also exhibit sensitivity to movement predictability. The present study addresses this question. We scanned twelve subjects using functional magnetic resonance imaging (fMRI) while they performed four motor-based tasks: predictable finger tapping, unpredictable finger tapping, predictable tongue movements, and unpredictable tongue movements. These motor tasks were guided by both visual and audio cues. In the finger task, subjects were shown a cartoon hand with an arrow pointing to which finger they should tap in time with a tone (one tone every 0.66 s). In the tongue task, subjects were shown a cartoon mouth pointing to where they should place their tongue in time with the tone. In the predictable condition, the arrows rotated clockwise. In the unpredictable conditions, the arrows appeared randomly, although still concurrent with the tone. Pre-processing and data analysis were completed using the AFNI processing stream. We performed a two-way ANOVA ($p < 0.001$, corrected for multiple comparisons) with Effector (finger, mouth) and Task (predictable, unpredictable) as factors. In line with known somatotopic organization, regions within right lobule V demonstrated stronger activation for finger vs mouth and regions within bilateral lobule VI had greater activation for mouth vs finger (Stoodley et al., 2012; Grodd et al., 2001). Our results were further confirmed by the meta-anayltic database Neurosynth which overwhelmingly associated hand keywords with our finger > tongue cluster and articulatory keywords with our tongue > finger cluster. Both effector-specific clusters demonstrated only weak sensitivity to predictability; instead, predictability modulated a separate region within left lobule VI. This region was significantly active in both tasks and had greater activity for unpredictable movements irrespective of whether subjects performed the movement sequence with their fingers or tongues. These results suggest that different loops within the cerebellum are sensitive to movement execution versus motor sequencing demands.

Disclosures: C.J. Lopresti: None. **T. Alvarez:** None. **J. Fiez:** None.

Poster

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Support: OTKA NF 105878

Janos Bolyai Research Fellowship of the Hungarian Academy of Sciences (KJ)

DAAD-MOB 29775

Title: Competitive neurocognitive networks underlying sequence learning

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Abstract: Human learning and memory depend on multiple cognitive systems related to dissociable brain structures. These systems interact not only in cooperative but sometimes competitive ways in optimizing performance. Previous studies showed that manipulations reducing the engagement of frontal lobe-mediated explicit, attentional processes could lead to improved performance in striatum-related procedural learning. In our study, hypnosis was used as a tool to reduce the competition between these two systems. We compared learning in hypnosis and in the alert state and found that hypnosis boosted striatum-dependent sequence learning. Since frontal lobe-dependent processes are primarily affected by hypnosis, this finding could be attributed to the disruption of the explicit, attentional processes. Our result sheds light not only on the competitive nature of brain systems in cognitive processes, but also could have important implications for training and rehabilitation programs, especially for developing new methods to improve human learning and memory performance.

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Poster

260. Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Title: A secondary task induces offline learning of motor sequences following a three-minute delay

Authors: *Y. DU, J. E. CLARK
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Abstract: Many have suggested that the acquisition of motor sequences in adults is driven by online and offline learning. Online learning requires iterative computations based on trial and error, but allows performance to improve quickly within a single learning session (i.e., the first few learning blocks). In contrast, offline learning leads to the improvement in performance after a latent period without practice, but it does not occur until at least 4 hours after the initial acquisition. However, in our previous study we observed when online learning is not effective (e.g., in children), offline learning takes place following a delay of 3 minutes. This evidence leads to the hypothesis that offline learning in the early stage of sequence acquisition is inhibited by online learning. To examine this hypothesis, two groups of adults were asked to perform a serial reaction time (SRT) task that requires foot stepping movements. There were eight learning blocks and a three-minute break was added between each two consecutive blocks. In the first five learning blocks (B1 - B5), one group performed the foot stepping SRT task alone (single-task group), while the other group concurrently performed the foot stepping SRT and a secondary tone-counting tasks (dual-task group). In additional three blocks (B6 - B8), both groups performed the foot stepping SRT task alone. Through the whole session, the stimulus followed the same pattern except in B5 and B7. The mean reaction time (RT) of B5 or B7 was compared to the mean RT of other blocks to assess learning. As revealed by the difference in RT between B4 and B5 and between B6 and B7, we found that the single-task group had greater learning than the dual-task group. In addition to the mean RT, we examined the progressive changes of RT within and between blocks. It was found that participants in the single-task group progressively reduced RT within each block. This online process, however, vanished in the dual-task group even after the secondary task was removed. Instead, we observed an offline RT improvement following each three-minute break. Furthermore, when both groups performed the foot stepping SRT task alone, the introduction of the novel sequence in B7 interfered with the learning in the single-task group, while the learning in the dual-task group was stable. These results suggest that a secondary task disrupts the online learning process. In addition, when online learning is impaired, offline learning that follows an interval of minutes emerges to secure effective learning and enable the performance to be more resistant to interference.

Disclosures: **Y. Du:** None. **J.E. Clark:** None.

Poster

260. Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Title: Effects of interference on implicit and explicit motor sequence learning

Authors: ***X. LI**, S. M. MORTON
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Abstract: Motor sequences can be learned explicitly or implicitly based on conscious awareness of the sequence. Retention of a learned motor sequence can be interfered with when two sequences are learned in quick succession. For instance, retrograde interference, or reduced retention of sequence A, happens when learning sequence A is followed immediately by learning sequence B. The goal of this study was to determine whether retention of implicit and explicit sequence learning was affected differently by retrograde interference. Thirty-two young healthy volunteers participated in two test sessions 24 hours apart. They were divided into two groups (control, interference) and two learning conditions (explicit, implicit). For all subjects, stimuli were presented as asterisks which appeared in one of four locations on a computer screen. Subjects were instructed to respond by pressing the corresponding key on a keyboard as quickly and accurately as possible. The sequence was a 12-item set of asterisk positions presented in a repeated blocked pattern, with random stimuli presented before and after. The interference group learned two different sequences on day 1 (sequence A, 5-minute break, sequence B); the control group did not learn a second sequence. On day 2, both groups were tested on sequence A. In the explicit condition, subjects were told there would be a period of time when the stimuli would be presented in a repeating sequence and the start of the sequence was indicated by a change in color of the asterisk. In the implicit condition, subjects were not told there would be any pattern to the presentation of stimuli and the asterisk colors always remained the same. Response times and accuracy were recorded for each trial. Skill level was measured as the difference in mean response times between sequence A and random trials at the end of each session. Retention was measured as offline gains, or the difference between skill levels on day 1 versus day 2. Results revealed a significant group × condition interaction, indicating that the amount of offline gain reduction caused by interference varied based on whether the sequence was learned explicitly or implicitly. Specifically, in the explicit condition, interference had no effect on offline gains. In contrast, there was a strong trend for interference to reduce offline gains if the sequence was learned implicitly. Overall, results suggest that sequence learning under implicit conditions is more susceptible to interference than explicit conditions. We postulate that explicit awareness of the sequence may engage additional memory systems during learning which reduces forgetting or facilitates consolidation.

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Poster

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Support: DAAD-MOB 29775

OTKA NF 105878

Janos Bolyai Research Fellowship of the Hungarian Academy of Sciences (KJ)

Title: The role of the prefrontal cortex in sequential memory formation and consolidation

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[1]Eotvos Lorand University, Inst. of Psychology, Budapest, Hungary; [2]Dept. of Clin. Neurophysiol., Georg-August Univ., Göttingen, Germany; [3]Inst. of Psychology, Eotvos Lorand Univ., Budapest, Hungary

Abstract: Sequence learning is crucial in everyday life from childhood to old age; it underlies the acquisition of motor, cognitive, as well as social skills. Previous studies have shown the involvement of the fronto-striatal circuits in this type of learning. The specific functions of the components of these circuits are, however, still debated. The aim of the present study was to investigate the role of the prefrontal cortex (PFC) in implicit sequence learning and consolidation. Healthy, young adults were trained on a probabilistic sequence learning task. Anodal transcranial direct current stimulation (tDCS) over the left or right dorsolateral PFC (DLPFC) was applied during the training in order to modify learning-related cortical plasticity in the targeted brain regions by increasing neural excitability. Performance was retested after a 2-hour stabilization period and a 24-hour retention period. We found a trend for the higher engagement of the right PFC leading to enhanced sequence learning. In contrast, the higher engagement of the left PFC induced by the anodal tDCS led to a weaker performance in the retention phase suggesting disturbed consolidation. These results highlight an interhemispheric competition between the underlying memory systems.

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Poster

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Support: Human Brain Project KT-2013 FET-F 604102(vi)

Title: Transient but consistent dynamic changes in motor cortex activity in repeating a trained and novel movement sequence

Authors: *E. GABITOV, D. MANOR, A. KARNI
Univ. of Haifa, Haifa, Israel

Abstract: An almost universally accepted tacit expectation is that practice-related changes and memory consolidation processes should be reflected in the average brain activity in brain areas relevant to task performance. Motor cortex (M1) plasticity has been implicated in motor skill acquisition and its consolidation. Nevertheless, no consistent pattern of changes in the average signal, related to motor learning or motor memory consolidation following a single session of training, has emerged from imaging studies. Here we explored the engagement of motor mnemonic processes as expressed in dynamic changes of on-line activity in M1. To this end, we scanned participants, using fMRI, during the paced performance of a finger-to-thumb opposition sequence (FOS), intensively trained a day earlier (T-FOS), and a similarly constructed, but novel, untrained FOS (U-FOS). Both movement sequences were performed in pairs of blocks separated by a brief rest interval (30 sec). Behaviorally, in addition to within-session 'online' gains expressed immediately after training, most participants expressed delayed, consolidation-phase, gains in the performance of the T-FOS on the day after training; the performance of the U-FOS on the second day was significantly slower, less accurate and more variable. Motor experience was not expressed in the average signal intensity evoked in M1. Surprisingly, although signal variability tended to decrease with performance, its magnitude and pattern did not differ for the two sequences. The two sequences were, however, differentiated in the pattern and magnitude of short-term brain activity modulations in response to task repetition. In M1, the execution of the T-FOS was characterized by a pattern of relative enhancement between blocks in a pair. Moreover, these across-blocks enhancement effects were positively correlated with the magnitude of each participant's overnight delayed gains and not with absolute performance levels. During the execution of the U-FOS there was no significant between-blocks modulation of the average signal but repetition had distinctive within-block effects; there were significant signal decreases within the first block, but not in the block following the brief rest interval. These

within-block effects reappeared in subsequent runs. Thus, task repetition, in different time frames, may uncover robust patterns of brief but recoverable changes in task-evoked neural activity reflecting local M1 dynamic processes related to levels of experience, learning and memory. Averaging over single events or blocks may not capture the dynamics of motor representations that occur over multiple time-scales.

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Poster

260. Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Title: Reduction of primary motor cortex cTBS excitability predicts benefit of feedback for retention of sequence knowledge

Authors: *A. D. STEEL[1], L. WILKINSON[2], E. MOOSHAGIAN[2], T. ZIMMERMANN[2], A. KEISLER[2], J. D. LEWIS[2], E. M. WASSERMANN[2]
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Abstract: Continuous theta burst transcranial magnetic stimulation (cTBS) can reduce excitability in motor cortex (M1) for up to sixty minutes. During this time, subjects have reduced motor evoked potentials (MEPs), a local effect on corticospinal neurons, and show impairment in performance on some cognitive tasks, which effect might be due to local or distant (network) effects. Despite widespread cTBS use, there are no reports on whether MEP reduction is related to cognitive impairments from M1 inhibition. We delivered cTBS over motor M1 before participants performed a serial reaction time task with and without monetary reward/punishment feedback. MEPs were collected before cTBS, immediately afterward, after learning (30 min), and at 45 min. cTBS significantly reduced MEP amplitude, which persisted at 45 min, and impaired mean group retention of task knowledge. There was no correlation between MEP amplitude reduction and retention of learning overall. However, subjects who showed more reduction in MEP amplitude at 45 minutes post-cTBS also showed more improvement at retention performance with task feedback ($r=0.81$; $p < .0001$). One explanation for this correlation is that susceptibility to local synaptic change (inhibition) from cTBS is associated with susceptibility to other drivers of plasticity, e.g., rewarding feedback. MEP reduction does

not reliably predict the intensity of changes in cognitive networks from inhibitory M1 stimulation.

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Poster

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Support: This work was supported by a grant from the Saarland University.

Title: Effects of moderate physical activity on learning a simple movement sequence

Authors: ***S. PANZER**[1,2], C. WEICH[2], F. MARSCHALL[2]
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Abstract: There is empirical evidence that physical activity (PA) induced an increase in the cerebral blood flow which is associated with the Brain Derived Neurotrophic Factor, a protein which supports nerve growth and neural plasticity as a basis function for learning. Learning a movement sequence involves a development of a memorial representation which is important for sequence execution. But until now, little is known about PA and the development of a sequence representation. The purpose of the present experiment was to determine effects of moderate PA on the development of a representation of a simple movement sequence. Participants were 38 students (mean age: 23.4 yrs; SD: 2.3 yrs; 19 females). All participants were instructed to perform a maximal step test on a bicycle ergometer, to determine maximal power output (MPO). From the MPO moderate PA was calculated 70% of the MPO. All PA was performed on a bicycle ergometer. In a learning and transfer design, participants were randomly assigned to one of five practice groups where continuous (70%) and intermittent (60% - 80%) PA was systematically varied before acquisition and/or before the retention and transfer tests between the groups. The learning task was to reproduce a 1300 ms spatial temporal sequence pattern of elbow flexions and extensions. After acquisition (99 trials), all participants performed a retention test and two effector transfer tests (9 trials each) to distinguish the dominant representation for sequence production. The mirror effector transfer test required the same pattern of muscle activation and limb joint angles as required during acquisition (motor representation). The non-

mirror transfer test required movements to the same visual-spatial locations experienced during acquisition (visual-spatial representation). The results indicated that participants of all groups increased their performance during acquisition, $F(10,320)= 97.19$, $p<.01$, regardless if they had previous PA or not. The analysis of the retention and transfer tests revealed a main effect of test $F(2,64)= 27.95$, $p<.01$. Post-hoc tests indicated that all groups performed the retention test superiorly compared to the two transfer tests. All other tests failed to reach significance. These findings showed that regardless if performers had moderate PA or not, or the positioning of PA, they rely on a visual-spatial and a motor representation for sequences production. Moderate PA had no beneficial and no detrimental effects on the development of a visual-spatial or motor sequence representation. The results provided a substantial amount of guidance in how the training of a movement sequence and PA can be scheduled.

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Poster

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Program#/Poster#: 260.13/QQ7

Topic: F.01. Human Cognition and Behavior

Support: FKZ 01GQ0951

Title: Anticipatory and carry-over coarticulation in sequential human arm movements

Authors: E. NOWAK, B. GRIMME, H. REIMANN, *G. SCHONER
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Abstract: One of the most typical properties of natural human movements is that one movement is not independent but involved in a sequence of other movements. A movement segment can affect its successor (carry-over coarticulation) or its predecessor (anticipatory coarticulation). In redundant motor systems coarticulation can be found at the level of end-effector trajectories and at the level of joint angles. As anticipatory and carry-over coarticulation influence movements at different points in time, a comparison between them can help us to understand the generation of movement better. To examine both kinds of coarticulations in sequential human arm movements two slightly different experimental setups were used. In each setup a cylindrical object was moved from a starting position to a position at the center (first sub-movement) and from there to a final position (second sub-movement). To analyze carry-over coarticulation six starting points

were arrange at 2, 4, 6, 8, 10 and 12 o'clock. The final targets were at 2, 6 and 10 o'clock. To examine anticipatory coarticulation the starting position was always arranged at 6 o'clock, while the final positions were at 3, 6, 9 and 12 o'clock. To ensure complete attention to the task, the targets were given by visual cues that had to be memorized and were removed before each trial. To uncover coarticulation at the level of joint angle configurations, we applied an analysis of motor equivalence based on the concept of the uncontrolled manifold. We decomposed the difference between joint configurations in different conditions into components that leave the position of the transported object invariant (UCM) and those that affect the position of the object (ORT). Motor equivalence is observed when this difference lies more in the UCM than in the ORT (same spatial trajectory, but moving through different joint configurations). Direction-dependent coarticulation was visible at the level of the end-effector and at the level of joint angles. However the preparation of a movement seems to be distinctly different from its post-processing. While carry-over coarticulation was mainly visible in the joint angles, it was different for anticipatory coarticulation. Here the influence at the level of the end-effector was more distinct.

Disclosures: **E. Nowak:** None. **B. Grimme:** None. **H. Reimann:** None. **G. Schoner:** None.

Poster

260. Sequence Learning

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 260.14/QQ8

Topic: F.01. Human Cognition and Behavior

Support: EPSRC

Title: A hierarchical model of human motor learning that combines high-level symbolic motor planning and low-level control of actuators

Authors: ***E. ABRAMOVA**[1], A. FAISAL[1,2,3]
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Abstract: Many real-world control problems involve complex, nonlinear and often unknown dynamics, such as those encountered in many engineering areas. In contrast to artificial systems, the human brain translates and learns across levels of representation optimally and with ease: at the behavioral level we often plan and specify tasks symbolically (e.g. "grab cup", "pour coffee")

(Daw et al., 2005), while at the level of actuation the brain continuously controls our 650 skeletal muscles (Todorov, 2004). It has been shown that mammals learn optimal action selection in symbolic serial decision making tasks by effectively implementing reinforcement learning algorithms (Schultz et al., 1997; Clascher et al., 2010). At the same time, the brain controls the redundant degrees of freedom of reaching movements in a manner well predicted by linear optimal feedback control theory (Todorov, 2004; Scott 2004). Given these findings, we propose a hierarchical, neurobiologically inspired, framework which enables us to learn optimal control of nonlinear systems with unknown dynamics while reducing the large computational costs and heuristic tuning that are often associated with numerical approximations. Our model - Reinforcement Learning Optimal Control (RLOC) - uses a top-level reinforcement learner (putatively located in the basal ganglia (Schultz et al., 1997)), which selects symbolic actions, each corresponding to a low-level locally optimal linear feedback controller (putatively implemented across M1, pMC and spinal cord (Scott, 2004)). RLOC uses low-level motor experience to learn the system dynamics for local optimal linear control, as encountered in human reaching movements. The learning loop between action selection and actuator control is closed with the high-level reward signal driven by the low-level optimal control costs, which enables the system to learn the global optimal sequence of local linear controllers. Our model can learn, starting from unknown task dynamics, the nonlinear optimal control of planar arm reaching movements and the pendulum-on-a-cart swing up and balance problem - producing a global policy for optimal task control for many starting states in a single learning run. The system learns quickly and finds solution rivaling/beating the performance of existing state-of-the-art nonlinear control algorithms (which have full knowledge of the dynamics) (Todorov et al., 2005), while outperforming monolithic reinforcement learning approaches (Doya, 2000). Our model demonstrates how experimentally established findings in symbolic reinforcement learning and linear optimal control of movements can be combined to learn nonlinear controls.

Disclosures: **E. Abramova:** None. **A. Faisal:** None.

Poster

260. Sequence Learning

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 260.15/QQ9

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01HD073147

Title: Motor learning in hemiparesis following stroke

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Abstract: It has been proposed that motor learning is an important component of the motor recovery process following stroke (Krakauer, 2006; Carr & Shepherd, 1989). However, it is difficult to determine whether stroke patients with hemiparesis have impairments in motor control alone (i.e. execution deficits), whether their motor control deficits affect their motor learning (i.e. because performance is poor learning is decreased), or whether patients have specific deficits in motor learning itself. This limited understanding is likely due to the performance confound as well as an overlap between the importance of regions lesioned in hemiparetic stroke in both motor control *and* motor learning. Previous studies examining this question have been unable to clearly dissociate the effects of impaired motor control on motor learning (Winstein et al., 1999; Platz et al., 1994; Takahashi & Reinkensmeyer, 2003; Schaefer et al., 2009). A task that accounts for differences in participant speed and accuracy (i.e. skill) could clarify how stroke affects motor control *and* learning. Here we examined motor learning in stroke survivors using a skill learning task that controls for deficits in motor execution. We hypothesized that motor learning would not be affected by impairment level if we controlled for execution deficits. We determined the functional impairment of 14 chronic unilateral stroke survivors using the Fugl-Meyer (FM) assessment. Then we split the participants into groups with mild impairment (mean FM=61±4) and severe impairment (mean FM=30±10). Participants sat with their affected arm supported by a Kinarm robotic exoskeleton and performed isometric elbow flexion contractions to move an on screen cursor to target locations. Initial skill was assessed by having participants perform the task at a range of tempos paced by a metronome, allowing us to produce a speed-accuracy function. Participants then completed four consecutive daily training sessions, in which they practiced performing the task as quickly and as accurately as possible. A final session examined post training skill by determining a second speed-accuracy function. Preliminary results indicate participants with mild impairment could successfully complete trials at faster speeds than participants with severe impairments. However, training led to error rate improvements of similar magnitudes in both groups. These data suggest that learning mechanisms following stroke are intact once we account for differences in execution. Therefore, differences in motor learning ability cannot explain why some stroke patients recover more than others.

Disclosures: R.M. Hardwick: None. **V.A. Rajan:** None. **P.A. Celnik:** None.

Poster

260. Sequence Learning

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 260.16/QQ10

Topic: F.01. Human Cognition and Behavior

Support: Intramural Research Program of NIMH

Title: Effects of short and long term motor learning on cortical structure

Authors: *A. TREFLER[1], C. THOMAS[2], E. AGUILA[1], M. KING[1], P. MODI[2], C. PIERPAOLI[2], C. I. BAKER[1]
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Abstract: A large number of studies have used magnetic resonance imaging (MRI) to detect structural changes in the adult brain following training programs that span from hours to weeks. However, the strength of the evidence from these MRI-based studies is often limited, particularly with regard to the specificity of any training effect on brain structure (Thomas and Baker, 2012). Here, we used a longitudinal within-subjects design to investigate the topography of short-term (1 hour) and long-term (1 hour/day for 1 week) training-dependent structural changes in a group of 18 healthy adults. To test the specificity of training-related changes in the brain, we used a lateralized motor-sequence learning task that required participants to master the ability to rapidly input a specific 8-digit sequence, using only the left hand. For each participant, we acquired 2 sets of T1-weighted structural MRI data, (a) before any training (baseline), (b) after a control task, (c) after short-term motor learning and, (d) after long-term motor learning. For each participant, we compared cortical thickness before and after training with cortical thickness before and after equivalent control time periods. Participants showed a strong effect of training in terms of reduced reaction time and increased accuracy that was specific to the trained hand and the type of motor sequence (trained versus untrained). Longitudinal analysis of cortical thickness revealed apparent changes in brain structure that included motor cortex in addition to other areas. However, the topography of the training-related changes differed between the short-term and long-term training programs and also differed between the different datasets collected in each scan session. Further, apparent changes in cortical structure were also observed in the control comparisons, particularly the long-term. These findings suggest that despite strong evidence for the behavioral effects of training, the evidence for concomitant changes in brain structure, as measured with structural MRI, do not appear to be robust.

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Poster

260. Sequence Learning

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 260.17/QQ11

Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant MOP-125869

QPRN student travel award

Title: Effect of experimental muscle pain on the acquisition and retention of locomotor adaptation

Authors: J. BOUFFARD[1], S. E. SALOMONI[2], K. TUCKER[2], W. VAN DEN HOORN[2], *C. MERCIER[1], J.-S. ROY[1], P. W. HODGES[2], L. J. BOUYER[1]
[1]CIRRIS - Laval Univ., Quebec, QC, Canada; [2]Univ. of Queensland, Brisbane, Australia

Abstract: Background and aims: It is well established that pain can influence motor control. However, the effect of pain on our ability to learn new motor tasks remains unclear. Previous studies have used different pain models and motor tasks, reporting conflicting results. We have shown that tonic CUTANEOUS pain undermines the retention of locomotor adaptation. The **aim** of the present study was to evaluate if similar effects are observed when pain is induced into a MUSCLE directly involved with the motor learning paradigm. **Methods:** Forty healthy participants walked on a motorized treadmill on two consecutive days while wearing a robotized ankle-foot orthosis on their right leg. Each day, participants walked for 5 minutes before (Baseline) and during (Adaptation) exposure to a force field that was applied during the swing phase and resisting ankle dorsiflexion. During the Adaptation period, participants were instructed to "overcome the perturbation and walk as normally as possible". On day 1, participants performed the task either without (n=23) or with (n=17) pain induced by infusion of hypertonic saline in their right tibialis anterior muscle (ankle dorsiflexor). To evaluate the retention of motor learning, participants repeated the force field adaptation task without pain on day 2. Movement error during swing was calculated as the absolute difference (error) between Baseline and Adaptation ankle joint angular trajectories. A three-way ANOVA (Day: 1 vs. 2; Time: early vs. late adaptation; Group: pain vs. no pain) was performed to compare the progression of movement error between groups, both during acquisition and retention. **Results:** Participants improved their performance with training and showed retention of the motor task (main effects of Time and Day; $p<0.01$). A significant Day x Group interaction ($p=0.045$) suggested a difference in the level of retention between groups. Despite similar movement errors for both

groups on day 1 (p=0.810), post-hoc analyses showed a trend for better performance of the pain-free group on day 2 (p=0.076). **Conclusion:** Consistent with our previous work using cutaneous pain, acute experimental muscle pain hinders the retention of a locomotor adaptation task, even though performance during acquisition is not significantly affected.

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Poster

260. Sequence Learning

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 260.18/QQ12

Topic: F.01. Human Cognition and Behavior

Title: Autonomic arousal during implicit associative learning predicts risky behavior

Authors: *A. B. FORMAN-ALBERTI, B. HINNANT
Psychology, The Catholic Univ. of America, Washington, DC

Abstract: Variability in the tendency to engage in risk-taking behavior exists, with some individuals being more risk-seeking and others being more risk-averse. The degree to which people are able to subconsciously detect and learn from somatic cues in different contexts may explain some of this variability. However, no research to date has explored whether individuals' autonomic arousal during implicit learning is related to their tendency to make risky decisions and their engagement in risky behavior. Thus, the current study examined whether young adults' autonomic activity at rest and during implicit associative learning predicted their ability to learn implicitly as well as their engagement in risky behavior and substance use. Parasympathetic activity (i.e. respiratory sinus arrhythmia or RSA) was recorded at rest and while participants completed three computerized tasks: the Triplets Learning Task (TLT; Howard, Howard, Dennis & Kelly, 2008), which assesses implicit learning, and the Iowa Gambling Task (IGT; Bechara, Damasio, Damasio, & Anderson, 1994) and the Balloon Analogue Risk Task (BART; Lejuez et al., 2002), which measure risky decision making and behavior. Participants also completed a self-report measure assessing frequency of substance use once the physiological data collection period ended. Results indicated that greater resting RSA predicted increased levels of substance use and poorer implicit learning. Furthermore, the interaction between resting RSA and RSA reactivity during learning predicted risky behavior on the BART, with those individuals who had low baseline RSA and less RSA withdrawal during implicit learning showing the greatest

amount of risky behavior. These findings help to fill an existing gap in the literature by illustrating that a relationship exists between parasympathetic activity, implicit learning, and engagement in risky behavior. Furthermore, these results demonstrate that levels of parasympathetic activity associated with advantageous behavior may differ depending on whether the information to be learned in a particular context is implicit or explicit.

Disclosures: A.B. Forman-Alberti: None. **B. Hinnant:** None.

Poster

260. Sequence Learning

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 260.19/QQ13

Topic: F.01. Human Cognition and Behavior

Support: NIH/NIA RO1AG036863

Title: Implicit sequence learning in Parkinson's disease: Intraindividual variability in reaction time

Authors: *K. R. GAMBLE[1], T. J. CUMMINGS, Jr[2], S. E. LO[3], J. H. HOWARD, Jr[4,5,1], D. V. HOWARD[1]
[1]Psychology, Georgetown Univ., Washington, DC; [2]Psychiatry, [3]Neurol., MedStar Georgetown Univ. Hosp., Washington, DC; [4]Psychology, The Catholic Univ. of America, Wasington, DC; [5]Neurol., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Behavioral research generally reports means or medians or interindividual standard deviation of performance to describe group data. However, it has been suggested that intraindividual variability may be a better indicator of cognitive function. Intraindividual variability is a measure of transient or state differences that may fluctuate within a task or even from task to task. While this variability measures short-term fluctuations, it has been shown to be a stable and reliable characteristic of individuals. Research suggests that this type of variability is related to brain structure or function, and may even be related to changes in neurotransmitters, such as dopamine. In the present study, we examined the differences in intraindividual variability between healthy older adults and people with Parkinson's disease (PD), a group known to have significant declines in striatal dopamine. To our knowledge, this is the first study to examine intraindividual variability in a sequence learning task in a group of people with Parkinson's disease. There were 27 medicated PD participants, in Hoehn and Yahr stages 1 to 2.5, and 30

age- and education-matched healthy older adults in this study. Participants completed three sessions of the Triplets Learning Task (TLT), an implicit sequence learning task that has a reduced motor component. We examined intraindividual variability in overall reaction time in the TLT using two measures, individual standard deviation (the standard deviation around each individual's mean RT) and coefficient of variance (an individual's standard deviation divided by their mean RT). In both measures of intraindividual variability, we found that response times in the PD group were more variable than in the control group, and this difference increased over the course of training. Thus, performance in a learning task was more variable in a PD than a control group, which may be due to a greater amount of fatigue in the PD group, or perhaps to dopamine decline over the course of training in PD participants as the effectiveness of their medication decreased.

Disclosures: K.R. Gamble: None. **S.E. Lo:** None. **J.H. Howard:** None. **D.V. Howard:** None. **T.J. Cummings:** None.

Poster

260. Sequence Learning

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Program#/Poster#: 260.20/QQ14

Topic: F.01. Human Cognition and Behavior

Support: the Intramural Research Program of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health

Title: Practice structure improves transitional memory through increased cortico-subcortical coupling with the premotor cortex

Authors: *S. S. SONG[1], S. J. GOTTS[2], E. DAYAN[1], L. G. COHEN[1]
[1]Human Cortical Physiol. and Stroke Neurorehabilitation Section, NIH/NINDS, Bethesda, MD; [2]Lab. of Brain and Cognition, NIH/NIMH, Bethesda, MD

Abstract: Learning relies on successful formation of transitional and ordinal memories (Conway and Christiansen 2001, Song and Cohen 2014, Terrace and McGoningle 1994). The influence of practice structure on these memories and the underlying systems-level neural substrates are not known. Here, we studied the influence of practice structure on learning of transitional and ordinal memories that compose skill. Human subjects were trained to learn motor sequences under varied (mixing sequences) or grouped (grouping sequences) practice schedules (Shea and

Morgan 1979) while they were scanned using functional magnetic resonance imaging (fMRI). Transitional and ordinal memories were assessed 30 minutes and one week after the training session. We report that: (a) Varied practice improved transitional but not ordinal memory, (b) coupling of the left dorsal premotor cortex with thalamus and right cerebellum, lingual and cingulate network increased during varied practice, and (c) across subjects, the greater the transitional memory, the stronger this coupling. Thus, practice structure benefits one type of memory but not another that composes skill and in proportion to coupling within a cortico-subcortical network linked to the premotor cortex. These results could contribute to understanding mechanisms by which different memories are influenced by practice schedules in neurorehabilitation after brain injury.

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Poster

260. Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01DC004855

Title: Oscillatory cortical dynamics of implicit learning in patients with schizophrenia

Authors: *L. B. HINKLEY[1], S. VINOGRADOV[2], M. FISHER[2], D. MIZUIRI[2], B. BIAGIANTI[2], S. NAGARAJAN[2]
[1]Radiology, UC San Francisco, SAN FRANCISCO, CA; [2]UCSF, San Francisco, CA

Abstract: An emerging hypothesis in the neuropathology of schizophrenia is that alterations in oscillatory activity contribute to cognitive and behavioral symptoms prevalent in the disorder. More specifically, high frequency neural activity (e.g. gamma) is thought to be impoverished in the condition, with these deficits contributing to cognitive impairments found in this population. Here, we use magnetoencephalographic imaging (MEGI) to test the hypothesis that impoverished oscillatory activity over frontal cortices impedes implicit skill learning in schizophrenia. MEG data was collected using a 275-channel biomagnetometer (VSM MedTech) during a modified serial reaction time task (SRTT) using manual or vocal movements. Individuals were instructed to respond to a short vowel (/e/, /i/, /o/, /u/) presented in the auditory domain at the beginning of each trial. Subjects either responded by speaking the vowel they have

heard (vocal), or pressing a button (manual) corresponding to one of four spatial locations. Stimuli were either presented randomly or in an eight-step movement sequence. Whole-brain oscillatory power changes were examined in the beta (12-30Hz), gamma (30-55Hz) and high gamma (65-115Hz) bands. Patients with schizophrenia failed to show learning effects for either response modality as a group ($p>0.05$) in comparison to healthy controls. Neurophysiologically, during the response phase, a decrease in beta power and an increase in high-gamma power localized to bilateral frontal cortex in healthy controls around movement onset. Deviations in oscillatory power in both frequency bands were observed in patients with schizophrenia during the learning phase over the same points in time. This data indicates that impairments in recruiting high-frequency neural synchrony translates into a deficit in cognitive learning at a rapid pace. These neuroimaging-based markers have the potential to track recovery following cognitive-based rehabilitative paradigms.

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Poster

260. Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: NIH R01AG036863

Georgetown University Undergraduate Research Fund

Title: Aerobic exercise and implicit learning in healthy young adults

Authors: C. G. WAMBACH[1], C. M. STILLMAN[1], J. H. HOWARD, Jr.[3], *D. V. HOWARD[2]
[1]Psychology, [2]Georgetown Univ., Washington, DC; [3]Psychology, The Catholic Univ. of America, Washington, DC

Abstract: Behavioral and neuroimaging data suggest that aerobic exercise benefits cognitive functioning. However, most studies have focused on executive functions in special populations, such as children and older adults. In this study we investigated whether aerobic exercise is associated with better implicit learning in healthy young adults. Implicit learning, which occurs without awareness or intent, relies on several brain substrates overlapping with those previously

shown to benefit from aerobic exercise. We thus predicted that exercise would be positively related to implicit learning in our sample. We tested 27 undergraduates from Georgetown University who did not participate in varsity sports. Participants completed the EPIC-Norfolk Physical Activity Questionnaire (EPAQ2), an extensive, validated self-report questionnaire that measures activity levels in total energy expenditure. Participants then completed the Triplets Learning Task (TLT), a measure of implicit sequence learning. In this task participants view four open circles on the screen that fill in sequentially red, red, then green and respond only to the green target. Unbeknownst to them, the first red cue predicts the location of the green target 80% of the time, forming a high probability (HP) "triplet". The remaining 20% of the time the target will appear in one of the three other locations, forming a low probability (LP) triplet. Implicit learning is measured by comparing reaction time to HP vs. LP triplets. To replicate previous experiments, participants also completed the Flanker Task, a measure of executive function, cognitive control. In this task, participants respond to the direction of a center arrow as quickly as possible. In each trial, the center arrow is flanked by stimuli that are pointing in congruent, incongruent, or neutral directions to the center arrow. Cognitive control is measured by subtracting reaction time to congruent trials from that of incongruent trials, with lower scores indicating better control. As predicted, we found a significant positive correlation ($r = 0.486$, $p = 0.018$) between activity levels and implicit sequence learning. However, we did not find the expected relationship between activity levels and cognitive control. This failure to replicate positive associations with cognitive control and the small sample size call for replication with a larger sample. Nonetheless, our results suggest that regular aerobic exercise may be associated with better implicit learning. Our findings also highlight the need to include measures of implicit learning in future randomized trials, to determine whether aerobic exercise also improves implicit forms of learning.

Disclosures: C.G. Wambach: None. **D.V. Howard:** None. **C.M. Stillman:** None. **J.H. Howard:** None.

Poster

260. Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: Biotechnology and Biological Sciences Research Council (BBSRC UK) Grant Ref BB/J017116/1

Title: Prefrontal and cerebellar contributions to oculomotor rule learning: An eye-tracking fMRI study

Authors: G. P. D. ARGYROPOULOS, J. L. MILLS, *N. RAMNANI
Dept. of Psychology, Royal Holloway Univ. London, London, United Kingdom

Abstract: Cerebellar Crura I/II are interconnected with prefrontal areas and may play important roles in skilled cognitive operations. In instrumental learning, memory-guided response selection is preceded by processes that establish rules through trial-and-error. Previous studies suggest that these may require, respectively, cerebellar and prefrontal circuitry. We used event-related fMRI to record activity timelocked to visual cues that arbitrarily instructed the selection of oculomotor responses. We isolated and compared rule-related activity evoked by cues that resulted in correct, memory-guided response selection, and cues that resulted in erroneous responses in which subjects had still not established the correct response through trial-and-error.20 subjects were scanned with fMRI (1100 whole-brain EPI images; TR=2s; 3T Siemens Trio) during trial-and-error learning (trial structure: visual cue; variable delay; 'Go!' trigger; saccade; feedback [positive; negative; null]). Variable delays permitted activity time-locked to cues to be isolated from 'Go!' response-related activity. Rules: arbitrary cue-location associations. Cues: Either fully (FR) or partially reinforced arbitrary symbols (PR; 20% of trials, ensuring sufficient incorrect responses), or arrows directly specifying target location (CTR). fMRI analysis: EPI images were preprocessed and analysed in SPM8. 1st-level GLM modelled cue-activity for correct responses to FR, PR, CTR, and incorrect ones to FR and PR; all other events, confounds. Following 2nd-level random effects ANOVA, SPM t-contrasts compared successful and erroneous response selection preceding saccades: FR,PR correct > FR,PR incorrect; FR,PR incorrect > FR,PR correct (FWE correction: $p<.05$). *Incorrect > correct rule processing:* Activations were found bilaterally in the caudal (BA8, frontal eye fields), and in mid-portions (BA46) of the middle frontal gyrus which extended into the inferior frontal gyrus on the left (BA45).There were no cerebellar activations. *Correct > incorrect processing:* Activations were present bilaterally in Crus I, but not in these frontal lobe areas. Cerebellar Crus I may play a role in memory-guided selection of oculomotor responses. Activity in middle and inferior frontal gyrus may be related to the requirement to select between competing responses when the correct one is unknown (the predominant cause of errors in this study). Activity in cerebellar Crus I may reflect selection following a constrained search through a set of well-learned associations.

Disclosures: G.P.D. Argyropoulos: None. **J.L. Mills:** None. **N. Ramnani:** None.

Poster

261. Consciousness and Neural Networks

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Topic: F.01. Human Cognition and Behavior

Support: NIH R01 NS055829

NIH 5T32NS00722429

NIH 5T32GM00720538

Title: Mechanisms of large-scale network switching in task engagement and conscious report

Authors: ***W. R. XIAO**[1], W. C. CHEN[1], R. E. WATSKY[1], R. KIM[1], E. A. B. LEVINSOHN[1], J. L. GERRARD[2], D. D. SPENCER[2], H. BLUMENFELD[1,2,3]
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Abstract: The mechanism by which perceived stimuli are processed and encoded into consciousness has been extensively studied with behavioral tasks. The addition of imaging and electrophysiology can elucidate the temporal and spatial location of the sequences necessary for the entire mechanism of consciousness. We believe that the mechanisms of global large-scale network-switching in the brain are similar to transient conscious events and with the transition that occurs between the passive resting state and active task engagement. We have developed a behavioral task paradigm in a mixed block and event-related design employing conscious report of a face stimulus titrated to threshold perception levels. In our task, each subject's subjective perception of the stimulus is validated by objective accuracy in locating the stimulus in one of four possible locations. The task takes place over either a distracting background involving a movie on marine life or a less distracting background of static. We also vary the duration of delay between stimulus presentation and probe questions. In using this task in normal subjects we saw no differences in overall performance with probe delay or with different backgrounds. As further validation of the behavioral task we found that even though only half of the presented stimuli were reported as perceived, nearly 100% of perceived stimuli were located accurately, while stimuli that were not perceived were located at chance levels. We have recruited 3 subjects with intracranial electroencephalography (icEEG) recordings to perform this task. We have confirmed event-related potentials and frequency modulations in response to the face stimuli and task transitions. Additional analyses are aimed at further elucidating the activity changes associated with transitions from rest to task, and during transient consciously reported events. In ongoing studies we hope to pair the behavioral task with additional icEEG recordings as well as with functional magnetic resonance imaging (fMRI) to determine the sequence of changes underlying and large-scale network switching during task engagement and conscious events.

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Poster

261. Consciousness and Neural Networks

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NIH F31 NS077540

NIH MSTP TG 2T32GM07205

Betsy and Jonathan Blattmachr Family

Title: Mechanism of impaired consciousness in typical childhood absence seizures

Authors: *J. N. GUO, R. KIM, S. JHUN, W. XIAO, E. FEENEY, X. BAI, M. NEGISHI, M. J. CROWLEY, L. C. MAYES, R. T. CONSTABLE, H. BLUMENFELD
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Abstract: Seizures of childhood absence epilepsy are marked by brief impairments of consciousness and by 2.5-4 Hz spike-and-wave discharges on electroencephalography (EEG). While previous imaging studies of absence seizures show a widespread involvement of cortical and subcortical areas, how these changes are related to ictal behavior is unknown. Prior studies with limited sample size have shown occasionally spared task performance during absence seizures. However, the mechanism for this variability in ictal behavioral impairment is not understood. To examine this, we performed two studies: 1) simultaneous behavioral, 32-lead EEG, and fMRI recordings in 36 patients and 2) separate out-of-magnet 256-lead EEG recordings with concurrent behavioral testing in 15 patients. Tasks included a repetitive tapping task and a more difficult continuous performance task requiring greater attentional vigilance. 579 seizures were captured in Study 1 and 150 seizures were captured in Study 2. Greater task difficulty and longer seizure duration were correlated with worse ictal behavioral performance. For both tasks, patients showed a bimodal distribution with most seizures exhibiting either fully spared or fully impaired performance. K-means clustering analysis of fMRI data revealed sequential activation of three known networks: 1. default mode network, 2. task-positive network, and 3. primary sensorimotor and thalamic network. Intensity of involvement for all three networks was larger in seizures with impaired task performance. Quantitative analysis of

high-density EEG revealed frontal predominance in seizure power within the 2.5-4 Hz range. Intensity of EEG power changes in anterior, middle, and posterior leads also were larger for seizures with impaired behavioral performance for both tasks. Finally, the larger amplitudes of both EEG and fMRI in seizures with poor performance occurred at or even preceding seizure onset, suggesting the pre-seizures state may determine seizure severity. Childhood absence epilepsy has significant affective and cognitive effects on patients and impairs their quality of life. Understanding the mechanism of loss of consciousness during childhood absence seizures may allow for development of improved therapeutics for this disorder.

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Poster

261. Consciousness and Neural Networks

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Topic: F.01. Human Cognition and Behavior

Support: PRESTO, JST

NIHR01EY015980

Title: Phenomenal consciousness created by decoded neurofeedback

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Abstract: Introduction: One of the serious controversies on consciousness is whether it is possible to identify the presence of so-called phenomenal consciousness or qualia, which is defined as contents of conscious experience independent of access to the experience via cognitive functions including top-down attention and working memory. Although several researchers claim that the neural representation for phenomenal consciousness is distinguishable from that for other cognitive functions (Lamme, 2003; Kanai and Tsuchiya, 2012), phenomenal consciousness has never been created or identified. Here we took a causal approach called decoded fMRI neurofeedback (DecNef) to create phenomenal consciousness. First, we induced

V1/V2 neural representation of color by DecNef without subjective report about perceived color. By doing this, we can isolate the neural representation independent of access. Then we tested whether the induced representation corresponds to phenomenal consciousness by asking subjects to report perceived color. Methods: After conventional retinotopic mapping, we built a decoder to discriminate stimulus color (red vs green) from V1/V2 fMRI signals. Then we conducted an fMRI DecNef experiment, in which subjects learned to regulate their brain activity during the presentation of an achromatic vertical grating based on the feedback disc. The disc size approximately represents the similarity between the V1/V2 activation patterns during the grating presentation and those corresponding to red grating. In a post-test stage, subjects indicated the perceived color of an achromatic vertical/horizontal grating. Results and Discussion: The mean red likelihood (i.e. magnitude of red representation) was significantly increased by DecNef training, indicating that subjects internally created association of vertical gratings with the neural activity corresponding to the red color. However, during the training, subjects did not spontaneously report any color. In the post-test stage after the training, subjects chose red significantly more frequently than green for the achromatic vertical grating. We confirmed that the red likelihoods for an achromatic grating during the DecNef induction were highly similar to those during the perceptual color test, and the only difference was whether subjects were asked to report color or not. These results clearly indicate that the neural representation corresponding to phenomenal consciousness of red color was created and retained in the early visual cortex. The representation was read out or accessed when subjects were required to report and therefore attend to the color.

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Poster

261. Consciousness and Neural Networks

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Topic: F.01. Human Cognition and Behavior

Title: The influence which rubber hand illusion gives the hand capacity

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Abstract: Background and aim of the study: The change in body ownership by the rubber hand illusion (RHI) has been reported to be due to a decline in skin temperature (Moseley GL, 2008) and a rise in the sharp pain threshold (Hegedus G, 2014). However, the relationship of hand capacity (HC) and the change in body ownership by RHI has not been investigated. The purpose of this study was to investigate the influence which RHI gives the HC. Methods: The subjects were 14 right handed healthy adults (mean age ± standard deviation : 27.5 ± 3.9 years). The subjects sat in front of a framework containing two identical compartments. In the right compartment, a left rubber hand (RH) was placed in an identical configuration. The experimental phase consisted of two blocks corresponding to the two experimental conditions (synchronous and asynchronous). Both the synchronous and the asynchronous conditions started with a 5-min stroking period, during which all of the fingers of the RH and the unseen left hand were stroked by two brushes; these strokes were either synchronous, to induce the RHI, or asynchronous. Before starting the experimental phase, a proprioceptive drift (PD) and HC were measured in all subjects. After the induction phase, PD and skin conductance response (SCR) were measured, and participants reported their perceptual experiences that were associated with stroking by answering a questionnaire. The evaluation of SCR, the amplitude of the largest SCR 0.03 greater than microsiemens that occurred 1-5 s from pinprick to the RH was scored as a response to that stimulus. In addition, uncomfortable feelings in each condition were measured with an 11-step numeric rating scale (NRS). The HC was compared before and after the experiment with paired t-tests. The HC variation difference was compared with an independent t-test for each condition. The Pearson product-moment correlation coefficient was used to evaluate the correlation between HC and uncomfortable feelings, PD, and SCR. Results: HC was not significantly different between the synchronous and asynchronous conditions ($p = 0.05$). Subjective illusion strength significantly differed between the synchronous and asynchronous conditions ($p = 0.01$). A significantly positive correlation was found between HC and uncomfortable feelings in the asynchronous condition ($r = 0.6$, $p < 0.04$). There were no significant differences in PD, SCR, or uncomfortable feelings between the two conditions ($p > 0.05$). Conclusion: These results suggested that the change in HC was related to emotions, such as uncomfortable feelings rather than a change in body ownership by the RHI.

Disclosures: **T. Yuda:** None. **M. Osumi:** None. **S. Morioka:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIMH Grant R01MH080833 awarded to EAK

Title: The effect of emotional valence on nonconscious visual memory activity

Authors: *S. KARK, S. D. SLOTNICK, E. A. KENSINGER
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Abstract: Most studies of recognition memory examine activity that is greater for correctly recognized items (hits) than for incorrectly forgotten items (misses) or correctly rejected lures (CRs). While these studies focus on the neural processes that support the ability to consciously recognize past events, there can also be unconscious influences from the prior study episode, as evidenced by repetition priming effects. Prior work has demonstrated priming effects in early visual regions (Slotnick & Schacter, 2006), the amygdala (Yang, Cao, Xu, & Chen, 2012), and the hippocampus (Hannula & Ranganath, 2009) - regions important in conscious recognition of emotional information (Keightley, Chiew, Anderson, & Grady, 2011). In the present functional magnetic resonance imaging (fMRI) study, we investigated the effect of emotion on nonconscious memory effects. Specifically, we examined whether repetition priming effects were modulated as a function of emotional valence. Seventeen participants (aged 19-35) studied International Affective Picture System (IAPS) images that had negative, positive, or neutral valence (i.e., they viewed each line-drawing outline immediately followed by the complete photo). After a 20-minute delay, participants were shown the line-drawing outlines of the previously studied photos and outlines of photos they never studied. For each item, participants made an old-new recognition judgment and a sure-unsure confidence rating. As repetition suppression effects are known to occur for forgotten items, priming effects were identified by comparing CRs to misses. The contrast of CRs > misses, collapsed across valence and confidence, revealed priming-related activity in the amygdala, early visual regions (BA18 and BA19), the insula, and the hippocampus, which is consistent with previous findings. A region-of-interest (ROI) analysis was conducted based on the magnitude of activity in each of these regions as a function of valence. We observed significant priming effects for negative items but not positive or neutral items in the amygdala and BA18 (there was also a significant Region x Valence x Response Type interaction). These ROI results suggest that the amygdala and BA18 mediate nonconscious memory for negative stimuli to a greater degree than positive or neutral activity. That is, even when participants have no conscious memory of previously viewing the stimuli, there are still influences of the prior stimulus exposure on sensory and affective processes, and some of these influences appear to be largest when stimuli are negative.

Disclosures: **S. Kark:** None. **S.D. Slotnick:** None. **E.A. Kensinger:** None.

Poster

261. Consciousness and Neural Networks

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Topic: F.01. Human Cognition and Behavior

Support: Experiment Crowdfunding Campaign

Title: Damage to the medial prefrontal cortex is associated with impaired autonomic responses to music-evoked autobiographical memories

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Abstract: Music is strongly intertwined with memories - for example, hearing a song from your wedding can transport you back in time, triggering the sights, sounds, and feelings of that night. Music-evoked autobiographical memories (MEAMs) are often vivid and rich with episodic details. Why does music evoke such vivid, detailed memories? We have posited that emotion is a critical part of the mechanism underlying the relationship between music and vivid memories. To investigate this issue, we studied patients with focal brain damage to the medial prefrontal cortex (mPFC), a key brain region for both emotion and autobiographical memory. Previous research has shown that individuals with damage to the mPFC have impaired emotional responses to many stimuli, including music (Johnsen et al., 2009). These individuals do not show autonomic responses to emotional events, findings that have been interpreted as reflecting impaired 'somatic markers' to emotional stimuli. Additionally, these individuals have impaired (less vivid) MEAMs. We therefore predicted that individuals with damage to the mPFC will have impaired autonomic responses to memory-evoking music. Participants with damage to the mPFC, brain-damaged and normal comparison participants listened to 30 songs that were popular during the years when the participant was between the ages of 15-30. After each clip, participants described memories that were evoked and rated how strongly they felt any emotions associated with these memories. During the experiment, skin conductance was recorded as a measure of emotional response. We found that normal and brain-damaged comparison subjects showed greater skin conductance response (SCR) during music that evoked memories as compared to music that did not evoke memories. This effect was not seen in the mPFC group. Additionally, the mPFC group had significantly lower SCRs to music that evoked memories than both comparison groups. However, when comparing the participants' ratings of emotional strength, we saw no significant group differences. That is, patients with mPFC damage had decreased emotional responses to MEAMs (as indexed by SCR) but did not show decreased

subjective emotional ratings. These findings can be taken to indicate that individuals with damage to the mPFC have an impaired "somatic markers" to memory-evoking music, despite reporting normal subjective ratings of felt emotions. This supports the proposal that the mPFC is a critical structure for autonomic responses to emotional stimuli, and that these responses are crucial for vivid music-evoked autobiographical memories.

Disclosures: **A.M. Belfi:** None. **D. Tranel:** None.

Poster

261. Consciousness and Neural Networks

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Topic: F.01. Human Cognition and Behavior

Support: NSF grant NSF IIS-1208203

Title: Pragmatic: A probabilistic and generative model of areas tiling the cortex

Authors: *A. G. HUTH, T. L. GRIFFITHS, J. L. GALLANT
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Abstract: In many regions of the cortex, functional representations appear to be organized into maps: retinotopic maps in visual cortex, tonotopic maps in auditory cortex, and somatotopic maps in somatosensory and motor cortices. However, because both anatomy and functional representation differ across individuals, it is notoriously difficult to define maps and areas consistently across subjects. Furthermore, only a small number of functional maps and areas have been discovered thus far, and much of cortex remains poorly understood. To solve these problems we developed PrAGMATiC: a probabilistic and generative model of areas tiling the cortex. This hierarchical Bayesian model assumes that the cortex is tiled with functional areas, and the precise organization of these areas in each subject is drawn from a single underlying probability distribution. Parameters of this distribution specify the functional properties of each area and the approximate anatomical distance between each pair of areas. This model avoids the lossy process of projecting each individual brain into a common anatomical space (e.g. MNI coordinates). Model parameters are learned using a Markov chain Monte Carlo technique similar to contrastive divergence learning for Boltzmann machines. Once the parameters have been learned, this generative model can be used to predict how functional representations are organized in new subjects whose data were not used for parameter estimation. Model

performance can then be assessed by comparing PrAGMATiC predictions to the actual data for the held-out subjects. We applied PrAGMATiC to datasets from two fMRI voxel-wise modeling experiments: one in which subjects watched natural movies, and one in which subjects listened to natural narrative stories. For both experiments, we estimated voxel-wise models that predict BOLD responses based on the semantic content of the stimuli. In both datasets, PrAGMATiC revealed dozens of cortical areas with consistent functional properties and anatomical locations across subjects. In the speech dataset, consistent functional areas tile much of the prefrontal, parietal, and temporal cortices. In the movie dataset, consistent functional areas tile anterior occipital cortex, posterior temporal cortex, and parietal cortex. Finally, the parameters of the PrAGMATiC models reveal which specific semantic categories_such as numbers, places, or people_are represented in each functional area. These results show that PrAGMATiC is a revolutionary new tool for identifying functional areas both at the individual and group level, and that it provides a powerful method for interpreting voxel-wise modeling results.

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Poster

261. Consciousness and Neural Networks

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Topic: F.01. Human Cognition and Behavior

Support: R01 MH069456

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1F31MH100958-01

Title: Using multivariate pattern analysis to investigate memory reactivation during sleep

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Abstract: To understand the role of sleep in memory processing, we aim to track reactivation of specific memories during sleep and to relate this reactivation to subsequent memory. The necessary technology to do this already exists in animal studies: Cellular recordings during sleep in rodents have demonstrated replay of specific neuronal patterns that were present during

learning. However, evidence for reactivation of specific memories in humans is currently lacking. To address this gap, we set out to track memory reactivation in humans by applying multivariate pattern analysis (MVPA) to EEG data. Specifically, we queried whether wakeful brain patterns re-emerged after presenting learning-related cues to participants during sleep. While awake, participants viewed 48 famous faces and scenes while EEG data were acquired from 64 channels, and event-related spectral perturbation (ERSP) values were used to train a face/scene classifier. Participants over-learned associations between those pictures and unique sounds, and then learned the location of those pictures on a spatial grid. Participants then took an afternoon nap. During slow-wave sleep, we presented half of the associated sounds from each category. After a 2.5-hr post-nap delay, participants took a recall test for all the picture locations. We predicted that sounds would trigger reactivation of associated faces and scenes during sleep, and that the degree of measured reactivation would influence subsequent recall. To investigate whether learning-related patterns re-emerged during sleep, we found the time (post stimulus onset) and frequency bins that yielded the best face/scene discrimination during wake, and we used data from these time / frequency bins to create spatial templates of ERSP feature values (across the 64 electrodes) for faces and scenes. We then matched these category-specific spatial templates to data from different time and frequency bins acquired during sleep. This approach instantiates the idea that face and scene processing are associated with distinct spatial patterns of activity that are preserved across wake and sleep, but these "signature" patterns might appear at different times post-stimulus-onset during wake vs. sleep, and they also might be manifest in different oscillatory frequencies. Preliminary results indicate that wakeful patterns re-emerge during sleep at above-chance levels. Further analyses will assess other approaches to measuring reactivation, and also the extent to which neural reactivation relates to subsequent memory retention on an item-by-item basis.

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Poster

261. Consciousness and Neural Networks

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Topic: F.01. Human Cognition and Behavior

Title: Default mode network activity precedes attention lapse in healthy subjects

Authors: *R. C. PHILLIPS, T. SALO, C. S. CARTER
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Abstract: During sustained attention tasks the mind tends to wander towards salient internal thoughts. Mind wandering has been associated with activity in the default mode network (DMN) but the evidence for how this network interacts with the Task-Positive Network (TPN) is mixed. Previous research has demonstrated clear anticorrelations in the BOLD signal of these networks during resting state, suggesting that the DMN may suppress the TPN, resulting in an attention lapse. However, co-activation of these networks has been observed during mind wandering, indicating the possibility that the TPN is active during executive processing of internal distractors. We sought to test whether attention lapses on a cognitive control task are correlated with DMN activity, and whether this activity is accompanied by suppression of the TPN. Twenty-two subjects were presented with the AX-Continuous Performance Task (AXCPT) while in a 3-Tesla MRI scanner. During AX/BY trials, there is no competing impulse that would tempt an incorrect response, and subjects generally display a high level of correct responses on these trials. Therefore, incorrect responses on these trials likely represent disengagement with the external task as attention turns to internal distractors. We used SPM8 to model lapse trials, as well as correct AX/BY trials. We contrasted lapse trials and correct trials in order to determine which areas of the brain displayed increased activity prior to a lapse response. We compared the BOLD signal time course from voxels in the DMN and TPN during our task to determine the degree of anticorrelation between these networks during task performance. We observed significant activation in the medial Prefrontal Cortex, a prominent hub of the DMN, at a threshold of $p<.05$, FDR. We also observed activation of the Posterior Cingulate Cortex at a lower threshold of $p<0.001$. We observed no suppression of TPN regions. Our time course analysis revealed that during correct trials, these networks are clearly anticorrelated, but not during lapses. These results suggest that default mode network activity plays a role in the focus of attention, which may be switched from an external task towards distracting internal thoughts. Our results indicate a further avenue of study in order to determine whether attention lapses only arise spontaneously, or whether they can be induced in response to salient internal distractors.

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Poster

261. Consciousness and Neural Networks

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Support: CAS Grant KSCX2-EW-J-8

Title: The effect of short-term meditation training on attentional blink

Authors: *F. LUO, S. WANG, J.-Y. WANG
Key Lab. Mental Hlth., Inst. Psychology, Chinese Acad of Sci., Beijing, China

Abstract: Back ground: Evidence have been provided that short-term meditation training could alter attention processing. Hence, the question arises as to whether the effect could also be manifested in the attentional blink task. The present study used a modified "target-mask, target-mask" (TM-TM) paradigm to test the effect of a 6-day meditation training on the reduction of the susceptibility to the attentional blink. Method: forty-one healthy meditation beginners participated in this experiment. They were assigned into a meditation training group (mean age 22, SD 1.62, 6 male and 14 female) or an attention task group (mean age 21.67, SD 1.56, 9 male and 11 female). The meditation group received meditation training (30 min per day) for 6 days. The control group was given an attentional task with a similar time schedule. All participants finished two "TM-TM" attentional blink tasks, one before and one after the 6-day training period. In the TM-TM paradigm, participants initiated each trial by pressing the space bar. The four-item sequence (T1, M1, T2, M2) began after a variable delay (100 ms, 200 ms, or 300 ms). We created three levels of T1 difficulty (easy, medium, and hard) by co-varying the the duration of T1 and M1 (45/45, 30/60, and 15/75 ms, respectively). The inter-stimulus interval between T1 and M1 was always maintained at 30 ms. T2 and M2 were fixed at the level of 30/60 ms. On each difficulty level, the stimulus onset asynchrony (SOA) between T1 and T2 varied between five levels (135 ms to 615 ms, in steps of 120 ms). Each of the 15 conditions of Difficulty × SOA were composed of 20 test trials. All trials were mixed and randomly presented in one block so as to avoid any predictive allocation of attention resource before a given trial. Thus, the change in attentional blink magnitude corresponding to T1 difficulty could only be attributed to the improved processing efficiency but not the changing of resource allocation for T1 or T2. The T2 accuracies were calculated at 15 conditions respectively on the trials that T1 were correctly identified and were analyzed with a 3 (difficulty) ×5 (SOA) × 2 (test time) × 2 (group) mixed ANOVA. Results: The results revealed that T2 accuracy of the 'hard' condition was significantly improved only in the meditation group, although it was enhanced in both groups under easy and medium condition. Post hoc test showed that under the 'hard' level, meditation training could significantly improve T2 accuracy when the SOA was 255 and 375 ms. Conclusion: These observations support that short-term practice of meditation could enhance the efficiency of information processing and help to overcome attentional limitation in the temporal domain.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Title: Risk-taking behaviors relate to functional connectivity in striatal and fronto-parietal networks in adolescence

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Abstract: Adolescence is a pivotal period of development, characterized by an increase in white matter integrity and decrease in regional grey matter thickness particularly in networks associated with higher-level functioning and complex cognitive processes. Among these are the fronto-parietal network involved in executive functioning and control, as well as the orbitofrontal-striatal network known to mediate reward sensitivity; both networks are instrumental in decision making, but they do not complete maturation until early adulthood. However, during adolescence individuals are already beginning to be held responsible for important decisions, and poor self-regulation may result in being exposed to riskier and potentially disadvantageous situations. Evidence shows that the development of the reward and executive control networks occur at different rates within healthy adolescents, resulting in a period characterized by an increase in reward sensitivity but a lack of executive control. Given this evidence, we investigated the correlations between functional connectivity in these networks and risk-taking behaviors within a group of healthy adolescents between the ages of 12 to 21. To characterize individuals' propensity for riskiness, we utilized the Balloon Analogue Risk Task (BART) for its consistent prediction of self-reported risky behaviors. We then identified regions of interest in both the striatal network (Di Martino 2008) and fronto-parietal network (Owen 2005) to pinpoint the reward and executive control circuits. With these regions of interest, we used resting state fMRI and a seed-voxel analysis with nine nuisance signals based on the

methods developed in the NITRC 1000 Functional Connectome project and covaried by age, sex, and mean performance in the BART. This initial analysis showed better performance in the BART was correlated with stronger connectivity in the executive control network. Likewise, ventral striatal seeds showed negative correlations with areas involved in cognitive control in individuals with better BART performance. This indicates that it is not just stronger connectivity in the fronto-parietal executive network, it is also the better coordination between this network and the orbitofrontal-striatal reward network that contributes to more beneficial risk behaviors. Due to the limited data on functional connectivity and its relationship to BART performance, continued assessment of self-reported behaviors and risk measures will assist in further understanding the relationships between these two networks and risk.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Title: Cautious decision making in obsessive-compulsive disorder: The role of perceptual uncertainty and implicit incentives

Authors: *P. BANCA[1], M. D. VESTERGAARD[2], V. RANKOV[1], S. MITCHELL[1], T. LAPA[1], M. CASTELO-BRANCO[3], V. VOON[1]
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Abstract: Introduction: Compulsive behaviours are typical symptoms of Obsessive Compulsive Disorder (OCD) that reflect difficulties to commit to ultimate decisions. They may be conceptualized as a means to accumulate sufficient evidence prior to a decision. Here we investigate the process of evidence accumulation in OCD in perceptual discrimination and

probabilistic reasoning, hypothesizing impairments in both decision types. **Methods:** Twenty-eight OCD patients and 35 healthy control subjects were tested with a low-level visual perceptual task (random dot motion task), whereby different coherent levels for motion were defined to measure high and low uncertainty, a probabilistic reasoning task (jumping to conclusions task) and two response conflict tasks as control tasks (flanker task and probabilistic selection task). Logistic regression analysis across all coherence levels (which accounted for visual detection threshold) and hierarchical drift diffusion modeling (HDDM) were used to characterize response strategies between patients with OCD and healthy controls in the random dot motion task. **Results:** OCD patients compared to healthy volunteers were more cautious in weighing the alternatives and accumulated more evidence particularly to high uncertainty in the visual perceptual but not in the probabilistic reasoning task (longer reaction time and response time intercept). This behaviour was consistent across behavioural and computational approaches and was more evident in patients with higher compulsivity scores. The HDDM analysis further showed higher decision threshold, or evidence needed to make a decision, in patients under high uncertainty and slower drift rate, reflecting poorer quality of evidence, under low uncertainty. With incentives to emphasize speed, patients decreased the decision threshold relative to healthy volunteers, accumulating less evidence, under low uncertainty, without compromising accuracy. These findings were unrelated to visual perceptual deficits and response conflict. **Conclusions:** This study extends the assessment of evidence gathering in OCD from probabilistic to perceptual decisions. Using both behavioural and computational approaches we highlight impairments in evidence accumulation in OCD and an influence of uncertainty. We further emphasize that OCD patients are sensitive to explicit salient incentives on the speed-accuracy tradeoff, improving evidence accumulation and shifting away from pathological internal monitoring. These findings may have relevance for therapeutic approaches.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Title: Decision making and dopamine variability in healthy human subjects

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Abstract: Impulsivity is a complex scientific construct that entails many behavioral proclivities. One important aspect of impulsivity is information sampling, sometimes referred to as reflection impulsivity. Previous work has shown that several groups with impulse control disorders show decreased information sampling in the beads (or urn) task and increased novelty preference in a reinforcement learning task in which novel options are occasionally introduced. Functional imaging of these tasks has implicated frontal-striatal circuits, and PET studies comparing patients with impulse control disorders to matched control groups have shown differences in the dopamine system. To further clarify the role of dopamine in these tasks, we have carried out behavioral studies in healthy subjects in whom we will also characterize dopamine signaling. We have focused on 3 information sampling tasks and the novelty task. The information sampling tasks include the beads task, the secretary task, and a perceptual inference task. Behavioral findings in the current sample (n=20; 11 female), replicate previously identified effects in patient groups separately tested on each task. In the beads task, subjects were less accurate ($F_{1,15}$=21.8, p<0.001), and sampled a greater number of items ($F_{1,15}$=20.5, p<0.001) when provided with less information. They also chose to sample a greater number of items under conditions in which they lost money ($F_{1,15}$=16.2, p=0.0011). In the secretary task, subjects sampled more items when the item list length was greater ($F_{1,19}$=7.07, p=0.0155). Also, there was an interaction between the variation of the items in the list and the list length ($F_{1,19}$=9.78, p=0.0055). The perceptual inference task revealed that subjects were more accurate ($F_{6,108}$=586, p<0.001) with smaller reaction times ($F_{1,15}$=46.0, p<0.001) under less ambiguous conditions. In the novelty task, participants selected novel choice options more often than the best alternative option. We will also characterize dopamine tone by measuring presynaptic DA stores and synthesis with fluorine-18 FluoroDOPA positron emission tomography (PET) in these same individuals, and examine correlations between dopamine signaling and behavioral performance in the tasks.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Title: Does working memory capacity mediate age and performance on the Balloon Analogue Risk Task (BART)?

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Abstract: Adolescence is a key stage of development of the executive control and reward circuits of the brain, two systems heavily involved in decision making. Previous studies show that decision making ability does not fully develop until after adolescence, resulting in increased risk taking during this period. The Balloon Analogue Risk Task (BART) is a computerized program used to measure risk taking propensity. Participants inflate balloons with different likelihoods of popping and must decide when to stop the inflation in order to earn the most possible points. This analysis aimed to determine if there is a relationship between age and BART performance, and if so, what specific cognitive functions contribute to that relationship. A sample of 34 healthy individuals between ages 8-21 recruited from an ongoing study at Zucker Hillside Hospital was administered the BART and two neuropsychological tasks targeting working memory: Wechsler Spatial Span and Letter-Number Span. BART performance was measured by the coefficient of variation in adjusted pumps, a measure of response consistency, to infer how well participants learn which balloons are more likely to pop and consistently apply that knowledge over the course of the task. A formal mediation model was used to test the impact of working memory on the relationship between age and BART performance. Using an approach by Baron and Kenny, a series of mixed effects regression models were performed: 1) whether age is associated with BART, 2) whether age is associated with working memory, and 3) whether age remains significantly associated with BART after adjusting for working memory. We found that age was no longer related to BART performance after controlling for working memory, indicating that the relationship between age and BART performance may be mediated by working memory capacity. The success of this mediation model implies that working memory has an impact on risk taking propensity. Future analyses will apply this model to a larger sample as well as test whether working memory mediates a relationship between age and real-life risk taking behaviors, and other aspects of BART performance. If working memory ability continues

to show a significant relationship with risk behavior, this knowledge may help inform the development of interventions to enhance working memory in particularly risk-vulnerable populations.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Title: Neural and behavioral indices of value- and urgency-dependent saccade prioritization in humans: Behavioral 'types' and conversion through implicit training

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Abstract: The representation of value, and its influence on behavior, is affected in several psychological disorders from depression to risk seeking behavior and addiction. We sought a neural marker of the spatial representation of the relative value of action targets using electrophysiology, eye tracking and psychophysics in humans. In the main experiment, we presented 15 subjects with two colored circle targets to the left and right of central fixation. After 800 ms delay, the fixation point changed color to that of one of the two targets, instructing to make a fast and accurate saccade to that target. Each color was associated with a different number of points (5/40 pts), awarded if the saccade was made within a 250-300ms deadline. On target presentation, we found a novel, transient positivity (~300ms) contralateral to the position of the higher-value target over lateral parieto-occipital scalp (PCP). The amplitude of this signal correlated with the difference in reaction time (RT) distribution between saccades to high and low value targets. Exploring individual differences revealed three distinct behavioral 'types.' Four subjects showed no effects of value on RT or accuracy (hereby labeled 'Conservatives'). Most of our subjects were 'Opportunists', displaying a clear RT difference between values and more erroneous saccades toward the high value target. Finally, 2 subjects were 'Extremists', who never acted towards the low value. Of these 3 groups, only Opportunists had the PCP signal.

Additional experiments in Opportunists showed that both a tight deadline and large value difference are necessary to elicit both the PCP and behavioral prioritization, which were absent when either was removed (no deadline or 20/25pts). Finer manipulation of these parameters in psychophysical tests with 2 subjects revealed that each could exhibit any behavioral type given appropriate parameters. Further, both Extremists and Conservatives could be induced to exhibit opportunistic behavior with urgency and/or relative value adjustments and, interestingly, exhibited a hysteresis effect whereby they retained such opportunistic behavior for the original parameters after training. They now exhibit a significant PCP for the original parameters. However, while Opportunists ceased exhibiting a PCP when converted to Conservatives, they still exhibited a strong PCP when induced to behave as extremists (deadline <200ms). This series of simple experiments provides a handle on value representation and its incorporation into processes underlying timely target-directed actions, and could lead to novel assays in psychological disorders associated with maladaptive decision making.

Disclosures: A. Blangero: None. **S.P. Kelly:** None.

Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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The National Research Foundation of Korea (2013R1A1A1010176)

Title: Distinctive resting-state brain activity in Internet gaming disorder: a comparison to alcohol use disorder and healthy control

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Abstract: Introduction: Internet Gaming Disorder (IGD) causes significant public mental health problems worldwide, especially in Korea. It is important to compare characteristics of IGD with those of substance addiction in order to elucidate the pathophysiology of IGD. In this

study, we explored the resting-state brain activity using regional homogeneity (ReHo) of functional magnetic resonance imaging (fMRI) among patients with IGD, those with Alcohol Use Disorder (AUD) and healthy controls. **Method**: Sixteen male patients with IGD (21.63 ± 5.92 years), 14 male patients with AUD (28.64 ± 5.92 years), and 15 healthy male controls (25.40 ± 5.92 years) were included in this study. All patients were seeking treatment at our clinics due to their excessive Internet game use or alcohol drinking. **Result**: The IGD group showed a significant ReHo decrease in the right superior temporal gyrus (STG) and increase in the posterior cingulate cortex (PCC) compared with healthy controls. The AUD group showed significant decrease in the anterior cingulate cortex (ACC) and increase in the PCC compared with healthy controls. **Conclusion**: These results showed neurobiological similarity and disparity of resting-state fMRI features among IGD, AUD and healthy controls. These findings may contribute to elucidate the pathogenesis and neurobiological underpinning of IGD.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Support: National Science Council Grant NSC 102-2410-H- 002-004-MY3

Title: Age-related striatal dedifferentiation and frontal overactivation during choice value processing

Authors: *Y.-S. SU[1], T.-L. TAI[1], J. O. S. GOH[1,2,3]
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Abstract: The dopaminergic system, which includes the ventral striatum and prefrontal cortex, is involved in value prediction during choice processing. Aging is known to decrease dopamine availability and efficiency in this fronto-striatal choice processing system. In this study, we postulated that this reduction in dopamine efficacy may lead to dedifferentiation or reduced selectivity of value encoding in older adults relative to younger adults. In tandem, there may be age-related overactivation in other frontal areas possibly reflecting compensatory processing for

reduced value encoding fidelity. We conducted a functional magnetic resonance imaging (fMRI) experiment using a lottery-choice task to investigate age-related neural differences related to choice value processing. 31 healthy younger adults (mean age: 23.75 yrs; SD: 2.11 yrs; 10 males) and 25 older adults (mean age: 68.46 yrs; SD: 4.05 yrs; 7 males) saw numbers depicting the probability (5 levels) of winning and losing a specified points value (3 levels). They chose whether to accept or decline each offer, then outcome feedback was provided. During the task, five functional EPI scans were acquired per participant with 218 volumes each scan, 2s TR, 38 axial slices, and 3.4375*3.4375*4 mm3 voxels. Behaviorally, older adults were more likely to accept choices coding low probabilities of winning compared to younger adults, with relatively longer response times in low probability conditions as well. As expected, whole-brain analysis of fMRI data revealed less sensitivity to increasing probability levels in older compared to younger adults in the ventromedial prefrontal cortex (VMPFC) and nucleus accumbens (NAcc). In addition, dorsal lateral prefrontal cortex (DLPFC) showed overactivation in older relative to younger adults particularly for responses to low probability conditions. Further correlation analysis showed that older adults with higher DLPFC responses to low relative to high probability conditions tend to accept more low probability conditions. Our findings show that older adults have more difficulty in effectively processing choice values, and specifically overvalue low probability choices. Importantly, our study uncovers a novel neural mechanistic link between age-related dedifferentiation in value encoding in the VMPFC and NAcc and overactivation in the DLPFC in relation to risky decision behaviors in old relative to younger adults.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Title: Attentional effects of stimuli induced craving in people with marijuana use disorders

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Abstract: Marijuana is the most frequently used illicit drug in US. As of 2003, an estimated 3.1 million people were daily marijuana users (Office of Applied Studies, 2003). It is important to understand what cues are more salient for psychological craving. A desire to smoke marijuana has been associated with exposure to marijuana-related cues (Gray et al., 2008; Gray et al., 2011; Nickerson et al., 2011). When presenting marijuana-related cues in a variety of sensory modalities, the cues elicited an increase in self-reported craving (Filbey et al., 2009). In a virtual reality study that exposed participants to multiple sensory and social cues, cannabis users reported higher craving levels and attention to cannabis cues in comparison to neutral cues (Bordnick et al., 2009). In fact, desiring marijuana was more prevalent when it was more difficult to obtain, implying increased craving due to the lack of availability (Shrier et al., 2012). The current study was a preliminary data analysis collected from a larger study on attentional bias and marijuana cue reactivity. The goal was to examine the relationship between measures of marijuana craving and various subscales on the Marijuana Craving Questionnaire (MCQ) for those with and without Marijuana Use Disorders (MUD). 36 participants who were matched on age, ethnicity, years of education, and gender were screened for eligibility and diagnosed using the Structured Clinical Interview for DSM-IV. They then completed a modified Flanker task, which presented three lines one after the other and asked the participant to decide on the orientation of the middle line while a positive, negative, neutral, or marijuana cue flashed with it. After completion, participants rated each marijuana and neutral cue on how much it makes them crave marijuana. Participants completed the MCQ pre and post experimental task. The repeated measures MANCOVA with the MCQ as a covariate revealed a significant interaction between cue type and group ($p<.001$, $R2=.80$) and a main effect of cue type on the craving ratings ($p<.001$, $R2=.86$). People with MUD showed an increase in craving level after viewing marijuana cues compared to neutral cues; control participants had no increase in craving after viewing either type of cues. The main effect of group on accuracy on the task was marginally significant ($p=.07$). People with MUD had overall lower accuracy on the task for both cues in comparison to controls. Results showed that craving for people with marijuana use disorder is influenced by viewing marijuana-related cues. For treatment, it may be important to help marijuana users who are attempting to quit to avoid cues that may trigger relapse.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: UPAEP 30108-1030

Title: Towards a study of compared EEG activity during logical reasoning tasks between champions of the Logic Olympiad and regular students

Authors: *V. REYES[1], J. CASTRO-MANZANO[2], J. CASTILLA-CASTILLA[3], C. PERALTA-UTRILLA[3], J. MEDINA-DELGADILLO[2]
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Abstract: Electroencephalogram (EEG) is a non-invasive, economical and reliable method that allows us to represent electrical brain activity with high temporal resolution. The EEG signal portraits cerebral changes on frequency bands associated to cognitive tasks. Based on the Neural Efficiency Hypothesis, which suggests that there is a lower cortical activation in subjects with higher levels of intelligence, in this work we registered the brain electrical activity of 20 regular undergraduate students and 20 champions of the International Logic Olympiad from the Master category. EEG activity was recorded over 14 scalp locations during rest (3 minutes) and during performance on 10 visually presented logical tasks (10 minutes). The latency and the accuracy of the responses were also measured. Preliminary results show an increase in posterior alpha power during the resting period in undergraduate students. There was also a decrease in lower alpha and an increase in beta activity in anterior brain areas during the solving of logical reasoning. This change is probably associated with attentional demands; however, we still need to evaluate the champions before advancing further conclusions. Results from the champions are being currently analyzed. Financial support UPAEP 30108-1030

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Poster

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The Wyncote Foundation

Title: Degradation of subjective value judgements in patients with behavioral variant frontotemporal dementia

Authors: *T. A. BISBING, C. T. MCMILLAN, J. P. POWERS, N. SPOTORNO, M. GROSSMAN
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Abstract: Orbitofrontal cortex (OFC) has been implicated in various aspects of decision-making, including the evaluation of value. The nature of value can be subjective (e.g., an individual prefers one item over another item) or objective (e.g., one item is more valuable than another item). In this study we evaluate the dissociation between subjective and objective value in behavioral variant frontomteporal degeneration (bvFTD) patients who have neurodegenerative disease in OFC. Subjective preference for junk foods, sports, vegetables, and flowers was assessed in comparison to objectively ordered stimuli such as circle diameter and line length. bvFTD patients (N=18) and demographically-comparable healthy controls (N=21) were asked to judge all possible pairs of seven stimuli from each of these six categories. For each pair, they were asked to select one of the images based on either their own preference (requiring the assignment of a subjective value to each stimulus). If participants can maintain a stable subjective value for each stimulus within a set, then a linear preference should emerge within each set. Patients with bvFTD performed more poorly than controls at consistently judging the subjective values ($U(37) = 31.5$, $Z = -4.485$, $p < .001$), however, they did not differ from controls in their ability to judge the relative magnitude of circle diameter and line length ($U(37) = 182.5$, $Z = -.396$, n.s.). We conclude that disease in OFC may selectively impair subjective value with relative preservation of objective value. Together these findings suggest that these two sources of value have distinct neuroanatomical correlates.

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Poster

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Title: Temporal discounting of future rewards in behavioral variant frontotemporal dementia and Alzheimer's disease

Authors: *K. RASCOVSKY[1], J. W. KABLE[2], R. KAZINKA[2], E. MORAN[1], C. OLM[1], J. GLASENBERG[1], A. BOLLER-HEWITT[1], C. T. MCMILLAN[1], R. CLARK[3], M. GROSSMAN[1]
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Abstract: Although patients with behavioral variant frontotemporal dementia (bvFTD) are known to make impulsive social and financial choices, few systematic studies have measured impulsivity in bvFTD using objective patient-based tasks. We administered the Delay Discounting Task (DDT) to evaluate temporal discounting of future rewards in patients with mild bvFTD (n=17, MMSE=24), Mild Cognitive Impairment / Alzheimer's disease (AD/MCI) (n=22, MMSE=24) and 20 matched elderly controls. Participants were asked to make 51 hypothetical choices between small immediate and larger delayed monetary rewards (e.g., "would you prefer $18 today or $30 in 67 days?"). Discount rates were estimated by fitting a logistic regression that assumes a person's decisions are a stochastic function of the difference in subjective value between the two options. We assumed that subjective value (SV) is a hyperbolic function of the reward amount (A) and delay (D): SV = A/(1+kD), where k is the participant's

discount rate (larger k values indicate greater discounting of future rewards). Mann-Whitney tests revealed that bvFTD patients had higher discount rates (k median=0.257) compared to elderly controls (k median=0.009, $p<.05$) and AD/MCI patients (k median=0.016, $p<.05$). Discount rates were not significantly different for AD/MCI patients and controls ($p>.05$). 10 bvFTD and 10 AD/MCI patients had high-resolution structural imaging within 1 year of the DDT. A non-parametric permutation analysis (RANDOMISE) constrained to regions of reduced gray matter in bvFTD related high discount rates to bilateral atrophy in orbitofrontal cortex, right insula, anterior cingulate and lateral prefrontal cortex. In conclusion, bvFTD patients showed greater discounting of future rewards compared to elderly controls and AD/MCI patients. These findings are consistent with the functional neuroimaging literature relating temporal discounting to a fronto-striatal network associated with value-based decision-making. Further studies should explore whether high discount rates in the DDT relate to real-life impulsive behaviors in bvFTD.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Title: Neuroanatomical changes in behavioral variant frontotemporal dementia correlate with risk-related behavior

Authors: *J. POWERS, C. T. MCMILLAN, K. RASCOVSKY, L. BURKHOLDER, M. GROSSMAN
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Abstract: OBJECTIVE: To evaluate neuroanatomical correlates of risk-related decision-making in behavioral variant frontotemporal dementia (bvFTD). BACKGROUND: BvFTD, a clinical syndrome associated with frontotemporal lobar degeneration, is characterized by changes in personality, social conduct, and executive functioning. Patients exhibit impulsive and inflexible behavior as well as impaired decision-making. The Balloon Analogue Risk Task (BART) assesses decision-making in a risk/reward paradigm. Little work has been done to investigate gray matter (GM) regions associated with BART performance in bvFTD. Furthermore, while bvFTD patients have considerable white matter (WM) disease, the relationship between BART performance and WM disease has not been investigated in bvFTD. METHODS: A modified version of BART was collected on 23 patients with bvFTD and 24 demographically comparable healthy seniors. For each trial in this computer-based task, the participant pumps up a virtual balloon to accrue points. The participant must continuously decide whether to end the trial by adding the accrued points to their bank or continue pumping to potentially earn more points. However, the number of pumps required to pop a balloon varies and is unknown to the participant, and no points are earned for trials in which the balloon pops. A Mann-Whitney U test was used to compare final bank totals between bvFTD and controls. Higher bank totals correspond with more successful decision-making. Also, pumping behavior was examined between earlier and later trials to assess learning. T1-weighted structural images and 30-directional diffusion-weighted images were acquired for these bvFTD patients and 36 demographically comparable healthy seniors. GM density and fractional anisotropy (FA) were assessed in bvFTD relative to healthy seniors and BART bank total was related to GM density and FA in bvFTD using regression analyses. RESULTS: BART bank total was decreased in bvFTD relative to behavioral controls, and while controls increased pumping behavior throughout the task, bvFTD patients showed no change. GM density and FA reductions were widespread in bvFTD throughout frontal and temporal GM and WM, respectively. BART bank total was associated with GM density in right orbitofrontal cortex and anterior insula and reduced FA in left superior longitudinal fasciculus and frontal body of the corpus callosum in bvFTD. CONCLUSIONS: Patients with bvFTD demonstrate impairments in risk-related decision-making and adaptive learning. BART performance in bvFTD is associated with frontotemporal network structures involved in risk-related decision-making and adaptive learning.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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McDonnell Center for Higher Brain Function

Title: Dopamine D2 receptors, reward discounting, and B-cell function in obese and normal-weight humans

Authors: *D. M. GREDYSA[1], S. EISENSTEIN[2], A. ARBELAEZ[3], J. KOLLER[2], J. PERLMUTTER[4], S. MOERLEIN[5], E. BIHUN[2], S. RANCK[2], A. BISCHOFF[4], T. HERSHEY[2]
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Abstract: Insulin acts in the brain to regulate dopamine (DA) tone and signaling and DA-mediated behaviors. While this relationship has been shown in animals, research in human populations is lacking. Obesity is associated with altered insulin sensitivity and pancreatic B-cell function, and it has been hypothesized that altered DA receptor levels in the brain may underlie obesity-related phenotypes, including altered reward discounting. Thus, our objective was to investigate whether obesity and B-cell function affect human brain reward circuitry and reward behavior via DA D2 receptors (D2R). Participants were obese (N=22; BMI>30) and normal-weight (N=15; BMI<28) adults without type 2 diabetes and other medical conditions. B-cell function was derived from an oral glucose tolerance test (OGTT) using the Disposition Index (DI), a composite measure of insulin sensitivity and secretion. Reward discounting was measured with the probabilistic (PRD) and delayed (DRD) reward discounting tasks. Participants underwent positron emission tomography scans using a D2-specific receptor ligand, [^{11}C](*N*-methyl)benperidol (NMB). D2R binding potentials (BPs) were computed in reward-related regions of interest (ROIs; putamen, caudate, and nucleus accumbens) using the Logan graphical

method with cerebellum as the reference region. All analyses controlled for age, gender, BMI and education. Results showed that obese and normal subjects differed only in BMI and B-cell function; there were no differences in reward discounting (PRD, DRD) or D2R levels in any ROI. In the total sample, DRD correlated with DI (r=.39, p=.031) and putamen D2R BP (r=–.37, p=.051), such that greater reward discounting related to lower B-cell function and higher D2R levels in putamen. In the obese group alone, DRD correlated with DI (r=.51, p=.037) and putamen D2R BP (r=–.57, p=.025), such that greater reward discounting related to lower B-cell function and higher D2R levels in putamen. When also controlling DI, PRD correlated with D2 BP in putamen (r=–.62, p=.019) and caudate (r=–.62, p=.017), such that greater reward discounting related to higher D2R levels in putamen and caudate. B-cell function was not related to D2R BP in any ROI in any group. These preliminary findings indicate that in obese and normal subjects, greater reward discounting was associated with higher D2R binding in the striatum and lower pancreatic B-cell function, independent of BMI. This suggests that individual differences in D2R levels and B-cell function may underlie altered reward behavior in humans, but that these phenotypes are not unique to common obesity as has been reported.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 262.14/RR5

Topic: F.01. Human Cognition and Behavior

Support: The Ministry of Education, Science, Sports and Culture

Title: The mechanism underlying quota-dependent modulation of risk-attitude and its deficit in the Pathological Gambling

Authors: *A. FUJIMOTO, H. TAKAHASHI
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Abstract: Patients with pathological gambling (PG) tend to continue gamble in spite of negative experiences and then provoke tragic consequences such as bankruptcy or family breakdown. Such puzzling risk-seeking in PG patients is considered to be based on the distorted cognition so-

called ‘chasing’, in which patients believe that the only way to recover financial loss is continuing gamble. Although many studies investigated the biased cognition in PG, the critical mechanism remains elusive. To elucidate the mechanism of ‘chasing’ from novel aspect, we tested the ability to modulate risk attitude depending on the quota severity in the PG group (n=21) as well as in the healthy control (HC) group (n=29) using a newly-developed gambling task under fMRI scanning. In this task, participants were required to choose between risky and safe options on every trial and achieve stage-quota that was assigned from 7 different levels in advance to each stage. Hence, participants needed to truck remaining point and trial number (trial-quota) and utilize sure and risky choices flexibly to maximize the chance of stage clear. The HC group tended to take sure (high expected-value) options when trial-quota was moderate and take risky (high magnitude) options when trial-quota was severe, suggesting quota-dependent modulation of risk attitude in healthy population. The PG group, on the other hand, chose risky option frequently when trial-quota was moderate, suggesting that PG patients had less ability to modulate risk attitude toward current quota adaptively. Thus, ‘chasing’ might be partially induced by inappropriate risk-taking due to disability of quota-dependent behavioral modulation. We also conducted fMRI experiments during task execution and showed that several areas including the dorsal anterior cingulate, insula and dorso-lateral prefrontal cortex reflected quota severity in HC group. This correlation was attenuated in PG group, implicated the neural bases of disability to modulate risk attitude depending on quota. Altogether, our results would reveal the mechanisms underlying ‘chasing’ in PG patients, and potentially provide new therapeutic target to recover ability to modulate risk attitude depending on the quota severity.

Disclosures: **A. Fujimoto:** None. **H. Takahashi:** None.

Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

Location: Halls A-C

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Program#/Poster#: 262.15/RR6

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust

National Health & Medical Research Council (NH&MRC) of Australia

Title: Dopamine mediates reward-dependent incentivisation of effort in Parkinson’s disease

Authors: *T. T.-J. CHONG, V. BONNELLE, S. MANOHAR, K.-R. VEROMANN, K. MUHAMMED, M. HUSAIN
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Abstract: Humans adapt the degree of effort they are willing to expend according to the rewards they expect to receive. Data from animal studies suggest that dopamine might be a key neurotransmitter involved in such incentivised decision-making. Here, we examined the extent to which the striatal neurodegeneration in Parkinson's disease (PD) disrupts the processing of effort and reward, and how dopamine may ameliorate such disruption. Patients with PD ($N = 22$) were tested ON and OFF their usual dopaminergic medication, and their performance was compared to healthy age-, gender- and education-matched controls ($N = 22$). Notably, none of our participants were apathetic or depressed, as assessed by standard clinical measures. In our task, participants had to decide whether they would be willing to exert one of six pre-specified levels of effort (as measured by force exerted on a hand-held dynamometer) to obtain one of six pre-specified stakes. For each participant, we estimated the effort level at which the probability of accepting a given stake was 50% (i.e., the effort 'indifference points' for each stake). Our key finding was that dopamine in PD exerts a motivating influence on choice behaviour. Specifically, patients ON medication were willing to invest greater effort for a given stake compared to when they were OFF. Importantly, this result could not simply be accounted for by differences in physical strength. Furthermore, compared with controls, the incentivising effect of dopamine was stake-dependent. For the lowest stake, patients with PD were less motivated than controls, regardless of whether they were on dopamine. However, this was overcome at higher stakes, when patients ON dopamine were willing to expend even greater effort compared to their healthy counterparts. Our results emphasise that motivational deficits may be present subclinically in PD, and independent of a syndrome of apathy or depression. In addition, we show that dopamine can enhance motivation towards effortful actions in PD, with its effects being most pronounced when the stakes are high. Notably, the incentivising effect of dopamine in our task was evident even prior to action initiation (i.e., during choice behaviour), suggesting that dopamine sets a variable gain on salience attribution depending on the rewards available. Overall, we conclude that motivational deficits are more prevalent in PD than previously thought, and that dopamine enhances the willingness to work for reward in a reward-dependent manner.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 262.16/RR7

Topic: F.01. Human Cognition and Behavior

Support: Univeristy of Missouri System IDIC Research Program

Title: Dissociable mechanisms of value-based decision-making in children: My own will or my Mom's choice?

Authors: *S.-L. LIM[1], A. S. BRUCE[1], J. B. CHERRY[1], N. HASS[1], S. N. BALAKRISHNAN[2], A. M. DAVIS[3]
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Abstract: Value-based decision-making is one of most important developmental tasks that children need to master. Through early learning experiences, children learn how to make optimal choices in a given environment with specific information. Particularly, food-related decisions in children are of great importance, because these can set up lifelong patterns of health-related behavior. However, healthy (optimal) food choices of children often require high-level cognitive regulation (e.g., choosing non-tasty but healthy lunch menu at school cafeteria), which may posit inevitable bounded rationality. The objectives of this project are to determine the computational and neural mechanisms underlying food decision-making processes in children using a human fMRI task. Seventeen healthy children between the ages of 8-14 made a series of food choices for 60 items (= eat or not) in two different conditions (children's own choice vs. guessing mom's choices for them) after providing separate taste and health ratings for each item. Our preliminary results (n=17) showed marked, dissociable behavioral and neural patterns between the two choice conditions. Child participants used primarily taste values when they made their own choices, which was accompanied by ventromedial prefrontal cortex (vmPFC) activations. Interestingly, when projecting what foods they believed their mother would choose for them, child participants used both taste and health values, which was accompanied by orbitofrontal cortex (OFC) and dorsolateral prefrontal cortex (dlPFC) activations. Understanding developmental mechanisms of value-based choices in children will have critical importance for improving health behavior choices in early development and in designing health behavior interventions accordingly.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

Location: Halls A-C

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Program#/Poster#: 262.17/RR8

Topic: F.01. Human Cognition and Behavior

Support: Center of Integrative Neurosciences Pool Project 2011-08 Tuebingen, Germany.

Fondo Nacional de Desarrollo Científico y Tecnológico Fondecyt (project nº11121153), Chile.

Title: Anterior insula self-regulation in obsessive compulsive disorder using real-time fmri-brain computer interfaces

Authors: *K. BUYUKTURKOGLU[1,2], H. RÖTTGERS[3], J. SOMMER[3], M. RANA[1,2], L. DIETZSCH[3], E. ARIKAN[3], R. VEIT[1], R. MALEKSHAHI[1,2], T. KIRCHER[3], N. BIRBAUMER[1,4], R. SITARAM[1,5], S. RUIZ[1,6]
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Abstract: Obsessive-compulsive disorder (OCD) is a common and chronic condition that can have disabling effects throughout the patient's lifespan. Typical obsessions include doubts, recurring thoughts with a connotation of violence, sexuality, religion and fear of contamination.

Actual or perceived threat of contamination can elicit several emotions, particularly disgust. Heightened disgust feelings towards disgust-inducing stimuli are commonly observed in self-report measures of OCD patients. A neural model based on recent studies of neuroimaging implicates that anterior insula has a role in disgust processing. Increased blood oxygen level dependent (BOLD) signal activity in the anterior insula could be implicated in the OCD psychopathology. In the current study, we applied real-time functional magnetic resonance imaging-brain-computer-interface (rtfMRI-BCI) to help OCD patients to achieve down-regulation of the abnormally high BOLD activity in anterior insula in the presence of disgust provoking stimuli. To explore the effects of rtfMRI-BCI-neurofeedback training in OCD symptomatology, several pre- and post-training measures were performed, e.g. confronting the patient with a disgust/anxiety inducing real-world object, ratings towards disgust provoking

pictures, physiological responses during these evaluations (heart rate, skin conductance, eye-tracking measurements) and general clinical evaluations. Preliminary results of the study (3 patients-2 female) show that OCD patients can gain self-control of the activity of anterior insula, albeit to different degrees with rtfMRI-BCI. In two patients who showed higher ability to regulate the activity of insular cortex, learned down-regulation led to significant positive changes in behavior: patients displayed less anxiety while confronted with a disgust inducing object ($p<0.01$, for both patients). Behavioral changes also were confirmed by a modification of the ratings towards symptom provocation pictures ($p<0.01$, $p<0.05$ respectively) and heart rate measurements inside the scanner ($p<0.01$, $p<0.05$ respectively) between baseline and down-regulation of insular cortex. The 3rd patient did not show a significant difference in any measurement between pre and post-tests. None of the patients showed significant difference in skin-conductance measurements in pre- or post-test/baseline-regulation conditions. These results confirm that insula down-regulation is possible in patients with OCD. A controlled decrease of insula activation could be used for symptom alleviation at least in some subtypes of OCD patients.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Program#/Poster#: 262.18/RR9

Topic: F.01. Human Cognition and Behavior

Support: German Federal Ministry of Education and Research (FKZ:01EO1001)

German Research Foundation (SFB 1052 Obesity mechanisms)

Title: Neural correlates of probabilistic reinforcement learning in individuals with obesity

Authors: *J. KUBE[1,2], L. KASTNER[1,2], A. HORSTMANN[1,2], A. HILBERT[2], A. VILLRINGER[1,2,3,4], J. NEUMANN[1,2]
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Leipzig, Leipzig, Germany; [4]Mind & Brain Institute, Berlin Sch. of Mind and Brain, Humboldt-University, Berlin, Leipzig, Germany

Abstract: Objective: Positive and negative reinforcement provide a powerful source of information to adjust behavior to changing environments. Recently, it has been reported that in addition to food-related alterations (Stoeckel et al., 2008) individuals with obesity also show changes in the neural processing of non-food reinforcement stimuli. Increased neural responses during the anticipation of monetary gains and losses in the ventral striatum, orbitofrontal cortex (OFC), and inferior frontal gyrus (IFG) point at more generalized alterations in reinforcement processing (Balodis et al., 2013). In this study we investigated if these alterations in the neural processing of non-food reinforcement affect feedback-based learning and behavioral adaptation. Methods:19 obese (BMI >=30, 10 female) and 23 lean (BMI 18.5-24.9, 11 female) right-handed adults matched for age and educational background performed a probabilistic reinforcement learning task during fMRI scanning. In separate gain, loss, and neutral monetary reinforcement conditions participants learned to choose between a high and low reinforcement probability option in order to gain money, avoid loosing money, or receive neutral feedback. Final score and the percentage of advantageous choices were examined as measures of learning performance. ROI analysis of the mean time courses of areas relevant for reinforcement processing (e.g. Liu et al., 2011) was employed to analyze neural responses during the outcome processing phase. Results:Individuals with obesity showed a significantly decreased learning performance in gain and loss trials. ROI analysis revealed that the processing of monetary gains and losses was associated with an increased activation of the IFG in obese vs. lean participants. Additionally, individuals with obesity also exhibited higher activation of the medial OFC in response to monetary losses. A greater differentiation between the two types of negative outcomes was found in obese participants, showing significantly higher activation to the receipt of monetary loss than the omission of monetary gain in the anterior cingulate cortex, IFG, and medial OFC than lean controls. Conclusion:Obesity is associated with a decreased reinforcement learning performance. Altered neural representations of negative reinforcement in the medial OFC and increased involvement of areas of executive control point at alterations in feedback processing potentially influencing associative learning in obesity.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

Location: Halls A-C

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Program#/Poster#: 262.19/RR10

Topic: F.01. Human Cognition and Behavior

Support: NIH NIDA DA01865-01

ARC FF 110100088

Title: Cannabis users failure to learn from errors associated with hypoactive dorsal ACC response

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Abstract: The chronic use of cannabis has been associated with attenuated error-related neural activity_particularly in the dorsal anterior cingulate cortex (dACC). Difficulty in modifying behaviour following an error is thought to be related to clinical impairments, such as perseveration, that are seen in substance abuse and other psychiatric disorders. Fifteen chronic cannabis users (four females, mean age = 22.40 years, SD = 4.29) and 15 control participants (two females, mean age = 23.27 years, SD = 3.67) were administered a visuospatial associative learning task that enabled participants to learn from their recall errors. Compared with controls, chronic cannabis users showed (1) lower recall error-correction rate and (2) hypoactivity in the dACC and left hippocampus during the encoding of error-related feedback. Furthermore, the difference in dACC activation between cannabis users and healthy controls varied as a function of error type, with the control group showing a significantly greater increase in activity from corrected to repeated errors than the cannabis group. The present results suggest that chronic cannabis users have poorer learning from errors, with the failure to adapt performance associated with hypoactivity in error-related dACC and hippocampal regions. The findings have implications for our understanding of the diminished capacity for learning from negative feedback seen in cannabis, and other forms, of dependence.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: NSF/NIH Collaborative Research in Computational Neuroscience (CRCNS) program and NIAAA (R01 AA018737)

Clinical Science Research and Development Service of the VA Office of Research and Development (I01CX000771)

Stress & Motivated Behavior Institute (SMBI), Rutgers-NJMS

Title: Post-traumatic stress disorder (PTSD) and generalization of learned associations: More severe re-experiencing symptoms are associated with increased generalization

Authors: *C. E. MYERS[1,2], R. J. SERVATIUS[1,2], M. W. GILBERTSON[3], S. P. ORR[4]
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Abstract: PTSD re-experiencing symptoms include intrusive memories of the traumatic event, which can be triggered by stimuli that reflect aspects of the traumatic event or can occur in the absence of external stimuli. At the same time, patients with PTSD often have impoverished memories for specific details of the traumatic event. One interpretation for this pattern invokes the concept of overgeneralization. Specifically, a traumatic memory is stored strongly but with minimal contextual and configural detail; later, other cues that would not normally evoke the memory now trigger recall. Contextual and configural information, which would normally help disambiguate memories, is unavailable. Re-experiencing symptoms may thus reflect a general memory bias against encoding contextual or configural information that is not limited to learning about traumatic events. In a prior study, we provided initial evidence for this possibility using an acquired equivalence task in which subjects learn cue-outcome mappings and then generalize learning about a subset of these cues to other "equivalent" cues. We found that veterans with more severe PTSD-related re-experiencing symptoms showed increased generalization. The present work examines a discrimination-and-transfer task in which subjects first learn to discriminate pairs of objects that differ in color or shape but not both. Subjects can either learn about configurations (e.g., choose one colored shape over a different colored shape) or can learn to ignore the irrelevant feature (e.g., given two objects of the same color, choose one shape over the other shape). Later, in an unsignaled transfer phase, irrelevant stimulus features are changed. Subjects who had learned to ignore the irrelevant features should perform well on transfer, while those who had learned about the configurations should perform near chance. We administered the discrimination-and-transfer test to 60 veterans (11.2% female, mean age 54.0 years) self-assessed for PTSD symptoms. Regression analysis revealed no relationships between PTSD

symptoms and initial learning; however, re-experiencing symptom scores significantly contributed to the prediction of transfer performance. Other PTSD symptom clusters (avoidance/numbing, hyperarousal) did not account for significant additional variance. These results suggest that re-experiencing symptoms reflect a general learning bias that promotes generalization at the expense of specificity. Future studies will be needed to determine whether this learning is a pre-trauma bias that confers risk for PTSD or emerges only after trauma exposure and PTSD symptoms development.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: NSF/NIH Collaborative Research in Computational Neuroscience (CRCNS) Program, by NIAAA (R01 AA018737)

Additional support from the Stress & Motivated Behavior Institute (SMBI)

Title: Exaggerated acquisition and resistance to extinction of avoidance behavior in treated heroin-dependent males (but not females)

Authors: *J. SHEYNIN[1,2,3,4], A. A. MOUSTAFA[1,5,6], K. D. BECK[1,2,3], R. J. SERVATIUS[1,2,3], P. A. CASBOLT[6], P. HABER[7], M. ELSAYED[7], L. HOGARTH[8,9], C. E. MYERS[1,3]
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Abstract: Addiction in general, and opioid addiction in particular, are often conceptualized as behavioral strategies for avoiding negative experiences. In rodents, opioid intake has been associated with abnormal acquisition and extinction of avoidance behavior. The current study tested the hypothesis that these findings would generalize to human opioid-dependent subjects, possibly in a sex-related fashion. Here, 27 heroin-dependent male and female patients treated with opioid medication (recruited from Royal Prince Alfred Hospital, Sydney, Australia) and 26 healthy control subjects were given a computer-based task to assess avoidance behavior. On this task, subjects controlled a spaceship and could either gain points by shooting an enemy spaceship, or hide in safe areas to avoid on-screen aversive events. Results show that while groups did not differ on escape responding (hiding) during the aversive event, heroin-dependent males (but not females) made more avoidance responses during a warning signal that predicted the aversive event. This group was also slower to extinguish the avoidance response when the aversive event no longer followed the warning signal. This behavioral pattern resulted in reduced opportunity to obtain reward without reducing risk of punishment. Results also suggest that differences in avoidance behavior cannot be easily explained by impaired task performance or by exaggerated motor activity in male patients. In sum, this study is the first to provide evidence for abnormal acquisition and extinction of avoidance behavior in opioid-dependent patients. Interestingly, data suggest abnormal avoidance is demonstrated specifically by male patients. Findings shed light on cognitive and behavioral manifestations of opioid addiction, and may facilitate development of therapeutic approaches to help affected individuals.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

Location: Halls A-C

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Program#/Poster#: 262.22/RR13

Topic: F.01. Human Cognition and Behavior

Support: NIMH/FIC R21MH095656 to MAG

Title: Learning from positive, but not negative, feedback is modulated by dopamine transporter genotype in patients with major depressive disorder and healthy subjects

Authors: *M. B. TAHA[1], H. Y. KHDOUR[1], I. T. MUGHRABI[1,2], J. Y. NATSHEH[1,3], H. M. DARWISH[4], M. M. HERZALLAH[1,3], M. A. GLUCK[3]
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Abstract: To better understand how depressive symptoms affect cognitive function in MDD, we evaluated two groups of subjects: medication-naïve patients with MDD and healthy control subjects. All were administered a category-learning task that allows for dissociation between learning from positive feedback (reward) versus learning from negative feedback (punishment). Medication-naïve MDD patients were impaired at learning from positive feedback as compared to healthy control subjects. However, there was no difference between MDD and healthy subjects in learning from negative feedback. Further, we examined the influence of the 3' variable number of tandem repeats (VNTR) polymorphism in the dopamine transporter gene (DAT1) on learning from positive and negative feedback in medicated patients with MDD. We grouped MDD and healthy subjects according to DAT1 VNTR genotype into 9-repeat carriers and 10-repeat homozygotes. MDD and healthy carriers of the 9-repeat allele, who presumably express less DAT1 and thus exhibit higher levels of dopamine, were more efficient in learning from positive feedback, whereas there was no difference between polymorphism carriers in learning from negative feedback. These findings highlight the importance of incorporating genetic profiles and studies of individual differences in future research on the relationship between psychiatric symptoms and cognitive function in MDD.

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: NIDA 1F32DA030017-01

NIDA 1F32DA033088-01

NIDA R21DA034954

NIMH 1R01MH090134

Title: The neural correlates of simulated drug choice in human addiction

Authors: *S. J. MOELLER[1], A. B. KONOVA[2], M. A. PARVAZ[1], M. MISYRLIS[2], K. E. SCHNEIDER[1], F. D'OLEIRE UQUILLAS[1], N. ALIA-KLEIN[1], R. Z. GOLDSTEIN[1]
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Abstract: Drug-addicted individuals often choose drug reinforcers over alternative reinforcers, a choice bias associated with addiction severity and its underlying neurobiological mechanisms. Yet, the neural correlates of drug-biased choice as related to value computation in the brain have not been identified. Here we investigate such value-based choice in individuals with cocaine use disorder (iCUD), using an fMRI task inspired by the neuroeconomics of simple choice. During this task, 10 iCUD (active and abstinent users) and 11 healthy controls (HC) viewed images depicting food (e.g., hamburger), threat (e.g., pointed gun), or cocaine (e.g., pipe); their preferences (*Strong No*, *No*, *Yes*, or *Strong Yes*) to view these salient images compared with a neutral reference image (wicker basket) were recorded on each trial. Choice ratings and reaction times (RT) (choice latency) were each analyzed with 3 (Picture: food, threat, drug) × 2 (Group: iCUD, HC) ANOVAs. Results showed that food choice ratings eclipsed cocaine- and threat choice ratings across groups (Picture main effect: $p<0.001$); iCUD also provided higher choice ratings than HC across pictures (Group main effect: $p=0.013$). Although the interaction did not reach significance in this preliminary sample, planned comparisons indicated that the group main effect was largely driven by iCUD's higher choice ratings for cocaine images ($p=0.096$; all other $p>0.25$) – particularly in active users (active>abstinent>HC; linear contrast: $p=0.021$). For RT, the longest RTs were on food trials, differing significantly from RTs on cocaine (but not threat) trials across groups (Picture main effect: $p<0.001$). A significant interaction ($p=0.021$) showed that whereas iCUD did not differ in RT between cocaine- and food trials, HC had slower RTs on food- than cocaine trials ($p<0.001$). Thus, all participants showed the highest preference and slowest RTs for viewing food images (indicative of their high subjective value), and iCUD also showed the expected cocaine choice bias relative to HC. Data collection is ongoing for a larger participant sample; further, fMRI analyses will model each picture type as modulated by the respective choice ratings, which will then be compared between groups. We expect that iCUD, during cocaine choice trials, will show activation in regions implicated in value computation [e.g., ventromedial prefrontal cortex (PFC)] and reduced activation in regions implicated in self-control (e.g., dorsolateral PFC). In sum, this fMRI task in iCUD is an important step forward (toward modeling real-world cocaine choice) from classical fMRI tasks of passive cue reactivity (which do not require evaluation of alternatives).

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Poster

262. Human Decision-Making and Learning: Individual Differences and Disorders

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Topic: F.01. Human Cognition and Behavior

Support: Studienstiftung des Deutschen Volkes (German National Academic Foundation)

Investissements d'avenir ANR-10-IAIHU-06, France

Title: Tight together: cross-level coupling during voluntary modulation of theta oscillations

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Abstract: The idea of voluntary control of neural activities has long been validated in human and animal studies. The modulation of oscillatory activity in the theta frequency range (4-8 Hz) has attracted particular interest due to its numerous possible applications in disorders or general cognitive boosting. Yet, we still lack understanding of the neurophysiological mechanisms entrained during such self-regulation of neural activity. To investigate how the modulations are organized at different spatiotemporal scales, simultaneous data from scalp, depth-, and micro-electrodes from 5 epilepsy patients undergoing presurgical evaluation were recorded while subjects were performing training of theta amplitude enhancement in the superior temporal cortex. Within successful sessions (test/baseline ratio > 1.2), periods with high theta power were detected for further analysis (> 3 SD of baseline). Spatial analysis has shown that modulatory effects were not limited to the training electrode but propagated to neighboring electrodes within 1-2 cm of cortex. The relationship between theta power and event duration was proportional, indicating that a certain temporal delay is needed to unfold maximal theta power. Subsequent spectrum and time-frequency representations revealed the concurrent presence of higher-frequent oscillations in the gamma range (30 - 40 Hz) occurring even prior to maximal theta power. Analysis of the cross-frequency-coupling showed that gamma responses were phase-amplitude

modulated on the ascending phase of the theta cycle. Similar responses were observed for multi-unit activity, where spike-timing was theta-phase-modulated only during successful trials. In accordance with recent proposals (1), we observed the interaction between large-scale (slow oscillatory activity) and local activity (higher oscillatory or cellular activity) in the form of theta-gamma coupling and phase modulation of cellular responses during trained modulation of theta activity. We argue that this tight coupling between spatiotemporal scales is a candidate mechanism for downward causation during self-regulatory processes of brain activity. (1) Bagdasaryan, J., & Le Van Quyen, M. (2013). Experiencing your brain: neurofeedback as a new bridge between neuroscience and phenomenology.Frontiers in human neuroscience, 7.

Disclosures: J. Bagdasaryan: None. **M. Valderrama:** None. **K. Lehongre:** None. **V. Navarro:** None. **M. Le Van Quyen:** None.

Poster

263. Functional Mechanisms of Attention I

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Program#/Poster#: 263.01/RR16

Topic: F.02. Animal Cognition and Behavior

Support: NIH MH060358

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Title: Thalamocortical alpha rhythms reflect attention and predict reaction times

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Abstract: Accumulating evidence suggests that oscillatory brain activity in the alpha frequency band (8-14 Hz) reflects functional inhibition (Haegens et al., 2011): decreased alpha facilitates processing whereas increased alpha functions to suppress task-irrelevant and distracting input. To examine this “functional inhibition” hypothesis, we analyzed data collected in monkeys trained to perform an intermodal (visual/auditory) selective attention task, which required them to attend to and discriminate stimuli within one modality, while ignoring stimuli in the other modality. Data consisted of intracortical linear array multi-electrode recordings (150 or 200 μm

inter-contact spacing) sampled from three macaque monkeys in the primary visual (V1) and auditory cortices (A1) and in the lateral geniculate nucleus (LGN). The analysis of laminar profiles of "local" field potentials (LFP) and multi-unit activity (MUA) was augmented by current source density (CSD) analysis of the LFP profile. We analyzed four conditions: (1) attend auditory, (2) attend auditory with visual distracters, (3) attend visual, and (4) attend visual with auditory distracters. As we have shown before (Bollimunta et al., 2011), alpha power in V1 LFP was modulated according to the attended modality: alpha was stronger when attending to auditory stimuli (and ignoring visual) as compared to attending to visual stimuli. Here we replicate this analysis for A1, and show lowest alpha power for attend auditory as compared to visual conditions. Recordings in the LGN showed increased alpha power during the visual distracter condition compared to the other conditions. As an additional step, we asked how (slow) fluctuations in ongoing alpha activity correlate with task performance in terms of reaction times to targets. We find that high alpha power in the non-attended modality (i.e., V1 for auditory and A1 for visual stimuli) corresponds with faster reaction times. This effect was most apparent in supragranular layers. These results support the notion that alpha reflects a mechanism of functional inhibition, with increased alpha in task-irrelevant regions suppressing distracting input, subsequently leading to improved behavioral performance. References Bollimunta A, Mo J, Schroeder CE, Ding M (2011) Neuronal mechanisms and attentional modulation of corticothalamic alpha oscillations. J Neurosci 31:4935-4943. Haegens S, Nácher V, Luna R, Romo R, Jensen O (2011) α-Oscillations in the monkey sensorimotor network influence discrimination performance by rhythmical inhibition of neuronal spiking. Proc Natl Acad Sci USA 108:19377-19382.

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Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Title: The role of low frequency oscillations in attentional modulation in area mt

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Abstract: Neural oscillations are prominent components of activity in brain areas, which are contributed mainly by non synaptic events. It has been proposed that these oscillations are recruited by attention, to modulate neuronal activities in different brain cortical areas. Different frequency bands of local field potentials (LFP) are representatives of these oscillations. Increase and reduction of oscillatory neuronal synchronizations in high and low frequency bands, respectively, has been suggested as main effects of attention, indicating the role of high frequency in expense of suppression of low-frequency oscillations. More recently, some experimental evidence challenged this finding with demonstrating a profound role of low frequency oscillations in modulation of neuronal activities. In our experiment, we recorded actions potentials and LFPs from the middle temporal area of a macaque monkey, during a spatial attention task. Two random dot patterns were presented simultaneously in either sides of a fixation spot. One of them was located inside the receptive field of recorded neurons and the other outside. While fixating, monkey had to attend to one of the stimuli, based on a given cue in the beginning of each trial, resulting in two attention conditions; attended and unattended. The task of the monkey was to detect a slight change in the target stimulus. All trials were split into different categories, based on their reaction times (RTs). After filtering the LFP signals in different frequency bands, instantaneous phase and amplitude of each signal were extracted using Hilbert transform. Time points within a window of 500 ms before stimulus onset were considered in our analyses. We tried to decode the RT category of each trial, in the two attention conditions, using linear SVM classifier. We also performed a time resolved analysis to find temporal patterns of the decoding performances. We assessed the performances by cross-validation. Our results demonstrate that phase of low-frequency bands, in particular theta (4-8 Hz) and alpha (8 - 12 Hz) predict the RT in the attended condition, better, in comparison with the unattended condition ($p<10\text{-}10$). Furthermore, the performances for these bands start to increase at about 300 ms before the stimulus onset in the attended condition, while we observed no specific temporal patterns in performances for the unattended condition. The resulted performances and temporal patterns indicate that attention changes state of the network just before the stimulus onset and therefore, assists the neural processing. These findings can be evidence for roles of low-frequency oscillations in attentional modulations of neural activities.

Disclosures: **M. Esghaei:** None. **M. Daliri:** None. **K. Maboudi:** None.

Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH060358

Title: Slow fluctuations underlying information processing in macaque monkeys

Authors: *C.-G. YAN[1], A. BARCZAK[1,2], S. HAEGENS[1,4], M. P. MILHAM[1,5], F. X. CASTELLANOS[1,3], C. E. SCHROEDER[1,4]
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Abstract: Resting state fMRI (R-fMRI) studies report slow hemodynamic fluctuations on scales not typically associated with behavior, as low as 0.01~0.1Hz. Although uncertainty exists about the precise neurophysiological bases of slow hemodynamic fluctuations, both R-fMRI fluctuations and their electrophysiological counterparts have clear impacts on perception, cognition and behavior. Here, we examine slow fluctuations in behavioral reaction time (RT) and activity rates, as well as their relationship with underlying neuronal activity. Two monkeys were presented with random streams of 'standard' (86%) and 'oddball' (14%) visual and auditory stimuli. The monkey pressed a switch to initiate stimulus presentation and released it upon detecting an oddball. In continuous good performance (<5% misses) of the intermodal task over 5-8 min periods, we find slow fluctuations in reaction time (RT) on the scale of the hemodynamic fluctuations noted in R-fMRI studies. RT varies over a range of ~200 ms (~40% of the mean RT), showing significant peaks (via permutation tests) throughout the low frequency range we sampled, with a cluster centering at 0.03Hz. Slow fluctuations in concurrently recorded multiunit activity (MUA) from V1 during the task, are positively correlated with the slow RT fluctuations in about half of the experiments, although this relationship is not significant in the remainder of the passes. To further explore the slow fluctuations in behavior and ongoing electrophysiological activity, we analyzed another data set with two monkeys performing a visual search task. The monkeys faced a grey video screen with four low contrast grey discs. A target (red dot) was hidden in one of the discs and only appeared when the monkey fixated in the central part of the "target" disc for at least 100ms. We observed slow fluctuations in the saccade rate (with a peak around 0.025 Hz) similar to the slow fluctuations seen in the first task. We also found a negative correlation between saccade rate and pupil diameter. MUA amplitude in V1 was also negatively correlated with pupil diameter but positively related to saccade rate. As the pupil diameter is considered to reflect sympathetic arousal, it would appear that both saccade rate and MUA decrease during higher arousal periods. This is consistent with the first finding that MUA decreases during fast RT epochs. Our findings underscore slow activity fluctuation effects in behavior and their underlying neurophysiological events occurring at faster scales, and support the view that the brain slowly shifts between dynamic states.

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Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Title: The role of fast-spiking parvalbumin interneurons and gamma rhythms in attention and behavior

Authors: *H. KIM, M. CARLÉN
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Abstract: Cortical fast-spiking parvalbumin (FS-PV) interneurons are central in certain network processes, including the generation of gamma oscillations. However, it is becoming increasingly clear that interneurons have functions beyond network coordination in local circuitry. It has recently been demonstrated that cortical FS-PV interneurons are recruited at specific behavioral events and serve a direct function in behavior. Attention is a central cognitive process enabling goal-directed behavior. Earlier work has indicated the presence of prominent inhibition in medal prefrontal cortex (mPFC) during attentional processes, but the source and the role of inhibition in attention are still unknown. We hypothesized that FS-PV interneurons are involved in attentional processes and conducted extracellular single-unit recordings paired with optogenetics in the mPFC of PV-Cre mice performing an attention task (3-choice serial reaction time task, 3-CSRTT). During the task, animals are required to attend to a brief cue presented pseudorandomly at one of three holes in an operant chamber and to report the stimulus location by nose poking. Single units were recorded and FS-PV interneurons were identified through opto-tagging. The 3-CSRTT was divided into several specific epochs pertaining to behavior (e.g. precue period, cue nose poke, reward consumption) and the population activities were analyzed in relation to the epochs. We found that the FS-PV population responded selectively at distinct epochs of the behavior, including the precue period and after cue nose poke, pointing to a role for FS-PV interneurons in attention. In summary, our results suggest a direct function of mPFC FS-PV interneurons in different behavioral events, including attentional processes and goal-directed behavior, and possibly also in reward and/or reward anticipation.

Disclosures: **H. Kim:** None. **M. Carlén:** None.

Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant EY020679

Title: Time course of attentional modulations in primary visual cortex

Authors: *X. LI[1], M. JANSEN[1], R. LASHGARI[1,2], H. SWADLOW[1,3], Y. BERESHPOLOVA[3], J.-M. ALONSO[1]
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Abstract: Spatial attention is known to modulate the activity of single neurons in primary visual cortex (area V1) but it remains unclear how these modulations change over time. To measure the time-course of V1 attentional modulations, we trained two rhesus monkeys to pay attention for 1-3 seconds to one of multiple drifting gratings cued at the beginning of each trial. The monkeys were rewarded to maintain their attention for the entire trial and release a lever as fast as possible after detecting a change in either grating color or orientation. V1 single neurons were recorded with a chronically implanted array of multiple ultra-thin sharp electrodes that were independently moved through the depth of the cortex (Swadlow et al., 2005). The spike trains of each neuron were converted into a continuous spike density function to calculate mean rate (F0) and response power within a low frequency range (LF) containing the stimulus frequency and nearest harmonics (1-7.5 Hz). Attentional modulations were measured as a percentage of response change when attention was cued inside versus outside of the receptive field and the attentional time course was calculated by sliding a temporal window of 500 msec (10 msec steps) over the peri-stimulus time histogram (PSTH) of the attentional modulations. In 61 neurons, we were able to perform enough attentional measures (average: 891 trials) to reliably track the attentional time-course and measure it at different trial durations. The attentional time-course of single V1 neurons could be accurately fit with a Weibull function and the fits were generally more accurate for F0 than LF (mean R2 for 2-sec trials: 0.939 for F0 vs. 0.761 for LF, n=61), however, some neurons were better fit with LF than F0 and some neurons could not be fit with either method (e.g. neurons with highly rectified PSTHs because the response was zero for half of the trial).

Our results demonstrate that spatial attention increases the mean firing rate of the average V1 population roughly linearly over time, at a rate of 20% per 0.1 seconds (percentage relative to maximum modulation). The attentional modulation peaks around 0.5-1 seconds after the stimulus onset and then starts decaying around 1.5-2 seconds at a rate of 5% per 0.1 seconds. These measurements indicate that the attentional modulations change continuously during the full duration of 1-second trials, increase and then saturate in 1.5-2-second trials and increase, saturate and start decaying in 3-second trials. Therefore, we conclude that the effect of spatial attention in V1 neurons changes over time and peaks at the median duration of the trial set used in the attention task.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG030646

Title: Assessing removal of illumination as a signal: Effects of loss of basal forebrain corticopetal cholinergic neurons

Authors: *J. A. BURK, D. OTOYA, C. LEONG, A. NG, C. T. KOZIKOWSKI
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Abstract: In the vast majority of experiments assessing visual attention in animal models, tasks are employed that require the detection of a stimulus that was previously absent. Relatively few experiments have employed procedures where the signal is the removal of a previously presented stimulus. In the present experiment, we modified a previously validated measure of a visual attention that required detection of a signal (illumination of a central panel light for 500, 100 or 25ms) from “blank” trials when the light was not illuminated. Loss of basal forebrain corticopetal cholinergic neurons has been shown to decrease signal detection in this task. We modified the task so that the central panel light was illuminated throughout the intertrial interval and a signal occurred when the light was turned off (4-s) whereas a blank trial occurred when the central panel light remained illuminated. Male FBNF1 hybrid rats were trained in this revised

attention task and then assigned to receive infusions of 192IgG-saporin or saline into the basal forebrain. Rats were retrained in the task after surgery and then received one session with a houselight flashing in the back of the chamber throughout testing and a second session with the signal decreased from 4-to 2-s. During presurgical training, we observed that animals required a longer signal to maintain stable task performance when the signal involved turning off the central panel light. Surprisingly, loss of basal forebrain corticopetal cholinergic inputs was associated with higher rates of signal detection compared with sham-lesioned animals, although this effect was attenuated with subsequent training. Flashing the houselight decreased accuracy on blank trials, but did not differentially affect lesioned and sham-lesioned animals. Signal detection accuracy significantly declined in both lesioned and sham-lesioned animals when the signal duration was decreased. Collectively, these results suggest that task manipulations appear to have similar effects whether the signal involves turning the central panel light on or off. However, the neural mechanisms that are engaged during these two types of tasks appear to be different. Future work in our laboratory will explore the role of basal forebrain noncholinergic neurons in performance of a task with turning the central panel light off serves as a signal.

Disclosures: **J.A. Burk:** None. **D. Otoya:** None. **C. Leong:** None. **A. Ng:** None. **C.T. Kozikowski:** None.

Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

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Title: Cholinergic modulation of frontoparietal activity during the orienting and disengagement of attention in rats

Authors: *V. LJUBOJEVIC[1], P. LUU[2], P. R. GILL[3], L.-A. BECKETT[1], K. TAKEHARA-NISHIUCHI[1], E. DE ROSA[1]
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Abstract: The frontoparietal cortices are thought to play a role in selective attention processes such as the orienting (OrA) and disengagement (DsA) of attentional focus (Thiel et al., 2004).

Yet, it is unclear how acetylcholine (ACh) input into the prefrontal (PFC) and posterior parietal (PPC) cortices modulates OrA or DsA. In the present study, we measured local field potentials (LFP) from the PFC and the PPC of rats performing a selective attention task. Further, we examined how cholinergic deafferentation of the PFC and the PPC affects both the behaviour and LFP activity associated with both aspects of attention. OrA and DsA were assessed using the Posner (1980) cuing task, a paradigm in which a subject responds to a target that is preceded by a spatially informative cue. OrA is isolated as speeding of reaction times (RT) after spatially informative cues (valid trial) compared to RT after non-informative cues (neutral trial) (Orienting Effect, OE=RTneutral-RTvalid). DsA is isolated as slowing of RT following the misleading cues (invalid trial) compared to RT after valid cues (Validity Effect, VE=RTinvalid-RTvalid). After the training on the CTD task male Long-Evans rats underwent either a selective cholinergic lesion reducing frontoparietal cortical ACh input (N=9), or a sham lesion surgery (N=8). Then, rats were implanted with cortical electrodes into the prelimbic PFC and the PPC and tested on the CTD. Histological analyses confirmed both the neurochemical and neuroanatomical specificity of the lesions and electrode placement respectively. We observed increased power of low-beta oscillations (12-18Hz) in the PFC after cue presentation that was associated with OrA (i.e. present on valid, but not on neutral trials). This pattern was observed in both groups of rats. Additionally, the size of the OE was equivalent between groups. We also observed increased power of high-beta oscillations (15-25Hz) in the PPC after target presentation that was associated with DsA (i.e. present on invalid, but not on valid trials). Interestingly, this DsA-associated brain activity was attenuated in lesioned rats. Also, lesioned rats had significantly slower RTinvalid and significantly higher VE than controls indicating behavioural deficit in DsA. In summary, this study uncovered distinct neural correlates of OrA and DsA. We found that the activity of neuronal population within the PPC was associated with the disengagement of attention from a spatial location, while the activity within the PFC was associated with the orienting of attention following spatially informative cues. Importantly, ACh input into PPC was necessary for successful and efficient DsA, while OrA was ACh-independent.

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Poster

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Title: Neural correlates of sustained attention in various modalities in rats

Authors: *D. WU, H. DENG, L. WEI, X. XIAO, Y. ZUO, Z. WANG
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Abstract: Sustained attention is a fundamental brain function, but our knowledge about its neural mechanisms is very limited. At the system level, previous studies have suggested that the anterior cingulate cortex (ACC) is a key brain region among those that generate the event-related potential of contingent negative variation (CNV) that is demonstrated to be caused by sustained attention. At the neuronal level, however, the limitations of the previous experimental strategies led to the lack of evidence for the potential causal relationship between ACC neurons and sustained attention. Thus, the present study aimed at the identification of the neurons responsible for sustained attention. We first developed a behavioral task to systematically study both visual and olfactory attention in rats. In our training procedure, rats should make a nose poke to a trigger aperture in front of the other three stimulus apertures to initiate the attention state that sustained for an appropriate time interval until the rats detected the location of the brief forthcoming stimulus. Then, using extracellular recording technology, we have sorted out a large number of neurons in the ACC and systematically studied response patterns of these neurons in both visual and olfactory sustained attention. The results showed that several types of neurons were involved in sustained attention. Generally, one type was excited and the other was inhibited during the time window of sustained attention despite of the modalities. More specifically, these neurons could be classified in terms of the onset of their responses to the sustained attention. Some neurons started to response before the trigger, some at the trigger and the others after the trigger. Also, the initiation time was related to difficulties of tasks: responses in difficult task were initiated earlier than in easy task. In terms of the offset of their responses, there also existed three types: response ends at the poke of food tray, at the poke of stimulus aperture, and between the poke of stimulus aperture and food tray. In terms of sensory modalities, some neurons showed different firing rates during visual and olfactory attention, respectively. At the population level, the fano factor was reduced, and the correlation coefficient between pairs of neurons was induced during the sustained attention. Based on these response patterns of ACC neurons, we deduced that these neurons played central role in the initiation and maintenance of sustained attention. More data are needed to support these hypotheses. The present study provides a new insight for investigation of the micro-neural circuits of sustained attention.

Disclosures: D. Wu: None. **H. Deng:** None. **L. Wei:** None. **X. Xiao:** None. **Y. Zuo:** None. **Z. Wang:** None.

Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

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Title: Emotional distracters and emotional state both influence spatial attention in macaque monkeys

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Abstract: Faces with emotional expression, even when irrelevant to the ongoing task, are known to act as potent distracters. The influence of affective stimuli is further modified by the emotional state. However it remains debatable whether this process is automatic and reflexive or dependent on the duration of exposure. Here we varied emotional content in face pictures used as distracters to test their influence on behavioral performance on a spatial attention task in monkeys. We sought to vary monkeys' emotional state by intranasal administration of the neuropeptides, Oxytocin (OT) and Vasopressin (VP). OT and VP are key emotional regulators implicated in producing opposing effect on anxiety and social stress. The task required monkeys to monitor one of two gratings for a subtle color change and were rewarded for making a saccade to target grating when the change occurred. Two irrelevant images of monkey faces with threatening, fearful or neutral expression appeared between fixation-spot and gratings. Presence of affective faces for duration greater than 200 ms significantly reduced accuracy (d') and shortened reaction time (RT). Both OT and VP slowed RT and improved accuracy in trials with emotional faces, however in VP trials this effect was less dependent on exposure duration, particularly in presence of threatening faces. We also tested whether monkeys looking behavior showed particular bias towards emotional expression in a free choice paradigm. When presented with faces of conspecifics with or without emotional expression, in 2/3rds of the trials, they made the first saccade towards neutral face, but on average spent significantly greater time scanning the threatening or fearful faces. When all three expressions were presented, they chose to scan

threatening faces for far longer duration. Thus while early saccades showed tendency for active avoidance, persistent presence of emotional images required redirection of attentional resources, possibly an adaptive mechanism for active scanning of the perceived threat.

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Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Title: Repeated sensory load post-weaning induces lasting alterations in attentional functions and BDNF expression

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Abstract: Attentional functions are known to change during the life of an animal, yet these changes remain poorly understood. More specifically, it is unclear whether sensory loading post-weaning could ultimately impact attentional functions in adulthood. Here we demonstrate the plastic nature of attentional functions during development. We show that exposing rats to an attention-engaging non-ecological environment during a presumed critical developmental window changes performance in attention-stringent tasks and influences the degree of neuronal plasticity of attention-related brain systems in adulthood. Specifically, we show that, rats exposed to a continuous regimen of changing and salient olfactory stimuli Post-weaning, acquire the five choice serial reaction time task (5-CSRTT) faster than control, yet found to be more distractible, impulsive, and have more variable reaction times. Moreover, these behavioral parameters were found to be correlated with BDNF levels in the hippocampus, nucleus accumbens and frontal cortices. This finding implies for a possible primary cause for attention-related deficiencies in humans as well.

Disclosures: I. Hadas: A. Employment/Salary (full or part-time):; Ben Gurion University in the negev, life sciences department. **Y. Amitai:** None. **A. Zangen:** None.

Poster

263. Functional Mechanisms of Attention I

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Smoking Research Foundation

Title: 3D video analysis of object exploration in rats

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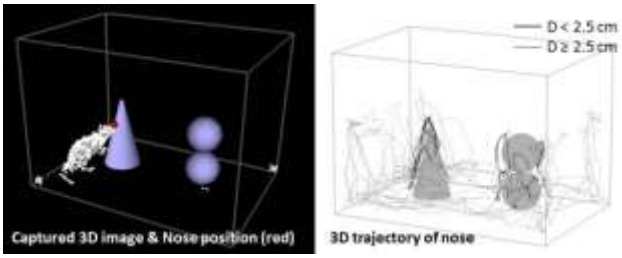
Abstract: Analysis of the trajectory provides insights into dynamic and complex brain activity responsible for spontaneous behaviors, as eye tracking has contributed to the investigation of the process underlying gaze control during scene perception. In this study, we developed a novel 3D video analysis system that could track the nose of a rat and applied it to analyze spatiotemporal patterns of object exploration. By means of this system, we analyzed object exploration of intact rats during a novel object recognition test, and compared the results in the intact rats with those in rats systematically injected MK-801. The 3D trajectory analysis revealed a specific pattern of object exploration in the sample phase of the novel object recognition test: intact rats first explored the lower parts of objects and then gradually explored the upper parts. The systematic injection of MK-801 suppressed changes in these exploration patterns. Comparison of these data with those analyzed by visual inspection of experts demonstrated that the system could precisely track the nose and detect the nose contact with an object. It is noted that the 3D system can reproducibly and accurately score the novel object recognition test, which represents an advance over previous scoring methods (visual observation and computerized 2D video analysis) in

which reproducibility and accuracy are incompatible. The results, along with those of previous studies, suggest that the changes in the exploration patterns reflect neophobia to a novel object and/or changes from spatial learning to object learning. These results suggest that the 3D system has the potential to facilitate future investigation of neural mechanisms underlying object exploration that result from dynamic and complex brain activity.



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Poster

263. Functional Mechanisms of Attention I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 263.12/RR27

Topic: F.02. Animal Cognition and Behavior

Support: The National Natural Science Foundation of China (No. 31372217)

The National Natural Science Foundation of China (No. 31270042)

Title: Rapid species discrimination and individual recognition associated brain areas and components of event-related potentials in frogs

Authors: ***G. FANG**, P. YANG, Y. TANG
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Abstract: Both species discrimination and individual recognition are crucial for survival and/or reproductive success. However, the neural mechanisms underlying these cognitive processes remained unclear. In this study we measured various components of the event-related potentials (ERPs) acquired from the telencephalon and mesencephalon of the music frog (*Babina daunchina*) during the non-reproductive and reproductive stages, elicited by conspecific calls and synthesized acoustic stimulus consisted of white noise (WN) with similar temporal structures as

the conspecific calls. Previous studies found that the males produce two types of advertisement call: calls from within nest burrows as highly sexually attractiveness (HSA) and outside as low sexual attractiveness (LSA). ERPs in response to stimuli showed that (1) amplitudes of N1 were significantly different between synthesized and conspecific sounds while no difference between HSA and LSA, indicating a fast process of auditory object discrimination at the species level around 100 ms; (2) amplitudes of P2 in the difference waves, defined as the amplitude elicited by one stimulus subtracted that by the other, between HSA and WN was significantly higher than that between LSA and WN in the right telencephalon, implying that individual recognition might be achieved in 200 ms; (3) latencies of P3 during reproductive stage were longer than those during non-reproductive stage in the left mesencephalon, suggesting the acoustic signal processing modulated by reproductive status. These results implied that both species discrimination and individual recognition might be accomplished very quickly in both the non-reproductive and reproductive stages and the related processes could be represented by the ERP components.

Disclosures: G. Fang: None. **P. Yang:** None. **Y. Tang:** None.

Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

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European Union Seventh Framework Program

Title: Effects of attention and working memory in the different layers of monkey primary visual cortex

Authors: *T. J. VAN KERKOERLE[1,2], M. W. SELF[2], P. R. ROELFSEMA[2,3]
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Abstract: Selective attention enhances the processing of relevant versus irrelevant stimuli. It is thought to rely on feedback from higher cortical areas to early sensory areas but direct evidence

for this is limited. Working memory on the other hand, maintains task-relevant information for short periods of time when the stimulus is no longer present. Working memory has been suggested to involve similar top-down mechanisms and may therefore take place in the same areas that are used for perceptual processing. However, robust working memory traces in primary sensory areas have so far only been found using fMRI. It is therefore unknown if these signals are caused by subthreshold synaptic activity or by spiking activity. We here set out to investigate whether working memory traces are present in the spiking activity of neurons in monkey primary visual cortex (V1) and whether selective attention and working memory involve top-down processing by recording from all cortical layers at the same time. We used a curve tracing task which generates robust object-based attentional modulation in V1, enhancing spiking activity for a target versus a distractor curve. We now measured attentional modulation and also required the monkeys to memorize the stimulus during a delay so that we could examine working memory in V1. We observed highly significant working memory traces in the spike rate of monkey V1, although they were weaker than the attentional effects. This memory activity was briefly lost if we presented a mask but it returned afterwards. Thus, the working memory trace in V1 is not robust to sensory interference but it can be restored and therefore does not depend on an afterimage. We used a control experiment to demonstrate that the memory trace was unrelated to eye movement preparation. V1 has a clear laminar organization with feedforward projections targeting mainly layer 4C and feedback projections targeting layers 1-3 and 5. Laminar recordings therefore have the potential to distinguish between bottom-up and top-down processing streams. We investigated both spiking activity and the current source density (CSD), which reflects the synaptic input to the different layers. The laminar profiles of attention and working memory were highly similar, showing signatures of feedback processing for the full duration of the trial. These findings show for the first time that a robust working memory can be found in the spiking activity of V1 neurons. Moreover, we provide direct evidence that feedback processing to V1 is involved in both selective attention and working memory, indicating that these two cognitive functions share cortical mechanisms.

Disclosures: T.J. Van Kerkoerle: None. **M.W. Self:** None. **P.R. Roelfsema:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Medical Research Council

Wellcome Trust

Title: Attention and normalization circuits in macaque V1

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Abstract: Attention affects neuronal processing and improves behavioural performance. In extrastriate visual cortex these effects have often been explained by normalization models, which assume that attention influences the same circuit that otherwise mediates surround suppression. While normalization models have recently been able to explain attentional effects, their validity has not been tested against alternative models. Here we investigate how attention and surround/mask stimuli affect neuronal firing rates and orientation tuning curves in macaque V1. We trained 2 macaque monkeys in a covert top-down spatial attention task where they had to detect a stimulus (luminance) change at a cued location. Stimuli were either single oriented bars, or orientated central bars, which were surrounded by multiple pseudo-randomly oriented bars. Surround/mask stimuli provide an estimate to what extent V1 neurons are affected by normalization, which was compared to the effects of spatial top down attention. We recorded 105 neurons in two monkeys (62 in monkey 1, 43 in monkey 2).We found that the strength of attentional modulation was often correlated with the strength of surround modulation, suggesting that attention and surround/mask stimulation (i.e. normalization) use a common mechanism. To explore this in detail, we fitted multiplicative and additive models of attention to our data. In one class of model, attention contributed to normalization mechanisms, in a different class of model it did not contribute to normalization. Model selection based on Akaike's and on Bayesian information criterion demonstrated that for most V1 neurons the effects of attention was best described by additive models. Moreover, in most cells the effects were best described by models where attention did not contribute to normalization mechanisms.

Disclosures: **A. Thiele:** None. **M. Sanayei:** None. **J. Herrero:** None. **C. Distler:** None.

Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Support: F32-EY023922

R01-EY019882

P30-EY008126

Title: Frontal eye field correlates of salient distractor suppression during visual search

Authors: *J. D. COSMAN[1], J. D. SCHALL[1], G. F. WOODMAN[2]
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Abstract: During visual search, the question of whether visual information that is salient, but task irrelevant, captures attention in a bottom-up manner has been long debated. Alternative views have proposed that this type of visual information can be effectively suppressed based on top-down goals. A great deal of behavioral and neuroscientific work has provided evidence for both views, suggesting that suppression and attentional capture may both be possible depending on task conditions or an individual's attentional state. In the current work, we examined visual responses of neurons in Frontal Eye Field (FEF), a region of prefrontal cortex thought to be critical for the instantiation of top-down control over attention, while macaque monkeys performed a visual search task in which an salient but task irrelevant singleton appeared on some trials. We observed that firing rates to salient distractors in a neuron's receptive field were suppressed relative to those for search targets, but not to the same extent as non-salient distractor items. This suggests that although salient but task-irrelevant visual signals are suppressed to some extent, this suppression is incomplete, which may be responsible for conflicting results in the cognitive science literature.

Disclosures: J.D. Cosman: None. **J.D. Schall:** None. **G.F. Woodman:** None.

Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG063646

Title: Interactions between noncholinergic basal forebrain neurons and muscarinic receptors in attentional processing

Authors: *C. T. KOZIKOWSKI, E. L. WOLFE, P. G. YANEV, J. A. BURK
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Abstract: Numerous studies have provided evidence that basal forebrain corticopetal cholinergic neurons are critical for normal attentional performance. However, the role of noncholinergic basal forebrain neurons in attention has not been well-characterized. Moreover, evidence regarding interactions between cholinergic receptor activity and noncholinergic basal forebrain neurons remains scarce. In the present experiment, rats (n=15) were trained in a two-lever sustained attention task that required to discriminate between brief illumination of a centrally located panel light (500, 100, 25 ms) from trials when the light was not illuminated. After reaching criterion performance, rats received infusions into the basal forebrain of saline (n=7) or the immunotoxin, GAT1-saporin (n=8), to lesion noncholinergic neurons. After re-establishing performance after surgery, all rats received systemic administration of the muscarinic receptor antagonist, scopolamine (0, 0.05, 0.20 mg/kg, ip). When attentional testing resumed after surgical recovery, lesioned animals' task performance did not significantly differ from sham-lesioned animals. However, following the highest dose of scopolamine, lesioned animals exhibited a larger decline in signal detection accuracy compared to sham-lesioned animals. Additionally, lesioned animals' omission rate was higher during injection sessions compared to sham-lesioned animals. These results suggest that noncholinergic basal forebrain neurons are not necessary for performance in a well-trained attention task. However, loss of these neurons renders animals' attentional performance more vulnerable to decreased cholinergic system stimulation. Finally, the lesion-induced increase in omissions may reflect a role for noncholinergic basal forebrain neurons in processes beyond attention.

Disclosures: C.T. Kozikowski: None. **E.L. Wolfe:** None. **P.G. Yanev:** None. **J.A. Burk:** None.

Poster

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Research to Prevent Blindness Unrestricted Grant

Research to Prevent Blindness Carrer Development Award

Eye and Ear Foundation of Pittsburgh

Title: Selective attention independently modulates both spiking correlations and EEG oscillations

Authors: *A. C. SNYDER[1], M. A. SMITH[1,2,3]
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Abstract: Selective visual spatial attention has been studied extensively at quite disparate scales of brain organization, from the large-scale networks observable using scalp EEG recordings in humans to the micro-scale networks of small populations of neurons observable using extracellular recordings with microelectrodes in monkeys. At the large-scale, retinotopic shifts in broad-band response and band-limited responses (e.g., power in the 8-12 Hz "alpha" band) have been linked to shifts in the locus of spatial attention. At the micro-scale, modulations of correlated trial-to-trial spiking variability ("spike count correlation"), consistent with an improvement in information processing, have been reported in monkeys performing a spatial attention task. To date these processes have only been studied independently. However, the fundamental mechanisms of spatial attention must involve the large-scale coordination of distant brain areas to balance the quality of processing in micro-scale circuitry and support goal-directed behavior. Thus, a comprehensive understanding of attention will require bridging these two scales. For this study we combined these two methods for the first time to study selective attention in monkeys, with the aim of relating the attention processes that have been independently studied across scales. We found that the relationship between EEG oscillations measured at the scalp and spike count correlation measured among small populations of neurons was not fixed, but instead varied depending upon the attention state of the animal. This suggests that the attention effects of EEG oscillations are not merely epiphenomenal reflections of underlying changes in spiking correlation, but rather that EEG oscillations and spike count correlation are indexing separable mechanisms of attention. For example, scalp EEG may reflect correlations among different subpopulations of neurons depending on the stimulus or the animal's cognitive state. In addition, the correlations among V4 neurons reflect only a small portion of the broader attentional network in the brain, which consists of vast numbers of neurons spread across numerous distant brain regions. Nonetheless, our findings support the

value of studying attention through the use of concurrent measurements of neural indices of attention across disparate scales.

Disclosures: **A.C. Snyder:** None. **M.A. Smith:** None.

Poster

263. Functional Mechanisms of Attention I

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Research to Prevent Blindness Career Development Award

Research to Prevent Blindness Unrestricted Award

Eye and Ear Foundation of Pittsburgh

Title: High-dimensional neural correlates of choice and attention in V4

Authors: *M. J. MORAIS[1,2], A. C. SNYDER[1,3], M. A. SMITH[1,2,3]
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Abstract: Complex cognitive tasks, such as making decisions in the face of noise and ambiguity, or allocating visuospatial attention, are necessarily accomplished by the concurrent activity of large populations of neurons. Nevertheless, these processes have historically been studied mostly by observing the first-order firing rate statistics of single neurons. In experimental paradigms designed to study movement planning and perceptual decision-making, researchers have recently begun to use high-dimensional analyses to elucidate the coding strategies employed dynamically in the brain during these processes. For this study, our aim was to assay how well these approaches can reveal the mechanisms of spatial attention in an earlier stage of sensory

processing. We recorded populations of neurons using microelectrode arrays in V4 of macaque monkeys in a spatial selective attention task. We then employed targeted dimensionality reduction, a hybrid of principal components analysis (PCA) and multiple linear regression (MLR), to orthogonalize the trial-averaged population data according to particular task variables. Using dimensions associated with the animal's choice along with cue and target characteristics, we showed that differing trial outcomes were associated with differing trajectories through this reduced subspace. For example, when the animal made an incorrect choice given valid cue information, the corresponding neural trajectory reflected less pronounced encoding of cue information. When the animal made a correct decision given invalid cue information, the corresponding neural trajectory diverted mid-trial to reflect nascent encoding of a different target and different impending choice. In this way, trial outcomes were defined not in terms of individual neurons but as states of a larger population. Selective attention coordinates and engages networks of neurons distributed in far-ranging brain regions; as such, the high-dimensional analysis used in this study generalizes to higher-order cognitive phenomena. This indicates that spiking correlates of attention can be investigated in their native high-dimensional spaces in order to more fully characterize how networks of neurons dynamically shift their resources to meet cognitive demands in real time.

Disclosures: **M.J. Morais:** None. **A.C. Snyder:** None. **M.A. Smith:** None.

Poster

263. Functional Mechanisms of Attention I

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Program#/Poster#: 263.19/RR34

Topic: F.02. Animal Cognition and Behavior

Title: Establishment of a touchscreen-based Variable Time Wait Task and Go/No-Go Task for mice to assess behavioral inhibition and sustained attention

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Abstract: Establishing reliable tests to assess behavioral inhibition and sustained attention in animal models is essential to elucidate the mechanisms of executive function deficits seen in patients with psychiatric disorders, such as attention deficit/hyperactivity disorder (ADHD). Here, we developed the 'Variable Time Wait Task' (VTWT) and 'Go/No-Go' (GNG) task using

a touchscreen device to assess behavioral inhibition and sustained attention in mice. Male C57Bl/6J (B6) mice and DBA/2J (D2) mice aged 10 weeks were tested in a chamber equipped with a touchscreen monitor. In the VTWT, touching a start cue (presented as white cross) initiated the trial, and a reward cue (presented as white panel) was displayed after a random variable blank period (1−3 sec, 3−5 sec, or 1−5 sec). In order to obtain a reward, mice had to withhold touching the start cue during the blank period and then touch the reward cue. In the GNG task, each mouse was assigned a Go or No-go stimulus. The stimulus consisted of either of the following visual cues; black and white stripes with upward motion and a still white windmill on a black background. Each trial is initiated with a start cue, and a Go or No-go stimulus was presented after a blank period. In order to obtain a reward, the mice had to touch the monitor in response to the Go stimulus and withhold touching during the No-go stimulus. In both of the tasks, the rate of correct response to the stimulus and the intra-individual variability of response latency to the reward cue were analyzed as an index of behavioral inhibition and that of sustained attention, respectively. In the VTWT, the B6 and D2 groups were able to wait for maximum duration of 5 sec with a final correct response of 70%. However, the intra-individual variability of response latency to reward cue in the D2 group was significantly greater than the B6 group, indicating decreased sustained attention in the D2 group. In the GNG task, the B6 and the D2 groups had scores from 0−10% (Session 1) to 70% (Final session) in the No-go correct response, while the Go correct response remained constant between 90 to 100% in both groups throughout the sessions. The intra-individual variability of response latency to the reward cue, i.e. Go stimulus, was again found to be significantly greater in the D2 group than the B6 group. In conclusion, our newly-developed tasks allowed us to assess the behavioral inhibition and sustained attention in mice, which are promising methods to elucidate the mechanisms of executive function deficits observed in patients with ADHD and other psychiatric disorders.

Disclosures: **A. Okada:** None. **T. Endo:** None. **C. Tohyama:** None. **M. Kakeyama:** None.

Poster

263. Functional Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Support: CNPq Grant 305082/2012-4

Title: Deficits on learing, memory, and attention in Wistar rats submitted to neonatal hypoxic-ischemia procedure

Authors: *L. BIZARRO[1], J. JABOINSKI[1], L. A. ALMEIDA[1], R. DECKER[1], P. MIGUEL[2], R. DIAZ[2], L. ORLANDI[2]
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Abstract: Hypoxic-ischemic (HI) events in neonates are one of the main causes of mortality and biological morbidity, it's implications comprise epilepsy, cognitive and motor deficits and cerebral palsy. The aim of this study was to investigate the effects of hypoxia-schemia on learning, memory and attention in rats. In this study were used Wistar rats (n=22) divided equally between two groups (HI=11 CTR=11). On the 7th post natal day (PND) they were submitted to the experimental HI procedure according to Levine-Rice's protocol. Between the 45th and 52nd PND the animals performed an object and a social recognition task. In the object recognition task we compared the time the animal spent investigating the familiar or the unfamiliar object, and for the social recognition task we compared the frequency of social behaviors directed to the familiar or the unfamiliar rat. From the 60th PND on, a 5-choice serial reaction time task (5CSRTT) was employed to assess visual attention. 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 by each rat. After that, four test sessions were performed with shorter stimulus durations (1s, 0.5 s) or manipulations in inter-trial-interval duration (7s, 2s), in a randomized order. HI group presented lower social recognition index ($t(20) = -3,147$, $p=0.005$), but not object recognition index ($t(20) = -1.366$, $p = 0.18$), although it spent less time investigating the objects than the CTR group ($t(20) = -2.885$, $p = 0.009$). The results in 5-CSRTT provided evidence of impairments in the performance of rats from HI group in acquisition of the task ($F(1,20) = 14.89$, $p = 0.001$). Also, in baseline and test sessions, the attentional performance of HI group was characterized by lower accuracy ($F(1,20) = 15.47$, $p = 0.001$), higher premature responding ($F(1,20) = 5.35$, $p= 0.031$) and increased latencies to respond correctly ($F(1,20) = 10$, $p = 0.005$) and to collect reward ($F(1,20) = 12.48$, $p = 0.002$). The only variables that showed main manipulation effects were total session lapse, trials and latency of reward, suggesting that the test conditions hindered the performance of both groups. The interaction between group and manipulation was showed only in omissions $F(4,80) = 3.15$, $p = 0.033$, in which the CTR group omitted more responses when the interval between trials was manipulated (shortened, in this case). The results suggest that neonatal HI events might produce deficits on social interaction, learning, and attention and increase impulsivity in adulthood.

Disclosures: **L. Bizarro:** None. **J. Jaboinski:** None. **L.A. Almeida:** None. **R. Decker:** None. **P. Miguel:** None. **R. Diaz:** None. **L. Orlandi:** None.

Poster

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Support: NSERC Discovery Grant

OMHF Research Grant

Title: Phasic and tonic activation of the locus coeruleus induces global remapping in the dentate gyrus

Authors: *S. L. GRELLA[1], J. NEIL[2], I. V. ODINTSOVA[1], G. MARTIN[2], C. W. HARLEY[2], D. F. MARRONE[1,3]
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Abstract: The role of norepinephrine (NE) in sculpting representations formed by the hippocampus remains poorly understood. Locus coeruleus (LC) neurons (the source of hippocampal NE input) are activated in response to novelty and induce both transient and long-term potentiation of the perforant path input to the dentate gyrus (DG), enhancing DG throughput. These effects suggest novelty-associated activation of the LC may induce a reset of representations in the DG (remapping), a possible mechanism for initializing new episodes. The distribution dynamics of the mRNA of immediate early genes Arc and zif268, allow us to map the activity history of individual neurons activated within the hippocampus of animals engaged in spatial processing using fluorescence *in situ* hybridization and confocal microscopy. Rats were placed in either (1) same context twice (A/A) or (2) two different contexts (A/B). Prior to placement in the second context, rats were infused bilaterally with glutamate in the LC (phasic activation). In addition, separate groups of rats were infused bilaterally with orexin A or bethanechol, (which increases tonic LC discharge) in order to assess remapping effects in the DG, CA1 and CA3 regions of the hippocampus. Preliminary data show that infusion of substances that up-regulate either tonic or phasic release of NE from the LC can drive remapping in the DG of animals in the A/A condition without affecting A/B animals, consistent with the notion of NE as a novelty "switch" for hippocampal circuits from retrieval to encoding.

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Poster

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Canadian anesthesiologists' Society

Fondation anesthésiologie réanimation du Québec

Title: Attenuation of high-frequency (50-200 Hz) thalamocortical EEG rhythms by propofol is more pronounced for the thalamus than for the cortex

Authors: S. J. REED, *G. PLOURDE
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Abstract: Background. Thalamocortical EEG rhythms in gamma (30-80 Hz) and high-gamma (80-200 Hz) ranges have been linked to arousal and conscious processes. We investigated the concentration-dependent effect of propofol on these rhythms to compare the relative sensitivity of cortex and thalamus. Methods. Adult male Long-Evans rats were chronically implanted with electrodes in somatosensory (barrel) cortex and ventroposteromedial thalamus. Propofol was given in target concentration mode. Spectral power was assessed during baseline, at four stable propofol plasma-concentrations (0, 3,6,9,12 μg/ml) and during recovery over four frequency ranges (30-50, 51-75, 76-125, 126-200 Hz). Unconsciousness was defined as complete loss of righting reflex. Multiple regression was used to model the change of power (after logarithmic transformation) as a function of propofol concentration and recording site. Results. Unconsciousness occurred at the 9 μg/ml concentration in all animals. For the 30-50 Hz range, propofol increased power in the cortex and decreased it in the thalamus. Cortical power in the 76-200 Hz range and thalamic power in the 51-200 Hz range decreased linearly as a function of propofol concentration. In all instances the concentration-effect slope for the thalamus was significantly steeper than for the cortex. Cortical power in the 126-200 Hz range and thalamic power in the 51-200 Hz range were significantly reduced during unconsciousness. The attenuation of cortical power in the 50-200 Hz range during unconsciousness was dependent on the phase of delta (0.5-3.0 Hz) power. Conclusions. Propofol causes a concentration-dependent attenuation of the power of thalamocortical rhythms in the 51-200 Hz range and this effect is far

more pronounced for the thalamus, which also provides a robust correlate of the hypnotic action of propofol.

Disclosures: S.J. Reed: None. **G. Plourde:** None.

Poster

263. Functional Mechanisms of Attention I

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Support: CNPq

CAPES

FAPESP

Title: Sustained auditory attention and response anticipation in a two-alternative choice task, in rats

Authors: *L. F. MARCHELLI, G. F. XAVIER
Departament of Physiologi, Univ. of São Paulo, São Paulo, Brazil

Abstract: This study investigated sustained auditory attention in rats using a 3-hole nose poke operant task. The animals were individuallty trained to insert and maintain their noses within the central hole of the apparatus along the presentation of 4 to 9 "basal" beeps (6 KHz, 70 dB), the number of basal beeps varied unpredictably from trial to trial. Then, upon presentation of a 100-ms duration target beep which frequency could be either 3 or 10 KHz (70 dB), the rats had to remove their noses from the central hole and insert it within one of the lateral holes, left or right, depending on the frequency. The Reaction Time (time elapsed from presentation of the target stimulus up to removal of the nose from the central hole, RT) and Movement Time (time elapsed from removal of the nose from the central hole up to its insertion in one of the lateral holes, MT) were recorded. The ANOVA revealed a significant reduction of the RTs as a function of the number of basic beeps ($F_{5,12}=8.43$, $p=0.00126$); this RT reduction was stronger when the target beep was 10 KHz as compared to when it was 3 KHz ($F_{1,12}=115.70$, $p<0.0005$), i.e., RTs for 10KHZ target beep are bigger than RTs for 3KHZ target beep when the number of basic beeps was smaller than 7 and when the number of basic beeps was greater than 6 RTs were more similar. Relative to the MTs, ANOVA revealed lack of significant differences ($F_{1,12}=1,131$

p=0.3954). In contrast, relative to the percentage of correct responses, ANOVA revealed a significant number of basic beeps effect ($F_{5,12}=3.86$, $p=0.02558$) showing that better performance was achieved when the number of basic beeps presented before the target beep was smaller. Not surprising, this effect is related to a significant increase in the number of anticipation errors as a function of the increase in the number of basic beeps before presentation of the target beep ($F_{5,12}=5.80$, $p=0.00595$). Relative to the number of commission errors, ANOVA revealed lack significant number of basic beeps effects ($F_{5,12}=1.53$, $p=0.25019$). Even though RTs for 3 KHz and 10 KHz target beeps varied differently as a function of the number of basic beeps, data on accuracy of performance indicate that the subjects discriminated these beeps. Together, these results suggest that different behavioral strategies were adopted by the rats depending on the number of basic beeps presented before the target beep. Data suggest that when the number of basic beeps was smaller than 7 the subjects did orient attention to the impending target stimulus. In contrast, when the number of basic beeps was greater than 6, the rats seem to have abandoned orientation of attention and anticipated their response to the impending target, lending to deterioration of performance.

Disclosures: L.F. Marchelli: None. **G.F. Xavier:** None.

Poster

263. Functional Mechanisms of Attention I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 263.24/RR39

Topic: F.02. Animal Cognition and Behavior

Title: Theta-gamma interactions in monkey visual cortex and their role in selective attention

Authors: *G. SPYROPOULOS[1], C. BOSMAN[2], P. FRIES[1,3]
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Abstract: The presence of theta oscillations in the primate visual cortex has been mostly linked to the successful maintenance of tokens in working memory (1,2). Their relation to attention remains, however, unknown. To investigate that relation, we used dense subdural electrocorticography grids (ECoG) in two awake rhesus macaques. In particular, we recorded local field potentials from early (V1) and intermediate (V4) visual areas of the subjects' left

hemispheres while they were performing a selective attention task. LFP power spectra showed a peak in the theta-frequency band (3-5 Hz), predominantly in V1. Attention to the contralateral as compared to the ipsilateral stimulus reduced this theta rhythm. Phase-locking spectra showed a corresponding theta synchronization between V1 and V4, which was reduced by attention in one animal. The phase of V1 theta was correlated with the strength of the local narrow-band gamma rhythm and the V1-V4 gamma locking. The local theta-gamma interaction was reduced by attention. Also, the theta phase modulated the probability of gamma-phase resets, and this relationship was again reduced by attention. Thus, theta-rhythmic resets of the gamma phase were more prevalent in the absence of attention. This pattern of results might be parsimoniously explained by the following scenario: Each stimulus induces local gamma-band activity in V1, which in turn entrains a gamma rhythm in V4 (3). The V1 gamma-band activity is reset rhythmically in the theta-frequency range. This reset temporarily reduces local V1 gamma activity, V1-V4 gamma phase locking, and the corresponding V4 gamma activity. The resulting theta-rhythmic modulation of the local gamma rhythm might actually constitute the theta peak in regular power spectra (similar to the way in which asymmetric alpha oscillations constitute slow potentials - 4). The theta-rhythmic reset affects predominantly the gamma induced by the unattended stimulus, which explains the negative attention effects on theta-rhythmic gamma phase resets, on theta-rhythmic gamma amplitude and thereby theta power itself. References: 1. Lee H, Simpson GV, Logothetis NK, Rainer G. (2005) Neuron 2. Liebe S, Hoerzer GM, Logothetis NK, Rainer G. (2012) Nat. Neurosc. 3. Bosman C et al. (2012) Neuron 4. Mazaheri A & Jensen O. (2008) J. Neurosc.

Disclosures: G. Spyropoulos: None. **C. Bosman:** None. **P. Fries:** None.

Poster

263. Functional Mechanisms of Attention I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 263.25/RR40

Topic: F.02. Animal Cognition and Behavior

Support: Howard University

University of Maryland at Baltimore

Title: Basal forebrain responses to odorants and modulation of olfactory bulb function in mice

Authors: *X. ZHAN[1,2], P.-B. YIN[3], T. HEINBOCKEL[4]
[1]Dept. Physiol. & Biophysics, Howard Univ. Coll. Med., WASHINGTON, DC; [2]Anat. and Neurobio., Univ. of Maryland at Baltimore, Baltimore, MD; [3]Inst. for Systems Res., Univ. of Maryland, College Park, MD; [4]Howard Univ., DC, DC

Abstract: The basal forebrain is thought to be a critical center for attention, but the mechanism remains elusive. We propose that the basal forebrain is responsive to peripheral sensory stimulation, which can in turn modulate primary inputs. We tested this idea by examining olfactory bulb and basal forebrain interactions in two conditions: 1) odorant responses in the nucleus of diagonal band (NDB) and 2) responses to electrical stimulation in the basal forebrain in anaesthetized and awake behaving mice. In anaesthetized preparations, stimulation of NDB induced increased spiking activity in MOB neurons. About half of the units with excitation exhibited rhythmic oscillations, whereas the other units did not. NDB stimulation can change the oscillation frequency. The NDB-responsive neurons were mainly located in the mitral cell, internal and external plexiform layers. Results obtained by recording in awake behaving mice were similar to those in anaesthetized animals, but the excitatory responses were more robust, and more inhibitory units were found. In several units, the effect was attenuated by scopolamine, a competitive mAChR antagonist. We also presented animals with Sharpie alcohol mixtures (n-propanol, n-butanol and diacetone alcohol), which induced excitatory and inhibitory responses. Field potential analysis revealed different oscillation frequency components after the onset of either odorant or electrical stimulation in anaesthetized and awake animals. As basal forebrain has robust projections to the prefrontal cortex, our findings suggest it contributes to a topdown mechanism of attention modulation. In olfaction, it modulates early olfactory processing.

Disclosures: X. Zhan: None. **P. Yin:** None. **T. Heinbockel:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.01/RR41

Topic: F.02. Animal Cognition and Behavior

Support: Z01 ES-100221

Title: Firing properties and immediate early gene mapping in hippocampal area CA2

Authors: *G. M. ALEXANDER, S. FARRIS, S. M. DUDEK
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Abstract: The function of hippocampal area CA2 in behavior has only recently begun to be assessed. Initial studies suggest that CA2 may function in social processing, as shown by the loss of social recognition memory in both the vasopressin 1B receptor knock-out mice (Wersinger et al., 2002) and in mice with CA2 output silenced by tetanus toxin expressed in CA2 pyramidal cells (Hitti and Siegelbaum, 2014). In addition, CA2 may function in spatial information processing, similar to CA1 pyramidal cells. Support for a role of CA2 in spatial processing comes from a knock-out mouse for regulator of G-protein signaling 14 (RGS14). In these mice, long term potentiation at the Schaffer Collateral-CA2 pyramidal cell synapse, absent from wild-type mice, is revealed. Perhaps as a consequence, these mice also display enhanced performance in the acquisition phase of a spatial memory task (Lee et al., 2010). Based on these findings, we hypothesize that CA2 functions in both social and spatial processing. To test this hypothesis, we have taken two independent approaches of querying CA2 pyramidal cell activity and exposed animals to various stimuli. First, we performed *in vivo* electrophysiological recordings from individual CA2 pyramidal cells in adult male Sprague-Dawley rats and asked how firing rate is affected by behavioral state as well as by spatial navigation, with or without exposure to social stimulation. Second, we exposed a separate group of male Sprague-Dawley rats to identical stimuli, and assessed brain sections for changes in immediate early gene (IEG) expression 5, 15 and 30 min after the exposure. Together, our data show that CA2 pyramidal neurons respond to spatial exploration and that additional social exposure does not enhance IEG expression or firing rate in CA2. Our data do suggest, however, that CA2 firing rate changes depending upon the behavioral state of the animal in a manner that differs from CA1. These results highlight the role of CA2 in processing spatial information but offer no further insights into its role in processing social information. Therefore, modulation of CA2 activity by social stimuli may be more subtle than a general increase in firing rate or may be affected during a later phase of consolidation or recall.

Disclosures: G.M. Alexander: None. **S. Farris:** None. **S.M. Dudek:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.02/RR42

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS079774

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Title: Hippocampal memory codes regulated by NMDA receptor synaptic plasticity

Authors: ***M. LI**[1], H. ZHANG[1,2], H. KUANG[1,2], G. CHEN[1], J. Z. TSIEN[1]
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Abstract: The CA1 region of the hippocampus is well known to be a crucial site for processing associative memories. From a structural perspective, memory is believed to be the storage of acquired information in a form of synaptic connectivity patterns via by NMDA receptor-dependent synaptic plasticity Here, by applying large-scale neural recording and dimensionality-reduction decoding algorithms, we investigate hippocampal CA1 activity patterns of trace fear conditioning memory code in inducible NMDA receptor knockout mice and their control littermates. Our real-time decoding analyses show that the conditioned tone (CS) and unconditioned foot-shock (US) can evoke hippocampal ensemble responses in control and mutant mice. Yet, temporal formats and contents of CA1 fear memory engrams differ significantly between the genotypes. The mutant mice with disabled NMDA receptor plasticity failed to generate CS-to-US or US-to-CS associative memory traces. Moreover, the mutant CA1 region lacked memory traces for "what at when" information that predicts the timing relationship between the conditioned tone and the foot shock. The degraded associative fear memory engram is further manifested in its lack of intertwined and alternating temporal association between CS and US memory traces that are characteristic to the holistic memory recall in the wild-type animals. We further investigate the electrophysiological properties of the pyramidal cell populations in control and mutant mice, the analyses reveal that the CA1 pyramidal cells in two genotypes show significant differences under various EEG oscillations. Thus, our study provide the first detailed description about how the content and temporal dynamics of real-time memory engrams are organized by the NMDA receptor-mediated synaptic plasticity, and how the responsive properties of hippocampal pyramidal cells change in NMDA receptor knockout animals.

Disclosures: **M. Li:** None. **H. Zhang:** None. **H. Kuang:** None. **G. Chen:** None. **J.Z. Tsien:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

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Program#/Poster#: 264.03/RR43

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant P41-EB001978

DARPA contract N66601-09-C-2081 (REMIND program)

Title: In-vivo predictive relationship from CA1 to CA3 in rodent hippocampus

Authors: *R. SANDLER[1], D. SONG[1], R. HAMPSON[2], S. DEADWYLER[2], T. BERGER[1], V. MARMARELIS[1]
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Abstract: Information flow through the hippocampus has traditionally been viewed in the context of the trisynaptic pathway. However, there has long been evidence to support the notion that CA1 may also causally influence CA3 via backprojecting interneurons and feedback connections through the Entorhinal cortex (EC). Many studies have shown this pathway to spread seizures in hippocampal slice preparations. Furthermore, this pathway has been shown to be modified in-vivo depending on the behavioral state of the animal. However, no work has been done to explore the role of this feedback connection on the single neuron level, in the in-vivo nonpatholgical, nonperturbed brain. In order to examine this hypothesis, rodent hippocampal activity was recorded using a multi-electrode array from areas CA3 and CA1 during a behavioral task. Spikes were sorted, time-stamped, and discretized. Multivariate autoregressive (MVAR) models were constructed for all recorded spike trains in both possible directions: from CA3 to CA1 and from CA1 to CA3. Monte Carlo methods were used to prune out all non significant input cells and to remove all non significant models. Altogether, 311 MVAR models were estimated across 7 animals and 9 sessions. Our results showed 121/166 (73%) of CA3→CA1 models and 96/145 (66%) CA1→CA3 models to be significant. These results confirm the existence of a predictive relationship from CA1→CA3 in rodent in-vivo hippocampus on a single neuron level. This predictive 'Granger' style relationship comes from both truly causal connection such as those from the EC and from common inputs such as those from the Septum. A bidirectionally connected hippocampus gives rise to the notion of a hippocampal 'closed-loop' model through which the well-known hippocampal resonant modes can emerge. The feedforward and feedback (autoregressive) parts of all estimated MVAR models were examined in the frequency domain. It was found that CA3 autoregressive kernels had more power in the theta band than those of CA1. This supports experimental findings that CA3 is an endogenous theta pacemaker within the hippocampus. Furthermore, it was found that CA3→CA1 feedforward

kernels had more power in the beta band which suggests that CA3 also transfers information through the beta/low-gamma band.

Disclosures: **R. Sandler:** None. **V. Marmarelis:** None. **T. Berger:** None. **D. Song:** None. **R. Hampson:** None. **S. Deadwyler:** None.

Poster

264. Hippocampal and Cortical Circuits I

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Topic: F.02. Animal Cognition and Behavior

Support: ONR grant N000141310672

Title: Memory consolidation during sleep slow oscillations

Authors: ***Y. WEI**, G. PRASHANTH, M. BAZHENOV
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Abstract: Sleep is critical for regulation of synaptic efficacy, consolidation of memories and learning. It has been proposed that synaptic plasticity associated with sleep rhythms could contribute to consolidation of memories acquired during wakefulness. In this study, we present a thalamocortical network model of the slow-wave sleep activity characterized by repeatable (< 1 Hz) transitions between active (Up) and silent (Down) states of the network, to study memory consolidation. The model consisted of thalamic relay (TC) and reticular (RE) neurons; cortical pyramidal neurons and inhibitory interneurons organized within three layers of a cortical column. All neurons were modeled based on the Hodgkin-Huxley kinetics. Spike-timing dependent synaptic plasticity (STDP) was implemented to regulate synaptic efficacy. The network displayed stage 3/4 sleep-like activity with Up and Down states; Up-states were initiated randomly by stochastic spontaneous release in synaptic terminals. When a weak repetitive external stimulation was applied to only a few cells in the cortical network, the spatio-temporal pattern of Up-state propagation became organized. Probability of the Up-states onset near the stimulation site increased even when the stimulus was applied less frequently than a slow oscillation frequency. Because of the refractoriness properties of the network, a probability of the next active state initiation was higher for the network site that initiated activity at the previous cycle of oscillation. When STDP was implemented, there was a net decrease in synaptic strengths and, at the same time, an increase in the strength of specific synapses. The change in

synaptic weights between any two neurons was determined by direction of active state propagation and by distance between the neurons. Our study proposes a mechanism of how interaction between cortically generated slow waves and sparse external input, possibly representing input from hippocampal formation, may lead to reorganization of synaptic strength during stage 3/4 sleep, therefore, influence memory consolidation processes.

Disclosures: Y. Wei: None. **G. Prashanth:** None. **M. Bazhenov:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: ONR Grant N000141310672

Title: Hippocampal ripples as inhibitory transients

Authors: *P. MALERBA[1], G. P. KRISHNAN[1], J.-M. FELLOUS[2], M. BAZHENOV[1]
[1]Cell Biol. and Neurosci., UC Riverside, Riverside, CA; [2]Psychology and Applied Mathematics, Univ. of Arizona, Tucson, AZ

Abstract: Sleep is known to be important for memory consolidation, and memories are thought to be stored in the hippocampus during wakefulness and "transferred" to cortex during sleep. Recently, memory replay - repeated sequences of pyramidal cell firing - has been demonstrated during sleep. Furthermore, sequence replay is associated with characteristic sleep oscillations, and tampering with replay can disrupt memory formation and consolidation. These results give rise to the hypothesis that replay may form the critical neural substrate of memory consolidation, but the mechanisms underlying sequence replay are still unknown. Among hippocampal-specific activity patterns, sharp-wave ripple complexes are brief high-frequency events, during which the firing sequences of previously activated cells are re-played. It is believed that sequence reactivation during ripples contributes to memory formation in the awake state and to memory consolidation during sleep. Ripples in the pyramidal layer of hippocampal area CA1 are believed to be triggered by a generalized excitatory event in area CA3. We develop a computational model of ripple generation to explore hippocampal sequence replay during sleep. Data suggests that during CA1 ripple events only a few pyramidal cells are recruited, and they spike at the peak of the event, while perisomatic interneurons are more broadly recruited and spike across the

duration of the event. In our model, broad generalized excitation from CA3 reaches CA1 cells and organizes the perisomatic interneurons in brief oscillatory transients, capable of sustaining high frequencies (150-200 Hz) only for about 50ms. Such high-frequency firing mediates high-frequency LFP oscillations in pyramidal neurons, and a subset of pyramidal cells is firing within the windows of opportunity left by the inhibitory activity. We compare and validate the properties of ripple generation in our model with data obtained *in vivo* using multi-tetrode recordings from non-anesthetized rats during awake and sleep states. In summary, our study proposes a novel mechanism of hippocampal ripple generation, consistent with *in vivo* data, and predicts a relationship between sequence replay in CA3 and CA1 hippocampal subregions.

Disclosures: P. Malerba: None. **G.P. Krishnan:** None. **J. Fellous:** None. **M. Bazhenov:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.06/RR46

Topic: F.02. Animal Cognition and Behavior

Title: Characterization of a continuous sharp wave ripple state in rat hippocampus under isofluorane anesthesia

Authors: *B. R. LUSTIG, Y. WANG, E. PASTALKOVA
Janelia Farm Res. Campus, Ashburn, VA

Abstract: The rat hippocampus has a long history as a model system used in research towards understanding memory. Hippocampal firing patterns during various states of behavior as well as under different forms of anesthesia provide valuable insight into the physiological mechanisms at work in this important brain structure. We found in rats under relatively deep isofluorane anesthesia (~2%), the hippocampus becomes entrained in a state of continuous sharp wave ripples (c-SWR). We carried out electrophysiological recordings using linear silicon probes with 32 recording sites spaced 50 microns apart to characterize the laminar profile of activity during this c-SWR state. During the c-SWR state SWR events in CA1 are composed of a signature sharp wave reversal across stratum radiatum as well as a high frequency ripples in the pyramidal layer. Interestingly these SWR events occur in a continuous repetitive oscillation at a frequency of 10-15 Hz. We found bursts of firing in CA3 preceding many SWRs, consistent with the idea that synchronous firing in CA3 drives SWRs in CA1. In order to explore the mechanisms

involved in the c-SWR state we perturbed the hippocampal circuit using optogenetics. We found optogenetic activation of neurons in area CA3 injected with AAV1-Syn-ReaChr-mCitrine abolished the continuous SWR state while also evoking strong gamma (~50 Hz) oscillations in the stratum radiatum and stratum lacunosum of CA1. These results are in agreement with the idea that precise synchronous firing of CA3 neurons is necessary for the generation of SWRs during the isofluorane anesthesia induced c-SWR. Our results and characterization of the hippocampus under deep isofluorane anesthesia gives new insight into hippocampus under a particular brain state and may provide a useful model preparation for the investigation of physiological mechanisms at work during SWR generation.

Disclosures: B.R. Lustig: None. **Y. Wang:** None. **E. Pastalkova:** None.

Poster

264. Hippocampal and Cortical Circuits I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH080318

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University of Minnesota Doctoral Dissertation Fellowship

Title: Hippocampal theta sequences reflect rats' spatial goals

Authors: *A. WIKENHEISER[1], A. D. REDISH[2]
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Abstract: Theta frequency (6-10 Hz) oscillations segment representations in the rodent hippocampus. Spatially-tuned pyramidal neurons (place cells) form temporally-structured spiking sequences bounded by theta oscillations (theta sequences). Theta sequences represent trajectories through space, suggesting an intuitive mechanism for planning and representing future actions. We tested if theta sequence look ahead (the distance that theta sequences extended beyond the rat's position) in dorsal CA1 was modulated by spatial goals. Rats performed a self-motivated foraging task, running laps around a circular track with three evenly spaced feeder sites, each with a unique delay length. Rats ran different patterns of trajectories between sites depending on their delay preferences; from any site, subjects could run to the next site (a one

segment trajectory), skip the next site and run to the feeder after that (a two segment trajectory), or skip the next two sites and complete a lap around the track, returning to the site initially departed from (a three segment trajectory). Theta look ahead was greatest for three segment trajectories, was shortest for one segment trajectories, and was intermediate for two segment trajectories. However, as rats neared their intended site, this effect was abolished, suggesting that look ahead tracked the distance to goals. Increased look ahead predicts that place fields traversed on longer trajectories should be larger, due to earlier activation of cells on approach to those fields. Place fields on three segment trajectories were significantly larger than those passed through on one segment trajectories. This effect also held on a trial by trial basis. The first spike on passes through fields during three segment trajectories occurred significantly earlier in the field than during shorter trajectory passes. The location of the final spike on passes through place fields was also modulated by goal destination, but the effect size was much smaller, suggesting that increased look ahead primarily affected the early portion of place fields, leaving the end intact. Many place cells undergo asymmetric expansion with experience. To test the interaction between expansion and look ahead modulation, we measured the relationship between lap number and place field center of mass, separately for each trajectory type. Expansion predicts a negative slope that is unaffected by current goal, while look ahead modulation predicts that line intercepts should vary with goal. Intercepts, but not slopes, were modulated by goal destination, suggesting that look ahead modulation occurred in tandem with, but separable from place field expansion.

Disclosures: **A. Wikenheiser:** None. **A.D. Redish:** None.

Poster

264. Hippocampal and Cortical Circuits I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH080318

JSPS grant 11J06508

Title: Systemic stimulation of α2 adrenergic receptors with clonidine affects oscillatory activity in the rat hippocampus during a spatial decision-making task

Authors: *S. AMEMIYA, A. D. REDISH
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Abstract: Hippocampal oscillatory activity is thought to reflect dynamic cognitive processes during decision making. The hippocampus receives innervations of noradrenaline (NA) from locus coeruleus and physiological studies have reported that NA manipulation affects hippocampal oscillations. These data suggest a potential role for NA in hippocampus during decision making, however, few studies have examined involvement of NA in regulation of hippocampal activity during decision making. We examined the influence of the α2 adrenergic autoreceptor agonist clonidine on local field potentials (LFP) recorded from the hippocampal CA1, CA3, and fissure regions from rats running a decision-making task. Rats ran a modified Hebb-Williams maze, consisting of a changeable central path, a final decision point, and rewarded return rails leading to the start of the loop. On each lap, only one side or the other was rewarded. Three reward-contingencies were used: turn left, turn right, or alternate for reward. During the analyzed probe trials, the rewarded rule changed approximately halfway through the session. On this task, particularly after the switch in contingency, rats sometimes pause and orient back and forth at the choice points, a process termed "vicarious trial-and-error" (VTE). To manipulate NA transmission, clonidine (30 μg/kg) or vehicle was delivered intraperitoneally 30 min before the maze session. Oscillations in the theta (4-12Hz), beta (14-29Hz), slow gamma (30-55Hz), mid gamma (60-90Hz), and fast gamma (90-140Hz) ranges were examined. Theta asymmetry has been hypothesized to reflect changes in the phase modulation of oscillations. To measure the asymmetry of each theta wave, we calculated the ratio of the ascending (trough to peak) and descending (peak to trough) parts. Consistent with previous studies, clonidine suppressed the occurrence of VTE-like events. Neurophysiologically, clonidine decreased power in all frequency bands. At the final decision point, control sessions showed a decrease of power in beta and slow gamma oscillations and an increase of power in mid gamma oscillations in VTE-laps compared to non-VTE laps. These changes were suppressed by clonidine. Asymmetry of theta waves also showed position-dependent changes, and theta was more symmetric on VTE laps than on non-VTE laps around the choice point. These differences were decreased by clonidine. Our findings suggest that NA modulates hippocampal processing during decision making.

Disclosures: S. Amemiya: None. **A.D. Redish:** None.

Poster

264. Hippocampal and Cortical Circuits I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA030672

Title: Task-dependent changes in local field potential (LFP) coordination between orbitofrontal cortex and ventral striatum on contrasting decision-making tasks

Authors: *J. J. STOTT[1], A. P. STEINER[1], Y. A. BRETON[2], A. D. REDISH[2]
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Abstract: Synchronous neural activity is essential for supporting cognition and behavior. Oscillations in local field potentials (LFP) reflect coordination within and between brain areas. In order to study the interactions between brain networks involved in decision-making, we recorded LFP signals simultaneously from dorsal hippocampus (dHC), ventral striatum (vStr), and orbitofrontal cortex (OFC) in rats running different decision-making tasks. One set of rats ran the Spatial Adjusting-Delay Discounting task, which required rats to make binary choices between a small immediate reward and a larger, delayed, reward. Another group of rats ran the Restaurant Row task, in which rats made sequential choices about whether or not to wait through a delay to receive differently-flavored rewards. Power spectral density analyses (PSDs) in vStr showed strong gamma band power in the 50 Hz (γ50) and 80 Hz (γ80) range, consistent with previous reports. PSDs from OFC showed a peak only at γ50 in the Delay-Discounting task, but showed peaks at both γ50 and γ80 in the Restaurant Row task. The dominant frequency in dHC, measured near the hippocampal fissure, was theta (5-12 Hz) in both tasks. Cross-correlograms of LFP power confirmed the presence of fundamental frequencies at γ50 and γ80 in vStr, γ50 in OFC on the Delay-Discounting task, and both γ50 and γ80 on the Restaurant Row task. Additionally, these analyses indicated positive correlations between γ50 power and power in the 25 Hz frequency range in both vStr and OFC, as well as an anti-correlation between γ50 and γ80 power in vStr, similar to that reported previously. Interestingly, the OFC showed a task-dependent difference in its LFP spectra in the γ80 range. While γ80 power was clearly present in OFC on the Restaurant Row task, it was not present on the Delay-Discounting task. As a whole, cross-correlogram plots looked similar between vStr and OFC on the Restaurant Row task, but clearly differed for animals that ran the Delay-Discounting task. These differing 'signatures' in the correlation between frequencies may indicate different cognitive processing modes in the Restaurant Row and Delay-Discounting tasks.

Disclosures: J.J. Stott: None. **A.P. Steiner:** None. **Y.A. Breton:** None. **A.D. Redish:** None.

Poster

264. Hippocampal and Cortical Circuits I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA030672

Title: Orbitofrontal inactivation blunts flavor preferences in the Restaurant Row task

Authors: *Y. BRETON[1], B. SCHMIDT[2], A. D. REDISH[2]
[1]Neurosci., Univ. of Minnesota, Saint Paul, MN; [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: We used the Restaurant Row task to infer flavor-based preferences in rats. In this task, rats made stay/skip decisions at each of 4 zones in daily, hour-long sessions. Rats ran clockwise around an octagonal loop with 4 spokes, at the end of which, a feeder provided 2 food pellets of unique flavor (cherry, banana, chocolate, unflavored) after a random, cued delay. Zones were only activated in sequence. As the rat entered each zone, a sequence of tones cued the delay and counted down until a reward was delivered or the rat proceeded to the next zone. Delays of 1 to 30 seconds were presented in pseudo-random order at each zone. The rats' stay/skip decisions at each zone provided a measure of how long they were willing to wait for each flavor. We defined the threshold delay as the delay at which animals were willing to wait for 50% of rewards delivered. We defined decision instability as the proportion of zone entries that were inconsistent with the threshold for each zone. The variance in zone-related thresholds in a session quantified the degree to which flavor impacted stay/go decisions. Designer receptors exclusively activated by designer drugs (DREADDs) provide a means to probe how different brain regions contribute to decision-making. The designer receptor can transfect a spatially restricted region, and the associated designer drug can functionally and reversibly inactivate the transfected cells. This allows animals with unique flavor and amount preferences to serve as their own controls. The role of the orbitofrontal cortex (OFC) in flavor-based decisions is unclear. To examine this question, we analyzed performance on the Restaurant Row task when inactivating lateral OFC with inhibitory DREADDs exclusively activated by clozapine-N-oxide (CNO). We transduced DREADDs to the lateral orbitofrontal cortex (OFC) of 4 Brown-Norway rats. Before every daily session, rats received either 5mg/kg CNO or vehicle control, in experimenter-blind pseudo-random order, for 20 days. Analysis of overall thresholds showed that inactivation of the OFC mildly reduced overall thresholds, but reduced high thresholds to a greater extent than low thresholds. As a result, variance in thresholds reliably decreased. Our analyses showed no significant effect on decision stability. When rats approached zones at which the decision to stay or skip was difficult, they occasionally paused and looked back and forth, a behavior known as vicarious trial and error (VTE). OFC inactivation slightly increased VTE behavior.

Disclosures: **Y. Breton:** None. **B. Schmidt:** None. **A.D. Redish:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.11/SS1

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA030672

Title: Silencing the rat medial prefrontal cortex decreases hesitation and impairs vicarious trial and error (VTE) behavior on the Restaurant Row task

Authors: ***B. SCHMIDT**[1], Y. A. BRETON[2], A. D. REDISH[2]
[1]Neurosci., Univ. Minnesota, Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: While at a choice point, rodents will often pause and orient towards potential routes, a behavior, termed vicarious trial and error (VTE), indicative of deliberative decision-making. During VTE, place cells in the hippocampus serially sweep forward towards the goal locations, neurophysiologically reflecting future options. Reward responsive cells in the ventral striatum also show firing at the choice point during VTE, neurophysiologically representing future potential rewards. The hippocampus and ventral striatum are both functionally and anatomically connected to the medial prefrontal cortex (mPFC). Although the hippocampus and ventral striatum show distinct neurophysiological activity during VTE, it remains unknown what role the mPFC plays during VTE. In order to examine the role of mPFC in VTE, we transduced DREADDs into the mPFC of rats and allowed them to run the Restaurant Row task. DREADDs are a state of the art, chemical-genetic method of silencing neurons by transducing a genetically modulated designer G-protein coupled receptor. DREADDs are solely activated by a systemic injection of the pharmacologically inert compound clozapine-N-oxide (CNO). DREADDs allows for a favorable temporal and spatial resolution for chronic, non-invasive, neuronal silencing. mPFC-DREADD transduced rats were trained on the spatial neuroeconomic task Restaurant Row. This task revealed individual preferences of reward flavor, the identification of value for each flavor and the cost the rat was willing to exert to receive it. On the Restaurant Row task rats ran on a circular maze with four evenly spaced spokes. At the end of each spoke, one of four different food rewards (cherry, banana, chocolate, unflavored) could be delivered. Each flavor was delivered at only one location. When the rat passed a spoke, a tone started to count down to

indicate the delay required before the reward would be dispensed. Delay at each encounter was selected independently from all other delays, spokes were only triggered in a clockwise order. The rats therefore encountered a serial set of choices of whether to wait out the delay for the food reward or skip the choice and move on to the next reward site. mPFC-DREADD transduced rats (n=4) given CNO showed reduced VTE behavior on the Restaurant Row task. Flavor preference was determined from the decision thresholds. CNO had no effect on flavor preferences for different food reward. CNO did increase decisive behavior by reducing the hesitation time before a decision was made. These behavioral data suggest that silencing the mPFC with CNO impairs deliberative decision-making in DREADDs transduced rats.

Disclosures: B. Schmidt: None. **Y.A. Breton:** None. **A.D. Redish:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.12/SS2

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA030672

NIH Training Grant HD007151

Title: Translating from rats to humans: A human foraging model of decision making

Authors: S. V. ABRAM[1], A. MACDONALD, III[2], *A. D. REDISH[3]
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Abstract: Translating animal models of decision-making for human use is difficult because of the different ethologies of different species. Typical temporal discounting paradigms require a forced-choice between two reward options of different value that are available at disparate time delays; however, these tasks fail to capture the dynamic nature of real-world scenarios that likely entail a series of stay/go decisions. Here, we extend the “Restaurant Row” task, in which rats encounter a series of flavored-food-locations (restaurants) and have the option of waiting out a signaled delay or skipping on to the next opportunity. Instead of food, human subjects foraged for information through an internet-like interface. This “Web-Surf” task allotted participants (N=38) 30 minutes to forage through four video galleries, each presenting a 4s clip from one of

four desirable categories (kittens, dance, bike-accidents, landscapes) as reward. Upon arrival at each gallery, subjects were informed of the delay before stimulus presentation. They then had the option to stay and wait for the video or skip on to the next gallery. Viewed videos were rated on a 1-5 scale with 5 being the highest. When transitioning between galleries, subjects clicked a series of "NEXT" buttons that were randomly positioned around the screen; this served as an analogue to the cost rats encounter when physically running a track between feeders. We assessed revealed preferences by calculating thresholds via fitting sigmoid functions. We then examined to what extent these thresholds (revealed preferences) correlated with average category ratings (stated preferences) to assess the face validity of the task. Revealed preferences corresponded with average ratings, with 78% of correlations above 0.50. Similarly, 68% of correlations between revealed preferences and post-test category rankings were above 0.50. Gender differences across certain categories were evident by thresholds and average category ratings: males consistently waited longer for bike-accident videos ($F[1,33] = 8.370$, $p < 0.01$) and landscape videos ($F[1,33] = 3.885$, $p = 0.057$). Similarly, males generally rated videos in these categories higher, bike-accident videos ($F[1,26] = 1.802$, $p = .19$), landscape videos ($F[1,26] = 16.118$, $p < 0.01$). Genders did not differ in preference for the animal or dance video categories.

Disclosures: S.V. Abram: None. **A. MacDonald:** F. Consulting Fees (e.g., advisory boards); Astellas Pharmaceuticals. **A.D. Redish:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.13/SS3

Topic: F.02. Animal Cognition and Behavior

Support: Univ. Stirling PhD Studentships

Title: Does place field repetition impair spatial learning?

Authors: R. M. GRIEVES[1,2], B. W. JENKINS[2], E. R. WOOD[2], *P. A. DUDCHENKO[1,2]
[1]Univ. Stirling, Stirling, United Kingdom; [2]Ctr. for Cognitive and Neural Systems, Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Hippocampal place cells fire when an animal is in a particular area of an environment and are generally thought to support spatial cognition. Recently, Derdikman et al. (Nat Neurosci 12:1325-32 2009) showed that in a hairpin maze composed of a series of parallel alleyways,

place cells (and grid cells) tend to fire in multiple alleyways and at approximately the same spatial location relative to the boundaries of each alleyway. Spiers et al. (Cereb Cortex 2013 doi: 10.1093/cercor/bht198) found similar "place field repetition" as rats explored a multi-compartment environment composed of 4 identical parallel boxes connected by a corridor. This raises the question as to whether, under conditions in which place field repetition between compartments is observed, animals can discriminate between the compartments, or be trained to do so. We tested whether rats (n=6) could learn a spatially-guided odour discrimination task in an environment similar to that used by Spiers et al., with 4 boxes arranged parallel to each other. A second group of rats (n=6) was trained in the same environment with the 4 boxes arranged in a semi-circular formation to provide directional information; in this environment we would not predict place field repetition. In each box, 4 bowls of sand, each scented with a different spice were available. One of the 4 bowls of sand in each box was baited with a reward, and a different bowl was rewarded in each of the 4 boxes. Training was carried out in 3 stages. In the first stage, 2 boxes and 2 scents were used, and the rats were trained for 12 trials a day (6 in each box) until they chose correctly on 5/6 trials in both boxes (83%) on 2 consecutive days. The second stage involved 3 boxes and 3 odours, and the final stage involved discriminating between all 4 boxes and all 4 odours. All rats trained in the semi-circular configuration learned the task readily, taking 27 ± 4.9 (mean ± SD) sessions to complete all 3 stages. In contrast, the rats in the parallel configuration were significantly impaired; after 50 days of training, only 1 rat had reached criterion at all 3 stages (taking 50 days to do so). A further 2 rats had reached criterion at the 2 and 3 box stages, and the remaining 3 rats had reached criterion only at the 2 box stage. These results suggest that rats have difficulty discriminating environments in which place field repetition occurs. This difficulty increases with the number of compartments; however, with extended training some discrimination is observed. In contrast, spatial learning occurs rapidly in compartments that are oriented in different directions. Future experiments will test whether learning in parallel compartments elicits a more global place cell representation.

Disclosures: **R.M. Grieves:** None. **B.W. Jenkins:** None. **E.R. Wood:** None. **P.A. Dudchenko:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.14/SS4

Topic: F.02. Animal Cognition and Behavior

Support: HFSP RGP0039/2010

BBSRC

Title: Exploring the effects of disrupting the nucleus reuniens in rats on performance of hippocampus-dependent tasks

Authors: E. ALLISON[1], T. RIPARD[1], G. YUKHNOVICH[1], P. A. DUDCHENKO[3,1], *E. R. WOOD[2,1]
[1]Ctr. for Cognitive and Neural Systems, Univ. of Edinburgh, [2]Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Sch. of Natural Sci., Univ. of Stirling, Stirling, United Kingdom

Abstract: The nucleus reuniens is thought to be a major route of information flow from the prefrontal cortex to the hippocampus because it is the site of a one synapse connection between the two areas. In addition, lesions or optogenetic inactivation of the nucleus reuniens cause a reduction in trajectory-dependent activity in hippocampal CA1 place cells (Ito et al. SFN 2013. Poster 769.11). Lesions of the nucleus reuniens might therefore block the prefrontal cortex input to the hippocampus, and reduce trajectory-dependent activity in the hippocampus. We tested whether the nucleus reuniens is necessary for acquisition and performance of a hippocampus-dependent task in which trajectory dependent place fields are observed. After lesioning the nucleus reuniens using ibotenic acid, we trained rats on a win-stay, lose-shift task in a double Y maze. The acquisition of this task is known to be hippocampus dependent. The reuniens lesion animals showed no differences in accuracy during acquisition or performance compared to sham operated control rats. This is surprising as it suggests that decreasing trajectory-dependent activity in CA1 place cells may not disrupt the ability of animals to learn the task. The double Y maze task could be solved either egocentrically or allocentrically, which may allow animals to compensate for any deficits. To test for deficits in allocentric navigation, we trained animals on a reference memory task in a water maze followed by a reversal of the platform location. Animals in the lesion group showed a significant initial impairment in learning the allocentric task but their performance was comparable to that of the control group after 3 days and there were no differences between groups during a reversal of the target location. Our findings are consistent with a role for the nucleus reuniens in the selection or performance of an allocentric navigation strategy, but suggest that it is not required for applying this strategy when the platform location is moved. Moreover, they suggest that any effects of nucleus reuniens lesions on the trajectory-dependent activity of place cells do not lead to impairments in a hippocampus-dependent win-stay, lose-shift task in which trajectory-dependent activity is normally observed. These findings suggest that prefrontal inputs to the hippocampus via the nucleus reuniens are not required for this task.

Disclosures: **E. Allison:** None. **T. Ripard:** None. **G. Yukhnovich:** None. **P.A. Dudchenko:** None. **E.R. Wood:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

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Program#/Poster#: 264.15/SS5

Topic: F.02. Animal Cognition and Behavior

Support: Max Planck Society

Spanish BFU2011-29089 and Spanish BFU2011-29286

Fritz Thyssen Stiftung

SFB636/A4

Title: Does memory retrieval depend on the precise synaptic organization?

Authors: M. T. HASAN[1], A. CARRETERO-GUILLÉN[2], M. A. GÓMEZ-CLIMENT[2], M. TREVIÑO[3], G. DOGBEVIA[4], M. ROßMANITH[4], *R. SPRENGEL[4], A. VLACHOS[5], A. GRUART[2], J. M. DELGADO-GARCÍA[2]
[1]Neurocure-Charité-Universitätsmedizin, Berlin, Germany; [2]Dpto. de Fisiología, Anatomía y Biología Celular, Univ. Pablo de Olavide, Seville, Spain; [3]Inst. de Neurociencias, Univ. de Guadalajara, Jalisco, Mexico; [4]Mol. Neurobio., Max Planck Inst. for Med. Res., Heidelberg, Germany; [5]Inst. of Clin. Neuroanatomy, Goethe-University, Frankfurt, Germany

Abstract: To investigate whether selective synaptic activities are required for the maintenance of a memory trace, we developed and applied a method called virus-delivered inducible silencing of synaptic transmission (vINSIST). Our vINSIST method is based on a novel destabilized tetanus toxin light chain variant (dsTeTxLC) with a very short half-life time (roughly 3 minutes for the new variant and roughly 6 days for the original one). The steady-state levels of dsTeTxLC are more than 2000-times lower than the original TeTxLC. We developed recombinant adeno-associated viruses (rAAVs) equipped with chemically-controlled genetic switches for inducible and reversible expression of the dsTeTxLC in the dentate gyrus (DG) granule cells of rabbits. Trace eyeblink conditioning consisting of a tone followed by an air-puff to the cornea of the eye with a 500 millisecond temporal interval resulted in robust memory formation for the conditioned eyeblink responses to the tone alone. *In vivo* electrophysiological recording of the DG-CA3 synapse in rabbits during the trace conditioning sessions, lasting several days, showed learning-dependent increases in both field excitatory field potentials (fEPSP) and conditioned eyeblink responses. After 'trace' eyeblink conditioning, we specifically silenced synaptic transmission (ST) of the DG mossy fiber (DG-MF) input onto CA3 neurons. As expected,

conditioned eyeblink responses to a tone were impaired after silencing of ST, and there were large decreases in fEPSP. However, after un-silencing of ST, we discovered that both fEPSP and memory retrieval were fully recovered. These results suggest that in spite of prolonged silencing of the ST, the previously formed memory trace was not lost; without evoked activity for a few days, the memory trace was fully recovered after unsilencing of ST. Consistent with published findings by the Delgado-Garcia lab, low TeTxLC expression induced a reduction in the pre- and postsynaptic markers of the DG-CA3 synapse; VGlut and PSD95 puncta corresponding to the DG-MF boutons and CA3 spines were both reduced by more than 30% and 20%, respectively. Preliminary results with long-term imaging of DG-MF boutons in organotypic cultures before and after silencing of ST show that the distribution of DG-MF boutons remain stable throughout a period of several days. With large reduction in VGlut and PSD95 puncta after silencing of ST, it is possible that there is a strong reduction in the synaptic weight parameters. It is thus tempting to speculate that after silencing of ST, memory trace for the conditioned eyeblink response can be re-assessed with a reorganized assembly of synaptic weights.

Disclosures: **M.T. Hasan:** None. **A. Carretero-Guillén:** None. **M.A. Gómez-Climent:** None. **M. Treviño:** None. **G. Dogbevia:** None. **M. Roßmanith:** None. **R. Sprengel:** None. **A. Vlachos:** None. **A. Gruart:** None. **J.M. Delgado-García:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.16/SS6

Topic: F.02. Animal Cognition and Behavior

Support: HHMI

Title: Large environments reveal the statistical structure governing hippocampal representations

Authors: ***P. RICH**, H.-P. LIAW, A. K. LEE
Janelia Farm, Ashburn, VA

Abstract: The rules governing the formation of spatial maps in the hippocampus have not been determined. We recorded hippocampal neurons from rats exploring a novel, 48-meter-long track, exposing the large-scale structure of place field activity. Single-cell and population activity was well-described by a simple, two-parameter stochastic model. Individual neurons had their own characteristic propensity for forming fields randomly along the track, with some cells expressing

many fields and many exhibiting few or none. Due to the particular distribution of propensities across cells, the number of neurons with fields scaled logarithmically with track length over a wide, ethological range. These features constrain hippocampal memory mechanisms, may allow efficient encoding of environments and experiences of vastly different extents and durations, and could reflect general principles of population coding.

Disclosures: P. Rich: None. **H. Liaw:** None. **A.K. Lee:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.17/SS7

Topic: F.02. Animal Cognition and Behavior

Support: DARPA N66001-09-C-2080

DARPA N66001-09-C-2081

Title: Hippocampal memory prosthesis: Enhancing nonlinear dynamical identification of spike-timing-dependent plasticity

Authors: *B. S. ROBINSON, D. SONG, T. W. BERGER
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Abstract: Insights into learning and memory can be gained with characterization of the activity-dependent requirements for persistent synaptic plasticity. There is evidence that the relative timing of pre-synaptic and post-synaptic spiking affects synaptic plasticity, however there are still many questions as to the nature of this relationship, especially in the mammalian brain during behavior. Here, we develop and analyze a framework to estimate the functional spike-timing-dependent plasticity (STDP) relationship from in-vivo behavioral spiking data. To assess the framework's estimation ability, we first simulate a model of a stochastic spiking neuron model with a pair-wise STDP learning rule. Subsequently, we analyze estimation feasibility of all model functions and parameters from spike timings. Resampled recorded hippocampal CA3 spiking data is used as input into the neuron model. For the STDP learning rule, the function of synaptic weight change vs. pre and post-synaptic inter-spike interval is not enough for full representation. In a STDP system with physiological input spike timing, the actual weight fluctuations and the stability of the system are dramatically affected by time course of plasticity

induction, weight saturation, and spike pairing scheme (all-to-all or nearest neighbor). We therefore additionally included a function for a realistic time course of plasticity induction, implemented a multiplicative weight saturation scheme, and simulated both all-to-all and nearest neighbor spike pairing schemes. All model functions and parameters were formulated and statistically estimated with Volterra kernels, basis function expansion and generalized linear model techniques from the modeled spike timings. Results demonstrate robust estimation of the pair-wise STDP function, as well as the plasticity induction time course. Accurate STDP function estimation was also reliable across spike pairing schemes, use of multiple inputs, and nonlinear multiplicative weight saturation. Furthermore, the amount of data necessary for the STDP function estimation was found to be three times as long for the nearest neighbor vs. all-to-all spike pairing schemes. The next logical step is to apply this estimation framework to in-vivo spike data from a learning task. Findings from such future studies could reveal insights into the activity-dependent nature of synaptic plasticity and be incorporated in the next-generation cortical prosthesis.

Disclosures: B.S. Robinson: None. **D. Song:** None. **T.W. Berger:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.18/SS8

Topic: F.02. Animal Cognition and Behavior

Support: DARPA (REMIND)

Title: Hippocampal memory prosthesis: Decoding and restoring hippocampal spatial memories with nonlinear dynamical modeling

Authors: *D. SONG[1], M. HARWAY[1], V. Z. MARMARELIS[1], R. E. HAMPSON[2], S. A. DEADWYLER[2], T. W. BERGER[1]
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Abstract: To build a cognitive prosthesis that can replace the memory function of the hippocampus, it is essential to model the input-output function of the damaged hippocampal region, so the prosthetic device can stimulate the downstream hippocampal region, e.g., CA1, with the output signal, e.g., CA1 spike trains, predicted from the ongoing input signal, e.g., CA3

spike trains, and the identified input-output function, e.g., CA3-CA1 model. In order for the downstream region to form appropriate long-term memories based on the restored output signal, furthermore, the output signal should contain sufficient information about the memories that the animal has formed. In this study, we verify this premise by applying regression and classification modelings of the spatio-temporal patterns of spike trains to the hippocampal CA3 and CA1 data recorded from rats performing a memory-dependent delayed nonmatch-to-sample (DNMS) task. The regression model is essentially the multiple-input, multiple-output (MIMO) nonlinear dynamical model of spike train transformation. It predicts the output spike trains based on the input spike trains and thus restores the output signal. In addition, the classification model interprets the signal by relating the spatio-temporal patterns to the memory events. We have found that: (1) both hippocampal CA3 and CA1 spike trains contain sufficient information for predicting the locations of the sample responses (i.e., left and right memories) during the DNMS task; and more importantly (2) the CA1 spike trains predicted from the CA3 spike trains by the MIMO model also are sufficient for predicting the locations on a single-trial basis. These results show quantitatively that, with a moderate number of unitary recordings from the hippocampus, the MIMO nonlinear dynamical model is able to extract and restore spatial memory information for the formation of long-term memories and thus can serve as the computational basis of the hippocampal memory prosthesis.

Disclosures: D. Song: None. **M. Harway:** None. **V.Z. Marmarelis:** None. **R.E. Hampson:** None. **S.A. Deadwyler:** None. **T.W. Berger:** None.

Poster

264. Hippocampal and Cortical Circuits I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 264.19/SS9

Topic: F.02. Animal Cognition and Behavior

Support: 09SYN-21-1003, TREAT-AD, co-financed by the European Social Fund (ESF) and the General Secretariat for Research and Technology, Greece

Title: Sex differences in the rat hippocampus and prefrontal cortex following rearing in an enriched environment

Authors: N. KOKRAS[1], I. SOTIROPOULOS[1,2], E. L. TZOUVEKA[1], D. P. BESSINIS[1], O. F. X. ALMEIDA[3], N. SOUSA[2], Z. PAPADOPOULOU-DAIFOTI[1], *C. DALLA[1]
[1]Dep. of Pharmacol., Med. School, Univ. of Athens, Athens, Greece; [2]Life and Hlth. Sci. Res.

Inst. (ICVS), Sch. of Hlth. Sciences, Univ. of Minho, Braga, Portugal; [3]Neuroadaptations group, Max Planck Inst. of Psychiatry, Munich, Germany

Abstract: Early life experiences affect brain development and the appearance of neuropsychiatric disorders, which are often characterized by sex differences. Environmental enrichment (EE) has beneficial effects on brain plasticity and has been proposed as therapeutic intervention. Although brain connectivity and function exhibit marked sex differences, little is known about sex-related responses to beneficial EE stimuli. Our previous studies show that early exposure of male and female rats to EE resulted in enhanced serotonergic and dopaminergic activity and histamine modulation in their visual system,1,2. Herein, we investigate sex differences on cognitive performance and underlying neurochemical and molecular changes related to neuronal and synaptic structure and function. Forty-nine male and female Wistar rats were reared in a standard or enriched environment from P0-P90 and then subjected to the open field and novel object recognition tests. Hippocampus and prefrontal cortex (PFC) were used for: i) evaluation of serotonergic, dopaminergic and glutamatergic neurotransmission by HPLC-ED2 ii) molecular analysis of different cytoskeletal and synaptic proteins by immunoblotting. While corticosterone levels were marginally increased after EE in males only, we found no EE effects in the open field. Following EE, both sexes showed increased preference for a novel object. EE decreased glutamate levels in the female hippocampus and increased the serotonergic 5-HIAA/5-HT ratio in both hippocampus and PFC in comparison to control females. Interestingly, these EE effects on glutamate and serotonin were not found in males. In addition, EE enhanced the hippocampal dopaminergic DOPAC/DA ratio in both sexes. Western blot analysis of cytoskeletal and synaptic proteins revealed significant Sex x EE interactions for phosphorylated isoforms of the cytoskeletal protein Tau and NMDA receptor 2B in males and for synaptic protein PSD95 and p35/25, the activator of kinase cdk5, in females. While males had higher hippocampal levels of 5-HT1A than females, EE reduced them in both sexes. Present findings provide new insights into sex-specific molecular and neurochemical adaptations that underlie the cognitive-enhancing effects of EE. References 1.D. P. Bessinis, C. Dalla, Z. Papadopoulou Daifoti, E.Tiligada. Histamine Involvement in Visual Development and Adaptation. Invest Ophthalmol Vis Sci 2012 53:7498-503 2.D. P. Bessinis, C. Dalla, N. Kokras, P.M. Pitychoutis, Z. Papadopoulou-Daifoti. Sex-dependent neurochemical effects of environmental enrichment in the visual system. Neuroscience 2013 254:130-40

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Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.01/SS10

Topic: F.02. Animal Cognition and Behavior

Support: NIH-NIAAA Grant R01AA007702

Vertex Scholar Award

Title: Ionotropic glutamate receptor activity within the ventral tegmental area is required for the expression of ethanol conditioned place preference

Authors: *M. M. PINA, C. L. CUNNINGHAM
Behavioral Neurosci., OHSU, Portland, OR

Abstract: Exposure to drug-associated cues triggers the activation of excitatory inputs to midbrain dopamine (DA) cells that are believed to drive motivated behaviors such as drug seeking. Previous studies have demonstrated that blocking ionotropic glutamate receptors (iGluRs) within the ventral tegmental area (VTA) impairs the expression of conditioned place preference (CPP) with several classes of abused drugs. However, few studies have evaluated the contribution of intra-VTA iGluRs in ethanol-induced CPP. The present study assessed the role of intra-VTA iGluRs AMPA and NMDA activation in ethanol CPP expression. Under stereotactic guidance, adult male DBA/2J mice (n = 96) were implanted bilaterally with guide cannula aimed at the medial VTA and given 3-5 days to recover prior to the start of conditioning procedures. A two-compartment unbiased place conditioning procedure was used, where ethanol (2 g/kg) was paired with a distinct tactile cue. Conditioning sessions were run twice daily, with saline (CS-) trials occurring in the morning and ethanol (CS+) trials in the afternoon. Trials were conducted across two days for a total of two sessions of each type (2 CS+ and 2 CS-) and the place preference test was administered 24 h after the final conditioning session. Antagonists selective for AMPA/kainate (DNQX) and NMDA (D-AP5) were co-administered into the VTA immediately before the preference test. CPP expression was disrupted in animals that received intra-VTA infusions of the DNQX and D-AP5 cocktail in two different dose combinations ($p < 0.05$ for each dose). Increased ambulation was observed only at the higher doses of DNQX and D-AP5 (0.005 + 0.5 μg/side; $p < 0.05$) and not at the lower doses (0.001 + 0.1 μg/side) compared to vehicle-infused control mice. These results suggest that disrupted ethanol CPP expression was not due to a nonspecific effect of iGluR antagonism on activity. Our findings indicate that activation of NMDA and AMPA receptors within the VTA modulates the expression of ethanol-

induced CPP. This work further supports the hypothesized role of intra-VTA glutamate signaling in conditioned reward and drug-seeking behavior. Combined with the literature, this work suggests that glutamatergic afferents of the VTA may be appropriate targets for treatments designed to reduce the cue-induced seeking behavior that contributes to relapse.

Disclosures: **M.M. Pina:** None. **C.L. Cunningham:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.02/SS11

Topic: F.02. Animal Cognition and Behavior

Title: Lycopene attenuates insulin resistance associated cognitive deficits in rats

Authors: *A. K. SACHDEVA, **K. CHOPRA**
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Abstract: BACKGROUND Growing evidence supports the concept that Alzheimer's disease is fundamentally a metabolic disease. Epidemiological studies on cognitive impairment in patients with diabetes found evidence of cross sectional and prospective associations between type 2 diabetes and moderate degree of cognitive impairment, both for memory and executive functions. Several studies suggest that consumption of a fat rich diet leads to peripheral insulin resistance and impedes cognitive performance. Thus, the present study was designed to elucidate the role of NF-κB pathway in neuroprotective effect of lycopene in an experimental paradigm of insulin resistance in rats. METHODS Six-week-old male Wistar rats were fed with 15% fructose in drinking water for 24 weeks. Body mass, food and water intakes were measured regularly as well as plasma insulin levels, blood glucose levels, glycosylated heamoglobin levels, HOMA-IR levels and lipid profile levels were measured to ensure development of insulin resistance . Insulin resistance associated cognitive impairment was measured by using Morris water maze test (computer tracking using EthoVision software) and elevated plus maze on 24th week. Treatment with lycopene (1, 2 and 4 mg/kg) was initiated after 6th week of fructose administration and continued till end of the study. At the end of study, animals were sacrificed for estimating biochemical and molecular parameters. RESULTS Insulin resistance was evident at 6th week and persisted till end of study (24th week) as demonstrated by significant increase in body weight, plasma insulin levels, blood glucose levels, glycosylated heamoglobin levels, HOMA-IR levels and deranged lipid profile. Cognitive deficit was significantly evident at 24th weeks.

Fructose-induced neuronal deficits were coupled with significant alterations in oxidative-nitrodative stress along with suppression of NF-κB and its downstream mediators as TNF-α, TGF-β1, caspase-3 and IL-1β levels. Treatment with lycopene (1, 2 and 4 mg/kg) dose-dependently ameliorated emergence of insulin resistance-induced memory impairment along with mitigation of oxido-nitrosative stress mediated alterations in cytokine levels. CONCLUSION These findings indicate that chronic fructose consumption generated a condition of neuronal insulin resistance coupled with oxidative stress and neuroinflammation leading to cognitive deficits. Lycopene dose-dependently ameliorated these changes possibly through inhibition of NF-κB signaling cascade.

Disclosures: **A.K. Sachdeva:** None. **K. Chopra:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.03/SS12

Topic: F.02. Animal Cognition and Behavior

Title: Effect of kappa opioid modulation on forebrain dependent trace associative learning: An eyeblink conditioning analysis

Authors: *R. LOH[1], S. SHAH[2], R. GALVEZ[3]
[2]Mol. and Cell. Biol., [1]Univ. of Illinois: Urbana-Champaign, Urbana, IL; [3]Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: There have been several findings indicating a primary role for the opioid receptor system in the modulation of learning and memory. Spatial tasks, fear conditioning, and y-maze spontaneous alternation have all demonstrated that opioid modulation can impair task acquisition. We have recently demonstrated that administration of the opioid antagonist, naloxone, prior to training also retards the ability of mice to acquire whisker-trace eyeblink (WTEB) associations. However, Naloxone, at the concentration used fully antagonized all opioid receptor subtypes, thus the specific opioid receptor mediating this effect for WTEB is not known. Much of the current literature investigating opioid effects in learning and memory have focused on the mu opioid receptor; however, several reports have indicated that there is a likely role for the kappa opioid receptor in various behavioral learning paradigms. The following analysis utilized WTEB to examine the role of the kappa opioid receptor in forebrain-dependent trace associative learning. In WTEB, animals are trained to associate whisker stimulation (CS) with a

salient unconditioned stimulus (US) that causes an unconditioned response. After several trials in which the CS is paired with the US, the animal begins to exhibit a conditioned response in anticipation of the US. Separating the CS and US with a stimulus free trace interval makes this paradigm forebrain dependent by recruiting higher brain structures such as the hippocampus and neocortex. In the following study adult male C57BL/6J mice were randomly assigned to either (0, 2.5, 5.0, or 10mg/kg) doses of the kappa opioid antagonist NorBNI, or (0 or 5.0mg/kg) dose of the kappa opioid agonist u50,488h. Mice were then trained on WTEB. The 10mg/kg of NorBNI significantly retarded learning relative to saline controls, suggesting that the kappa opioid receptor is involved in the acquisition of forebrain-dependent associative learning. Interestingly, kappa agonist u50,488h did not show a significant effects on learning. This could likely be due to one of several factors unrelated to kappa modulation; such as potential limitations in the behavioral paradigm used. These data suggest that the kappa opioid receptor plays a role in acquisition of forebrain dependent trace associative learning. Further research will focus on determining the specific brain region and mechanism by which kappa is driving this effect.

Disclosures: **R. Loh:** None. **S. Shah:** None. **R. Galvez:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.04/SS13

Topic: F.02. Animal Cognition and Behavior

Title: Can environmental enrichment alleviate adult learning impairments caused by adolescent nicotine exposure in rats?

Authors: *S. M. RENAUD, M. E. MILLER, S. B. FOUNTAIN
Psychology, Kent State Univ., Kent, OH

Abstract: Research has shown that adolescent nicotine exposure causes learning impairments in adult rodents. For example, adolescent nicotine exposure in rats produces sex-specific impairments of serial pattern learning in the serial multiple choice (SMC) task in adulthood (Pickens, Rowan, Bevins, & Fountain, 2013). Environmental enrichment, on the other hand, has been shown to reduce behavioral and cognitive deficits caused by aging or neurotoxins (Frick & Fernandez, 2003; Mesa-Gresa, Ramos-Campos, & Redolat, 2014). This study was designed to determine if exposure to enrichment could alleviate learning deficits in the SMC task caused by

adolescent nicotine exposure. To this end, female rats were randomly assigned to one of three conditions: no nicotine and no enrichment (control), nicotine with enrichment, and nicotine without enrichment. On postnatal days 21-89 (P21-89), rats experienced either an enriched environment or a standard environment. Environmental enrichment consisted of access to a running wheel, small hiding places, and toys to manipulate and chew. Enrichment was varied weekly so that cages always had 3 different toys and the same toys would not be encountered again for 3 weeks. The standard environment was never changed. On P25-59, all rats received twice-daily injections of 1.0 mg/kg nicotine or saline. Beginning on P90, enrichment was discontinued. On P90-95, rats were shaped to nose-poke for water. On P96-141, in the SMC task rats learned to nose poke receptacles on the 8 walls of an octagonal chamber in the pattern, 123-234-345-456-567-678-781-818, where digits represent the clockwise position of successive correct receptacles in the circular array and dashes indicate brief pauses. Rats had 90 minutes to complete up to 20 patterns daily for 900 total patterns. The pattern consisted of three element types, namely, within-chunk, chunk-boundary, and violation elements, used to assess different learning mechanisms. Adolescent nicotine exposure impaired chunk-boundary element acquisition. No effect of enrichment was found for any element type. Planned comparisons showed that nicotine with enrichment rats performed significantly worse on chunk-boundary elements than nicotine without enrichment rats on days 3-5, 7, 29, and 32 of acquisition. Thus, enrichment that did not facilitate pattern acquisition in rats not exposed to nicotine (see accompanying poster) also did not alleviate the deficits in serial pattern learning caused by adolescent nicotine exposure. It is possible that under conditions where enrichment does facilitate learning in rats not exposed to nicotine, enrichment might then alleviate nicotine effects.

Disclosures: **S.M. Renaud:** None. **M.E. Miller:** None. **S.B. Fountain:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.05/SS14

Topic: F.02. Animal Cognition and Behavior

Title: Interactions of behavioral training and ketamine administration on changes in parvalbumin positive neurons

Authors: *M. M. BOLTON, C. F. HEANEY, A. S. MURTISHAW, M. A. LANGHARDT, J. W. KINNEY
Psychology, Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Ketamine is a high affinity non-competitive antagonist of the ionotropic N-methyl-D-aspartate (NMDA) glutamate receptor. Several previous investigations in our laboratory using chronic (15 days) subanaesthetic administration of ketamine have demonstrated learning and memory deficits in rodents. We have also repeatedly observed an increase in the number and altered position of parvalbumin (PV) positive neurons in the CA3 field of the hippocampus in ketamine treated animals. In our previous investigations, animals were being tested in behavioral tasks over successive days of ketamine administration. In order to determine if behavioral engagement during ketamine administration is necessary for the increase in PV neuron number or altered position, we performed no behavioral testing over 15 days of treatment and evaluated PV number and position. In addition, our timeline of the change in PV neuron number may be related to recent data indicating an antidepressant role of ketamine. Numerous recent clinical studies have demonstrated a rapid-acting antidepressant effect of subanaesthetic ketamine. There is also a growing literature that indicates the antidepressant effects of ketamine are lost following multiple infusions. There are limited data on the mechanisms responsible for both the antidepressant effects as well as the loss of these effects. In the current study, we were interested in if the change of PV neuron number and position observed in previous studies may influence antidepressant like behavioral changes due to ketamine. We performed the forced swim test to the groups of rats receiving 15 days of saline or ketamine. Results indicate that chronic ketamine administration without behavioral testing did not result in an increase in the number of PV neurons. Similarly, no differences in PV neuron position was observed in these studies. These data indicate that behavioral engagement throughout the course of ketamine administration is necessary in order to alter PV neuron number and position. In addition, our data demonstrate that in the absence of the change of PV neuron number chronic ketamine increased struggle time in the forced swim test versus controls.

Disclosures: **M.M. Bolton:** None. **C.F. Heaney:** None. **A.S. Murtishaw:** None. **M.A. Langhardt:** None. **J.W. Kinney:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.06/SS15

Topic: F.02. Animal Cognition and Behavior

Support: FAPESP

Title: Relationship between astrogliosis in hippocampal formation and neural plasticity induced by acute treatment with Ginkgo biloba and acquisition of fear memory

Authors: M. A. S. TILGER, C. R. ZAMBERLAM, R. F. SILVA, *S. M. CERUTTI
Biol. Sci., Univ. Federal De Sao Paulo, Sao Paulo, Brazil

Abstract: The brain's ability to adapt and change its structure, connectivity and functional properties over time characterizes neural plasticity, which can be investigated using different approaches. Memory formation has been recognized as a stimulant of brain plasticity, which is often characterized by neuronal and glial adaptations. Our group has been evaluated neural plasticity by means of the analysis of behavioural and morphological correlates. The present study investigated the astroglial reaction employing glial fibrillary acidic protein immunohistochemistry (GFAP-IR) in the hippocampal formation. (CA1, CA3 and GD) with one dose before fear conditioning of EGb (250 mg/Kg, 500 mg./Kg and 1000 mg/Kg),vehicle (Tween® and Saline), agonists (10.0 mg/Kg Buspirone or 10mg/Kg N-methyl D-aspartate), antagonists (0.3 mg/Kg (S)-WAY 100135 or 3.0 mg/Kg Ro25-6981) of the 5-HT1ARs or NMDARs/GluN2B and antagonists+EGb ((S)-WAY+EGb or Ro+EGb) (n=3/group). Further, negative control groups (saline 0.9% i.p. and Tween 80 12% v.o.), no-footshock group (CS) and conditioned group (CS-US association) were used. Twenty-four hours after the behavioral experiments were performed, the rats (n=3/group) were deeply anesthetized and perfused with 4% paraformaldehyde. After decapitation, the brains were removed, post fixed, cryoprotected in 10% sucrose, frozen and stored at -80ºC until use. Adjacent serial 20-µm-thick coronal brain sections were obtained with a cryostat and mounted on gelatin-coated slices. These sections integrated two levels including formation hippocampal rostral and caudal (coordinates -2.04 to-4.20 mm from bregma). At each region, 4 fields were analyzed bilaterally. Images were acquired using AxioVision software (Carl Zeiss, Axion imager A2). ImageJ software was employed for quantification of the immunoreactive labeling in CA1, CA3 and GD. An increased number of GFAP-IR astrocytes were found in the CA1, CA3 and GD ($P<0.05$) of the EGb groups. GFAP-IR was increased in the CA3 and GD in Buspirone group ($P<0.05$). However, no significant changes were observed to NMDA groups ($P>0.05$). Additionally, the overexpression of GFAP was reversed by treatment with (S)-WAY 100135 or Ro25-6981 prior EGb. Our results provide further evidence that astroglial reaction in the hippocampal formation may be involved in neuroplastic response in those regions. The fear memory/treatment-dependent changes suggest that EGb might be effective for memory enhancement through its effect on the astrocytes cells which seems to be modulate by NMDA-type of glutamate as well as by serotoninergic-type 5HT1A receptors.

Disclosures: **M.A.S. Tilger:** None. **S.M. Cerutti:** None. **C.R. Zamberlam:** None. **R.F. Silva:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.07/SS16

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 24730642

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The Naito Foundation

Japan Foundation for Aging and Health

Title: Maintenance of memory functions by chronic administration of cilostazol, a phosphodiesterase 3 inhibitor, in aged mice models

Authors: ***S. YANAI**, K. KOJIMA, T. ARASAKI, S. ENDO
Tokyo Metropolitan Inst. of Gerontology, Tokyo, Japan

Abstract: The balance between adenylate cyclase and phosphodiesterases (PDEs) controls concentration of cAMP to modulate a variety of physiological functions including learning and memory. Thus, PDE inhibitors enhance cAMP actions through elevation of intracellular cAMP concentration. Inhibitors specific for PDE isoform have been developed for diseases. One of these inhibitors, cilostazol, a PDE3 selective inhibitor, is clinically effective to treat the heart failure and intermittent claudication. Recently, we reported that cilostazol enhanced hippocampus-dependent memory in young mice (Yanai et al., in press), however, its effects is unknown for the age-related memory impairments. We examined the effect of chronic administration of cilostazol as a potential therapeutic intervention for memory impairment in aged population by using two animal model; 23-month old C57BL/6J and 8-month old

senescence accelerated mouse prone 8 (SAM P8). For C57BL/6J mice, cilostazol (0, 0.3%, or 1.5%) had been administered for 10 months and they were subjected to the behavioral test battery at 23 months of age. Non-cilostazol-administered age-matched group showed impaired performance in the Morris water maze task compared to young control mice, however, the performance of 1.5% cilostazol-administered mice was similar to that of the young mice. No major influence of cilostazol was observed in the open field test, suggesting that chronic cilostazol administration does not have adverse effects on locomotor activity and anxiety. For SAM P8, cilostazol was administered for 3 months before the behavioral experiments. At the age of 8 months, they were subjected to the Pavlovian fear conditioning task. The 1.5% cilostazol-administered SAM P8 exhibited significantly higher conditioned freezing than the SAM P8 without cilostazol in both cue- and context-dependent memory tests. The performance of 1.5% cilostazol-administered SAM P8 was comparable to that of the control normal aging mice. In addition, the cilostazol-administered SAM P8 showed a significant increase in the number of phospho-CREB positive cells in hippocampal dentate gyrus. The results suggest that chronic cilostazol administration may prevent the age-related memory decline via modulating cAMP pathway including the phosphorylation of CREB.

Disclosures: **S. Yanai:** None. **K. Kojima:** None. **T. Arasaki:** None. **S. Endo:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.08/SS17

Topic: F.02. Animal Cognition and Behavior

Support: NINDS Grant P01NS045260-01

NINDS Grant R01NS057128

Title: A calpain-2 selective inhibitor enhances learning and memory through prolonged ERK activation

Authors: ***Y. LIU**, Y. WANG, G. ZHU, X. BI, M. BAUDRY
Western Univ. of Hlth. Sci., POMONA, CA

Abstract: While activation of calpain-1 is necessary for the formation of long-term potentiation (LTP) elicited by theta burst stimulation (TBS) in field CA1 of hippocampus, activation of

calpain-2 during the 1 h-consolidation period after TBS limits the extent of synaptic potentiation, and its inhibition results in enhanced LTP. In LTP induction, calpain-1 activation results in SCOP truncation followed by ERK activation, whereas in the consolidation period, calpain-2 activation stimulates mTOR-dependent SCOP protein synthesis through calpain-mediated PTEN degradation, which limits the duration of ERK activation. To further evaluate the role of calpain in learning and memory, we injected mice intraperitoneally (i.p.) with different doses of a selective calpain-2 inhibitor (Z-Leu-Abu-CONH-CH2-C6H3(3,5-(OMe)2) (C2I), from 0.1 mg/kg to 10 mg/kg) 30 min before the training session. Treatment with C2I significantly increased freezing times in both context-and tone-elicited fear-conditioning paradigm at 0.1-0.3 mg/kg, and inhibited performance at doses above 1mg/kg, as compared to vehicle-treated mice. The differential effects of low and high doses of C2I on learning and memory match their inhibitory effects on calpain-2 and calpain-1. SCOP levels were significantly decreased and ERK phosphorylation significantly increased in dorsal but not ventral hippocampus 5 min after training, and levels of these two proteins returned to basal level 50 min after training. However, in mice pretreated with 0.3 mg/kg C2I, SCOP levels remained decreased and ERK activation elevated up to 50 min after training. These results demonstrated that a calpain-2 selective inhibitor enhances learning and memory by prolonging ERK activation in dorsal hippocampus.

Disclosures: **Y. Liu:** None. **Y. Wang:** None. **G. Zhu:** None. **X. Bi:** None. **M. Baudry:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.09/SS18

Topic: F.02. Animal Cognition and Behavior

Support: DAINIPPON SUMITOMO PHARMA

Eli Lilly Japan

Title: Effect of an N-methyl-D-aspartate receptor antagonist in animal models of schizophrenia: An immunohistochemical study in the rat

Authors: H. MATSUO[1], T. NISHIMORI[1], H. ABE[1], A. KURAMASHI[1], G. KOGANEMARU[1], H. FUNAHASHI[1], K. EBIHARA[1], T. IKEDA[2], *Y. ISHIDA[1]
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Abstract: The antipsychotic agent is considered to have more desirable effects on the cognitive deficits in patients with schizophrenia, while dysfunction of the N-methyl-D-aspartate (NMDA) receptor has been associated with negative and cognitive symptoms in schizophrenia. Perospirone is an atypical neuroleptic with potent 5-HT2A and D2 receptor blocking actions, and can improve both positive and negative symptoms of schizophrenia (Ohno et al., 1997). To elucidate pharmacological profiles of perospirone, we examined the ameliorating effect of perospirone on methamphetamine-induced disruption of latent inhibition (LI) and the expression of c-Fos in rats. The disruption of LI was ameliorated in rats following administration of haloperidol or perospirone, and they significantly increased the number of c-Fos-positive cells in the nucleus accumbens shell, but not in the prefrontal cortex, the nucleus accumbens core and the amygdala. Moreover, the enhanced expression of c-Fos in the nucleus accumbens shell was furthermore increased by pretreatment with MK-801, an NMDA receptor antagonist, indicating that the nucleus accumbens shell plays a crucial role in the effect of antipsychotic agents through the NMDA receptors.

Disclosures: H. Matsuo: None. **T. Nishimori:** None. **H. Abe:** None. **A. Kuramashi:** None. **G. Koganemaru:** None. **H. Funahashi:** None. **K. Ebihara:** None. **T. Ikeda:** None. **Y. Ishida:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.10/SS19

Topic: F.02. Animal Cognition and Behavior

Title: Fear reducing effects of Souroubea Spp. and Platanus occidentalis in rats: Potential relevance for post traumatic stress disorder

Authors: *K. DELCELLIER[1,2], C. CAYER[3,2], P. KENT[3,2], J. ARNESON[3], Z. MERALI[3,2]
[1]Carleton Univ., Hull, QC, Canada; [2]Inst. of Mental Hlth. Res., Ottawa, ON, Canada; [3]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: The prevalence of anxiety disorders is rising rapidly in today's society, impacted greatly by recent times of civil unrest and war throughout the world. Many of the currently prescribed drug therapies for anxiety disorders are associated with aversive side-effects and lose efficacy with repeated administration. Ethnobotanical research has shown that anxiolytic properties of certain plants are of therapeutic relevance in treating anxiety symptoms. The

objective of the present study was to investigate the anxiolytic effects of a botanical blend ethanolic extract of a 1:1 Souroubea sympetala and Platanus occidentalis (Sycamore tree), which contains 15% of the previously identified anxiolytic principle; betulinic acid (BA). The three main facets of conditioned fear: fear memory expression, extinction and reconsolidation were investigated using the Fear Potentiated Startle (FPS) and Conditioned Emotional Response (CER) paradigms, based on their translational relevance to post traumatic stress disorder (PTSD) as exaggerated startle and avoidance are primary symptoms. In the case of fear memory expression, extinction and reconsolidation, the FPS and CER of the rats was measured through comparing the effects of the botanical blend ethanolic extract (7mg/kg), to diazepam (5mg/kg) or vehicle administration. Significant decreases in fear responses were observed throughout the three fear memory processes upon treatment of the botanical blend extract, effects similar to those observed with diazepam. These findings are consistent with previous pilot results whereby the dried raw plant materials of the botanical blend significantly reduced fear- and anxiety-like responses in rats. Although more research is needed in order to further the understanding of its mechanism of action, these results are promising and may offer a safe clinical alternative to treating anxiety disorders including PTSD.

Disclosures: K. Delcellier: None. **C. Cayer:** None. **P. Kent:** None. **J. Arneson:** None. **Z. Merali:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.11/SS20

Topic: F.02. Animal Cognition and Behavior

Support: CNPQ

FAPESC

The University of Texas Health Science Center at Houston

Title: Folic acid prevented cognitive impairment in Experimental pneumococcal meningitis

Authors: *T. BARICHELLO[1], J. S. GENEROSO[2], L. R. SIMOES[2], D. DOMINGUINI[2], A. MOREIRA[2], P. FERRARI[3], C. GUBERT[4], F. KAPCZINSKI[3], L. C. JORNADA[2], L. G. DANIELSKI[5], F. PETRONILHO[6], J. BUDNI[2], J. QUEVEDO[6]

[1]Psychiatry and Behavioral Sci., The Univ. of Texas Hlth. Sci. Ctr. At H, Houston, TX; [2]Univ. do Extremo Sul Catarinense, Criciuma, Brazil; [3]Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil; [4]Univ. Federal do rio Grande do Sul, Porto Alegre, Brazil; [5]Univ. do Sul Catarinense, Tubarao, Brazil; [6]Psychiatry and Behavioral Sci., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: *Streptococcus pneumoniae* is a common cause of bacterial meningitis, with a high mortality rate and neurological sequelae. In contrast, folic acid plays an important role in neuroplasticity and the preservation of neuronal integrity. In the present study, we evaluated the influence of folic acid on memory, oxidative damage, enzymatic defence, and brain-derived neurotrophic factor (BDNF) expression in experimental pneumococcal meningitis. Male Wistar rats received either 10 µl of artificial cerebral spinal fluid or an equivalent volume of *S. pneumoniae* suspension at concentration $5x10^9$ UFCol/ml. The animals were divided into six groups: control, control/folic acid at 10 mg/Kg, control/folic acid at 50 mg/Kg, meningitis, meningitis/folic acid at 10 mg/Kg, and meningitis/folic acid at 50 mg/Kg. Folic acid was dissolved in distilled water and administered orally by gavage at a dose of 10 or 50 mg/Kg once per day for 7 days, at the same time the animals received ceftriaxone (100 mg/kg body weight given s.c., during 7 days) starting 18 h after the induction of pneumococcal meningitis. After 10 days, the animals were free from infection. We performed blood cultures that were all negative in this period and the animals were randomised and subjected to behavioural tests: open-field task and step-down inhibitory avoidance task. The animals that received folic acid at a dose of 10 or 50 mg, there was a reduction in both crossing and rearing during open-field task when compared with the training session, demonstrating habituation memory ($p<0.05$). During the step-down inhibitory avoidance task, there was a difference between the training and the test sessions, demonstrating aversive memory ($p< 0.05$). In the untreated meningitis group, there was no difference between the training and the test sessions, demonstrating impairment of habituation and aversive memory in this group. In the hippocampus, BDNF expression decreased in the meningitis group; however, adjuvant treatment with 10 mg/Kg folic acid increased BDNF expression in the meningitis group ($p<0.05$). Folic acid decreased lipid peroxidation, protein carbonylation, nitrate/nitrite levels, and myeloperoxidase activity and increased superoxide dismutase activity in the hippocampus ($p<0.05$). There is substantial interest in the role of folic acid and related pathways in nervous system function and in folic acid's potential therapeutic effects. Here, adjuvant treatment with folic acid exerted protective effects in experimental pneumococcal meningitis.

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Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.12/SS21

Topic: F.02. Animal Cognition and Behavior

Support: VA Merit Review

VA Career Scientist Award

Title: Influence of norepinephrine, corticotrophin releasing hormone and corticosterone, individually and in conjunction, on the expression of hippocampal synaptic plasticity in tissue obtained from stressed versus non-stressed rats

Authors: G. E. FARMER[1], M. F. MIDKIFF[1], C. R. PARK[1], *D. M. DIAMOND[1,2]
[1]Psychology, Univ. South Florida, TAMPA, FL; [2]Res. and Develop., VA Hosp., Tampa, FL

Abstract: The onset of stress is accompanied by an increase in levels of multiple neuromodulators, each with a different time course in its effects on the hippocampus. Whereas norepinephrine (NE) and corticotrophin releasing hormone (CRH) are rapidly released in the hippocampus, corticosterone (CORT) exhibits a more delayed time course of action. The effects of NE, CRH and CORT on hippocampal plasticity are ordinarily assessed in isolation, but *in vivo*, these neuromodulators interact, potentially in a manner which is not predicted from their individual actions. Therefore, we assessed their effects, individually and in concert, on the hippocampus, *in vitro*. We also evaluated the influence of acute stress *in vivo* on the effects of these neuromodulators on synaptic plasticity, *in vitro*. Pairs of male adult Sprague-Dawley rats were transported to a holding room where one of the two rats was immediately anesthetized and decapitated (Group 1) followed by conventional hippocampal slice processing. The second rat remained in the holding room for 2.5 hr prior to slice preparation (Group 2). Long-term potentiation of the field EPSP in dorsal CA1 stratum radiatum was induced by primed burst (PB) stimulation, which consisted of one pulse followed 170 ms later by a 6 pulses at 100 Hz. Bath application of NE (10 μM) and/or CRH (125 nM) was initiated 5 min prior to PB stimulation and remained present for 10 min post-PB stimulation, for a total of 15 min. Bath application of CORT (30 nM), alone or in conjunction with NE or CRH, was initiated 2 min prior to PB stimulation and remained present for 10 min post-PB, for a total of 12 min. The magnitude of PB potentiation (PBP) was greater in Group 1 versus Group 2, which is consistent with previous work demonstrating the sensitivity of CA1 to acute stress. Bath application of NE blocked the reduction of PBP in Group 2. CRH had no effect on the differential expression of PBP in Groups

1 vs 2. CORT reduced PBP in all groups, alone or in conjunction with NE or CRH. NE + CRH produced an effect that was equivalent to NE, alone. This work illustrates that: 1) brief stress just prior to decapitation can inhibit hippocampal plasticity; and 2) an assessment of combinations of neuromodulators in conjunction with acute stress may yield insight into the dynamics, and complexity, of stress-plasticity interactions in the behaving animal.

Disclosures: G.E. Farmer: None. **D.M. Diamond:** None. **M.F. Midkiff:** None. **C.R. Park:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.13/SS22

Topic: F.02. Animal Cognition and Behavior

Title: Chronic effects of the 'biased' 5-HT1A receptor agonists F15599 and F13714 on object pattern separation and core body temperature

Authors: *B. T. VAN HAGEN[1], N. P. VAN GOETHEM[1], R. SCHREIBER[2,3], A. NEWMAN-TANCREDI[4], M. VARNEY[4], J. PRICKAERTS[1]
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Abstract: Pattern separation, or the formation of distinct representations out of similar inputs, is an important hippocampal process implicated in memory formation. Impairments in pattern separation could underlie the cognitive symtoms of multiple disorders like PTSD and schizophrenia. Based on the distribution of the serotonin 1A receptor (5-HT1A¬R) in the brain, and the molecular and cellular mechanisms of this receptor, we hypothesized that it plays a role in pattern separation. Our previous research showed that the new, 'biased' 5-HT1A agonist, F15599, which preferentially activates postsynaptic cortical heteroreceptors, acute treatment improved rat performance in a new spatial object pattern separation (OPS) task. In contrast, F13714, which preferentially activates presynaptic autoreceptors, impaired performance in this task (Schreiber et al., 2013, Soc. Neurosci., #865.18). Herein, we investigated the effects of chronic treatment with F15599 and F13714 on spatial OPS performance in rats. We hypothesized that, through desensitization of presynaptic autoreceptors by F13714, the OPS performance impairment would ameliorate due to increased activation of postsynaptic receptors. Core

temperature measurents were included as a mechanistic readout of postsynaptic 5-HT1A¬R activation. An increase in hypothermia by F13714 treatment would reflect a shift to activation of more postsynaptic 5-HT1A¬Rs. 16 animals per condition were treated daily with i.p. injections of 0.32 mg/kg F15599, 0.02 mg/kg F13714 or vehicle (PBS). OPS performance was measured at day 1, 8 and 14 of treatment. Core body temperature was measured daily, 30 min after drug administration. The impairment in OPS performance with acute treatment of F13714 was replicated and subsequently a gradual increase of performance was found, resembling vehicle treatment at day 14. Body temperature dropped by one degree celcius from treatment day 4 onwards. F15599 showed an enhancement in OPS performance compared to vehicle acutely, and this was maintained through day 14. Body temperature initially dropped, but then rose with one degree compared to day 1. These results imply possible desensitization of presynaptic 5-HT1A¬Rs by F13714, leading to stimulation of postsynaptic 5-HT1A¬Rs which could explain the drop in temperature. The temperature data even suggest desensitisation of postsynaptic receptors, but this is not reflected in the behavioral data. Further studies, e.g., determination of the number of 5-HT1A¬Rs, are ongoing to better understand the potential desensitization of pre- and/or post-synaptic receptors.

Disclosures: B.T. Van Hagen: None. **N.P. van Goethem:** None. **R. Schreiber:** None. **A. Newman-Tancredi:** None. **M. Varney:** None. **J. Prickaerts:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.14/SS23

Topic: F.02. Animal Cognition and Behavior

Support: Institutional grant KA-2012

Title: Evaluation the effect of tempol on learning and memory impiarment induced by sleep deprivation

Authors: *O. F. KHABOUR[1], K. H. ALZOUBI[2], A. S. ALBAWAANA[2]
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Abstract: Sleep deprivation has been associated with learning and memory impairment through induction of oxidative stress so damaging the neurons and prevent firing. On the other hand, Tempol, a small membrane permeable redox-cycling nitroxide compound, that promotes the

metabolism of many reactive oxygen species (ROS), has antioxidant and neuroprotective effect that may antagonize this oxidative stress. In our study we hypothesize that chronic administration of tempol can prevent learning and memory impairment induced by sleep deprivation. This hypothesis was evaluated and possible molecular targets for tempol action were determined. Sleep deprivation was induced in rats using modified multiple plate form model. Tempol was administered to rats via oral gavage. Behavioral studies were conducted to test the spatial learning and memory using radial arm water maze. And at the end of the experiment, the hippocampus was dissected; BDNF protein, TBARS levels and antioxidant markers (GSH, GSSG, GSH/GSSG ratio, GPx, SOD, and catalase) were assessed. The result of this project revealed that chronic sleep deprivation impaired both short and long term memory ($P<0.05$), while Tempol treatment prevented such effect. Furthermore, Tempol normalized chronic sleep deprivation induced reduction in the hippocampus activity of Catalase ($P<0.05$), GPx ($P<0.05$), and SOD ($P<0.05$). Tempol also enhanced the ratio of GSH/GSSG in chronically sleep deprived rats treated with tempol as compared with only sleep deprived rats ($P<0.05$), without any effect on BDNF activity ($P>0.05$) or TBARS activity ($P>0.05$). In conclusion chronic sleep deprivation induced memory impairment, and treatment with Tempol prevented this impairment probably through normalizing antioxidant mechanisms in the hippocampus.

Disclosures: O.F. Khabour: None. **K.H. Alzoubi:** None. **A.S. Albawaana:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.15/SS24

Topic: F.02. Animal Cognition and Behavior

Title: SUVN-I1312004, Novel M_1 muscarinic acetylcholine receptor positive allosteric modulator: Potential for the treatment of Alzheimer's disease

Authors: *R. ABRAHAM, R. MEDAPATTI, R. KARTHIK GADI, V. RAVIRALA, S. YATHAVAKILLA, R. SUBRAMANIAN, R. CHOWDARY, M. ABDUL RASHEED, N. MUDDANA, S. GAGGINAPALLI, P. JAYARAJAN, R. NIROGI
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Abstract: Muscarinic receptors namely M_1 is being actively pursued as target for improvement of cognition in elderly patients suffering from Alzheimer's disease (AD). Due to the conserved nature of the muscarinic receptors at the orthosteric site, allosteric site is being explored .

Targeting the less conserved allosteric site provides selectivity and advantage of obtaining pharmacological agents with lesser side effects. SUVN-I1312004 is selective M_1 muscarinic receptor positive allosteric modulator with no agonist like activity. SUVN-I1312004 was inactive at orthosteric receptor sites of M1-M5 protein, with % inhibition values <20% at 10 uM. In the functional reporter gene assay at human M_1 muscarinic receptor, SUVN-I1312004 shifted acetylcholine dose response towards leftward with a 57 fold shift of acetylcholine potency at 10μM and an EC_{50} (inflection point) of 337 nM and the effects were blocked by a selective antagonist, which proves that SUVN-I1312004 is a potent positive allosteric modulator of human M_1 muscarinic receptor. The pharmacokinetic and brain penetration properties were investigated in rats. SUVN-I1312004 had favorable pharmacokinetic profile and adequate brain penetration in male Wistar rats. Efficacy of SUVN-I1312004 was assessed in various cognitive tasks such as object recognition task (ORT) and fear conditioning assay. SUVN-I1312004 enhanced the discriminative index when administered alone and/or in combination with donepezil in ORT. SUVN-I1312004 enhanced the duration of freezing in the fear conditioning test, memory deficit being induced by scopolamine. It was found to be non- mutagenic in bacterial reverse mutation assay. SUVN-I1312004 is a novel, potent, selective, orally bioavailable and efficacious M_1-PAM that holds promise in the treatment of AD and dementia. SUVN-I1312004 is being further evaluated for various safety parameters.

Disclosures: **R. Abraham:** A. Employment/Salary (full or part-time):; employement. **R. Medapatti:** A. Employment/Salary (full or part-time):; Employment. **R. Karthik Gadi:** A. Employment/Salary (full or part-time):; employement. **V. Ravirala:** A. Employment/Salary (full or part-time):; Employment. **S. Yathavakilla:** A. Employment/Salary (full or part-time):; Employment. **R. Subramanian:** A. Employment/Salary (full or part-time):; employment. **R. Chowdary:** A. Employment/Salary (full or part-time):; Employment. **M. Abdul Rasheed:** A. Employment/Salary (full or part-time):; employment. **N. Muddana:** A. Employment/Salary (full or part-time):; employment. **S. Gagginapalli:** A. Employment/Salary (full or part-time):; employment. **P. Jayarajan:** A. Employment/Salary (full or part-time):; employment. **R. Nirogi:** A. Employment/Salary (full or part-time):; Employment.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.16/SS25

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant R15DA015351

Title: Responsiveness to restraint stress and scopolamine in simple learning and memory

Authors: *I. M. WHITE, Z. ABBOTT, J. B. DUTY, W. WHITE
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Abstract: The present study examined the interaction between stress and scopolamine in simple learning and memory. One group of Wistar rats was shaped to lever-press to receive food pellets, some subjects were exposed to a stress manipulation (restraint) whereas others were not, and then all subjects received training on a fixed ratio 5 (FR5). A second group was trained on FR5 until subjects reached behavioral criteria, they received either scopolamine or saline, all subjects received restraint-stress for a 30-min period, and then they received additional testing on FR5. The third group of subjects was trained on FR5, they received cortisol, yohimbine, or saline, and then they were tested on FR5. Performance measures included the mean latency of the first lever-press, the mean time required for five lever-presses (runtime), and the mean latency of food-retrieval. Stress disrupted acquisition of FR5, without affecting food retrieval or consumption. Scopolamine and stress disrupted maintenance of FR5 by increasing the first response latency, compared to saline control. Food retrieval was affected by scopolamine, but not by stress. Exposure to stress in conjunction with scopolamine injection produced a greater impairment in performance, compared to other conditions. Neither scopolamine nor stress affected runtime, whereas the combination of the two markedly increased runtime. Runtime was impaired by yohimbine-induced stress, but not by cortisol. Our data suggest that stress differentially modulates scopolamine-induced deficits and motivational state.

Disclosures: I.M. White: None. **Z. Abbott:** None. **J.B. Duty:** None. **W. White:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.17/SS26

Topic: F.02. Animal Cognition and Behavior

Support: UHCL Faculty Research Support Fund

Title: Scopolamine impairs latent working memory in the water maze

Authors: *S. Y. JABITTA, J. J. IZYGON, D. M. NGHIEM, M. M. HENCEROTH-CHOMIAK, W. T. WILLIAMSON, D. H. MALIN
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Abstract: The Morris water maze is routinely used to explore neural mechanisms of working memory. Humans can often acquire working memory relevant to performing a task without having to actually perform the task. This can be modeled in the water maze through direct placement on the escape platform, followed by assessing the subject's performance in swimming back to the platform. This sort of learning was studied in the water maze through a revised direct placement task with novel pre-training and control procedures. Muscarinic cholinergic mechanisms, which are inactivated by scopolamine, are essential to memory for standard working memory paradigms in the water maze. This experiment determined whether this would also be true for latent learning based on direct platform placement. The subjects were 18 Long-Evans hooded rats. All rats were pre-trained for three days in the escape procedure by swimming progressively longer distances back to the platform where they had been placed. During four days of working memory testing, 12 rats were trained by direct placement on the platform in a different quadrant within the maze each day. Six sham-trained rats were placed on a platform in a small cylinder in a different room. Five minutes later, all rats were placed in the full-sized water maze and assessed for path-length and latency to reach the escape platform. On two days, rats were injected i.p. thirty minutes before training with 1 mg/kg scopolamine hydrobromide in saline, and on the other two they received saline alone. The order of drug and saline injections was counterbalanced among subjects. ANOVA with one-repeated measures variable (drug) revealed a significant effect of drug, $p = 0.002$ and of training vs. sham-training, $p = 0.026$. Post-hoc pairwise comparisons indicated significantly lower escape latencies, $p < 0.01$, in the training plus saline condition than in the training plus scopolamine, sham-training plus saline and the sham-training plus scopolamine conditions. Accuracy scores (distance to platform/subject's path-length) were also significantly better in trained rats without scopolamine, $p < 0.01$ than all other conditions. There was a probe trial with the platform removed after the daily direct placement. Trained rats without scopolamine traveled a significantly, $p < 0.01$, longer path-length within the target quadrant and spent significantly, $p < 0.01$, more time there than trained rats with scopolamine and sham-trained rats without scopolamine. The overall results suggest that muscarinic cholinergic systems are essential to spatial learning by mere visual observation, as distinguished from trial-and-error learning.

Disclosures: S.Y. Jabitta: None. **J.J. Izygon:** None. **D.M. Nghiem:** None. **M.M. Henceroth-Chomiak:** None. **W.T. Williamson:** None. **D.H. Malin:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.18/SS27

Topic: F.02. Animal Cognition and Behavior

Title: Modafinil as a neuroenhancer

Authors: *H. M. MURPHY[1], C. H. WIDEMAN[2]
[1]Dept Psychol, [2]Biol., John Carroll Univ., Cleveland, OH

Abstract: Modafinil, a schedule IV psychostimulant approved by the FDA to treat narcolepsy, has become popular among students as a neuroenhancer. It has been suggested that it facilitates cognitive functions, with pro-mnemonic effects and problem solving improvements. The purpose of the present experiment was to examine the influence of modafinil on working memory in rats. Six control and 6 experimental rats were placed into individual cages equipped with a running wheel to monitor activity. The study was divided into three different periods; 1) habituation - 1 week; 2) experimental - 3 weeks, in which a 64 mg/kg dose of modafinil was administered to experimental animals in a condensed milk treat; and 3) withdrawal - 5 days. During the experimental and withdrawal periods, the spatial working memory of the rats was tested utilizing the Morris Water Maze. A 2 hour delay period followed drug administration and a protocol for spatial working memory was employed. Three testing sessions were given during the experimental period and one testing session was given during the withdrawal period. Animals were given two trials in each session. The first was a sample trial in which animals discovered the location of the platform by trial and error and the second was a test trial in which animals were required to recall the location of the platform. Platform placement and starting place location of the rat were changed every session. Performance of control animals during the sample trial and the test trial for the four sessions of exposure to the maze showed no difference in time to reach the platform. Performance of experimental animals demonstrated faster attainment of the goal during the test period. During withdrawal, there was no significant difference in time between the two trials for animals that were previously given modafinil. Concerning wheel running, although both groups were similar during the habituation period, activity of the experimental group was significantly lower than the control group during the rest of the experiment. The results of this study support the hypothesis that modafinil has a positive effect on working memory in rats, specifically to increase short term, trial-dependent learning. It is suggested that benefits of modafinil can extend beyond its prescribed usage. Due to the significant decrease in activity, modafinil could become an advantageous medication to treat hyperactivity disorders and, because of lack of withdrawal symptoms, it could become a safe replacement for other psychostimulants. A cautionary note, however, is that the use of modafinil is extremely relevant in discussions related to ethical concerns associated with neuroenhancers.

Disclosures: **H.M. Murphy:** None. **C.H. Wideman:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.19/SS28

Topic: F.02. Animal Cognition and Behavior

Support: Smoking Research Foundation (Japan)

Title: Involvement of BDNF in doxorubicin and cyclophosphamide-induced spatial cognition in rats

Authors: ***Y. KITAMURA**[1], S. YONEDA[1], I. MIYAZAKI[2], M. ASANUMA[2], T. SENDO[1]
[1]Dept. Clin. Pharm., Okayama Univ. Hosp., Okayama, Japan; [2]Dept. Brain Sci., Okayama Univ., Okayama, Japan

Abstract: We previously showed that chronic treatment with doxorubicin and cyclophosphamide chemotherapeutic agents (AC regimen) impairs spatial cognition in rats. The chronic administration of the AC regimen decreased serum brain-derived neurotrophic factor (BDNF) (but not hippocampal BDNF) protein levels and BrdU-positive cells in the dentate gyrus of the hippocampus. These behavioral changes may be attributed, in part, to decreased hippocampal neurogenesis, and chemotherapy may lead to these impairments by altering neurogenesis via peripheral BDNF. In the serum BDNF is stored in platelets and can bind to platelet receptors. On the other hand, romiplostim is a second generation thrombopoietin receptor agonist (TPO-RA) that mimics the function of TPO. TPO is the key regulator of the platelet production. We hypothesized that romiplostim blocked the decreasing effect of hippocampal neurogenesis and spatial cognitive impairment, based on treatment with romiplostim inhibiting the decrease in serum platelets caused by the AC regimen. [METHODS] Male Wistar rats were administered doxorubicin (2 mg/kg, i.p.) and cyclophosphamide (50 mg/kg, i.p.) once per week for 2 weeks. Romiplostim (30 μg/kg, s.c.) was administered once per week for 2 weeks before the AC regimen, and with the co-administration of the AC regimen. The novel place recognition task was performed in the 3th week. [RESULTS and DISCUSSION] Saline-treated rats spent significantly more time exploring an object moved to a new location than exploring the same object left in the old location in the novel location recognition test. This behavior was impaired following treatment with the AC regimen. Romiplostim improved the impairment of spatial cognition. Serum platelet counts and BDNF were decreased with the AC regimen. Romiplostim

increased these factors in normal rats. However, these factors significantly decreased with the AC regimen. [CONCLUSION] Our results show that chronic treatment with the AC regimen impairs spatial cognition in rats. These behavioral changes may be attributed, in part, to decreased hippocampal neurogenesis. In this study, we demonstrate that spatial cognition impairment is a possible mechanism by which the AC regimen decreases the effect of serum BDNF.

Disclosures: **Y. Kitamura:** None. **S. Yoneda:** None. **I. Miyazaki:** None. **M. Asanuma:** None. **T. Sendo:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.20/SS29

Topic: F.02. Animal Cognition and Behavior

Support: NSERC Grant 400176

Title: Severe crossmodal object recognition impairment in ketamine-treated rats: Amelioration by α4β2 nicotinic receptor stimulation of the GABAergic system

Authors: ***J. M. CLOKE**, B. D. WINTERS
Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstract: The neural bases of multisensory integration impairments in schizophrenia are not well understood. Rats treated sub-chronically with NMDA receptor antagonists (e.g., ketamine), which model symptoms of schizophrenia, are impaired on a tactile-to-visual crossmodal object recognition (CMOR) task; this deficit is reversed by systemic nicotine, which can also attenuate cognitive impairment in patients with schizophrenia. Furthermore, cortical gamma oscillations mediated by parvalbumin-containing GABAergic interneurons (PV-INs) may be deficient in schizophrenia, potentially contributing to aberrant multisensory processing. PV-INs contain nicotinic acetylcholine receptors (nAChR). The current study assessed the receptor specificity of the ameliorative effect of nicotine in the CMOR task, as well as the potential for nAChR interaction with GABA and glutamate. Male Long-Evans rats were treated sub-chronically for 10 days with ketamine (30 mg/kg) or saline and then tested on the CMOR task after a 10-day washout. Systemic nicotine (0.2 mg/kg) given before the sample phase of the CMOR task reversed the ketamine-induced impairment, but this effect was blocked by co-administration of

the $GABA_A$ receptor antagonist bicuculline at a dosage (0.5 mg/kg) that itself did not cause impairment. Pre-sample systemic co-administration of the NMDA receptor antagonist MK-801 (0.001 & 0.01 mg/kg) did not block the remediating effect of nicotine in ketamine-treated rats. Finally, the selective α7 agonist GTS-21 (0.01, 0.03 & 0.1 mg/kg) and α4β2 nAChR agonist ABT-418 (0.06, 0.1 & 0.6 mg/kg) were tested, with only the latter reversing the ketamine impairment; bicuculline also blocked this effect. These results suggest that nicotine-induced agonism of α4β2 nAChRs ameliorates CMOR deficits in ketamine-treated rats via stimulation of the GABAergic system. Ongoing work is assessing similar interactions in the distributed network implicated in CMOR task performance, including the orbitofrontal, perirhinal, and posterior parietal cortices. Cortical dysfunction in these regions may contribute to the severe CMOR deficit apparent in ketamine-treated rats. The findings of this research may have important implications for understanding the nature and potential treatment of cognitive impairment in schizophrenia.

Disclosures: J.M. Cloke: None. **B.D. Winters:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.21/SS30

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 25293124

Title: Novel cognitive enhancer SAK3 enhances T-type voltage-gated Ca^{2+} channel current in Neuro2a cells expressed cav3.1 gene

Authors: *Y. YABUKI[1], M. WAKAMORI[2], K. FUKUNAGA[1]
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Abstract: The T-type voltage-gated Ca^{2+} channels (T-VGCCs) are involved in the pathophysiology of epilepsy, pain and sleep. We recently elucidated the mechanism underlying cognitive enhancement of a novel Alzheimer disease drug candidate, ST101 which stimulates T-VGCC in mouse cortical slices and neuro2A cells over-expressed Cav3.1 (Moriguchi et al., J Neurochem 2012;121:44-53). We successfully synthesized another series of spiroimidazopyridine derivatives (SAKs), which are much stronger than ST101 in the stimulation of Ca^{2+} conductance of Cav3.1 channel. Therefore, acute ST101 treatment in the

hippocampal slices failed to potentiate the hippocampal long-term potentiation (LTP), whereas SAK3 treatment significantly enhanced the hippocampal LTP. The LTP enhancement by SAK3 was associated with an elevation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity in the hippocampal slices. We also confirmed that acute SAK3 administration enhanced hippocampal acetylcholine (ACh) release in olfactory bulbectomized (OBX) mice. Consistent with these observations, acute administration of SAK3 improved impairments of memory-related behaviors in OBX mice. The enhanced ACh release via T-VGCC is closely associated with CaMKII activation in the hippocampus, thereby improving cognitive impairments in the Alzheimer disease-like models. Taken together, SAK3, cognitive enhancer acting on T-VGCC can be a novel candidate of therapeutics for Alzheimer disease.

Disclosures: **Y. Yabuki:** None. **M. Wakamori:** None. **K. Fukunaga:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.22/SS31

Topic: F.02. Animal Cognition and Behavior

Support: Deanship of Research, Jordan Uni of Sci and Tech

Title: The effect of waterpipe tobacco smoke exposure on learning and memory functions

Authors: *K. H. ALZOUBI[1], O. F. KHABOUR[2], E. A. AL HARAHSHAH[2]
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Abstract: Tobacco smoking is a global health hazard that kills about 5 million peoples annually. Cigarette smoking and waterpipe smoking are the most popular methods of tobacco consumption. Waterpipe smoking is spreading noticeably especially among youth. In this study, we investigated if waterpipe smoking impairs learning and memory in the hippocampus. Additionally, possible molecular targets for expected learning and memory impairment were determined. Animals were divided into two groups; waterpipe tobacco smoke, and control. The waterpipe tobacco smoke group was exposed to smoke from the waterpipe by whole body exposure system 1hr, 5 days/wk, for one month. Behavioral studies were conducted to test the spatial learning and memory using the Radial Arm Water Maze (RAWM). Additionally, hippocampal BDNF protein levels and oxidative stress biomarkers (catalase, GPx, SOD,

TBARS, GSH, GSSG, GSH/GSSG ratio) were assessed. The result revealed that waterpipe tobacco smoke impaired short- and long- term memories. Moreover, result showed that waterpipe tobacco smoke induced reduction in hippocampal activity of catalase, SOD, GPx, GSH, and GSH/GSSG. The GSSG was significantly increased in waterpipe tobacco smoke group. No effects on the levels of BDNF or TBARS were observed. In conclusion, waterpipe tobacco smoking exposure induces memory impairment, which was correlated with significant changes in oxidative stress biomarkers.

Disclosures: K.H. Alzoubi: None. **O.F. Khabour:** None. **E.A. Al Harahshah:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.23/SS32

Topic: F.02. Animal Cognition and Behavior

Title: A negative allosteric modulator selective for the NR2b subtype of NMDA receptors impairs cognition in cynomolgus monkeys

Authors: *M. R. WEED[1], M. BOOKBINDER[1], J. POLINO[1], D. KEAVY[1], R. N. CARDINAL[3,4], J. SIMMERMACHER-MAYER[2], F.-N. LUAN[2], L. J. BRISTOW[1]
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Abstract: Antidepressant activity of NMDA antagonists and negative allosteric modulators (NAMs) has led to increased investigation of their behavioral pharmacology. One well-characterized effect of non-selective NMDA antagonists is impaired cognition in multiple species. A few cognition studies with subtype-selective NAMs have reported mixed results with NR2b NAMs in rodents including increased impulsivity, no effect on cognition or even improvement of some cognitive tasks. In contrast, only one study of cognition in nonhuman primates was found using a selective NR2b NAM administered directly into the dorsolateral prefrontal cortex of macaques, and the compound impaired working memory performance [Wang et al., 2013, Neuron 77(4) 736-749]. The current study tested a selective NR2b NAM, CP 101,606 in two tests of cognition in cynomolgus monkeys. CP101,606 produced a selective impairment in memory in the NHP CANTAB list delayed match to sample (list-DMS) task.

Doses of CP 101,606 at 1, 3, 5.6 and 10 mg/kg impaired performance on trials with 20 and 45 second delays without affecting performance on trials with 2 sec delays. List-DMS impairment waned with longer pretreatments and lower plasma exposure with performance after 1 mg/kg returning to vehicle levels after 5 hours. In a separate cohort, CP 101,606 impaired performance on NHP CANTAB visuo-spatial Paired Associates Learning (vsPAL) test. vsPAL performance was impaired after 1 mg/kg on both initial accuracy (memory) and eventual accuracy (learning) components of the task with selective impairment of more difficult conditions. Whether selective NR2b NAMs impair cognition in humans has not been well studied, although supra-therapeutic doses of CP 101,606 have been reported to produce adverse events consistent with impaired cognition. Additional clinical studies should address this aspect of NR2b NAM pharmacology. The results of these studies clearly show that systemic administration of selective NR2b antagonists can cause transient cognitive impairment in multiple cognitive domains.

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Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.24/SS33

Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 grant (1-R01GM084979-01, 3R01GM084979-02S1.)

March of Dimes Birth Defects (12-FY08-167),

Title: Long term dantrolene treatment reduced intraneuronal amyloid in Alzheimer triple transgenic mice

Authors: ***H. WEI**[1], Z. WU[1,2], B. YANG[1,3], C. LIU[1], M. ECKENHOFF[1], W. LIU[4], S. PICKUP[4], Q. MENG[1], Y. TIAN[2], S. LI[3], G. LIANG[1]
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Abstract: Our previous study suggested that early dantrolene treatment reduced amyloid plaque burden and nearly abolished memory and learning loss in a triple transgenic Alzheimer's disease (3xTgAD) mouse model. In this study, we further investigated the long term treatment of dantrolene on amyloid pathology in the brain and on cognitive function. Fifteen month old 3xTg-AD mice and wild type controls were treated with oral dantrolene at 5 mg/kg or vehicle control twice per week for 6 months. Learning and memory were examined using the Morris Water Maze at 21 and 22 months of age. After the behavioral testing, hippocampal and cortical brain volumes were calculated with magnetic resonance imaging and motor function was evaluated using the rotorod. . The amyloid burden in the hippocampus was determined using immunohistochemistry. We found that dantrolene significantly decreased intraneuronal amyloid accumulation in the hippocampus. No significant differences could be detected in hippocampal or cortical brain volume, motor function or in cognition among all experimental groups, indicating that the mice were still pre-symptomatic for AD. Thus, long term dantrolene treatment significantly decreased the amyloid burden in aged 3xTg-AD mice prior to significant changes in brain volume, or cognition.

Disclosures: H. Wei: None. **Z. wu:** None. **B. yang:** None. **C. liu:** None. **M. Eckenhoff:** None. **W. liu:** None. **S. Pickup:** None. **Q. Meng:** None. **Y. Tian:** None. **S. Li:** None. **G. Liang:** None.

Poster

265. Learning and Memory: Pharmacology I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 265.25/SS34

Topic: F.02. Animal Cognition and Behavior

Title: Interferon-β1a-induced effects on memory and depression in model animals

Authors: *A. VAZQUEZ-ALVAREZ[1], D. PINEDA-VÁZQUEZ[2], E. LEON-CRUZ[2], I. TIBURCIO-DOMINGUEZ[2], R. MIRELES-LOPEZ[2], R. VAQUERO-GARCIA[2], C. REYES-VAZQUEZ[1]
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Abstract: Both, neurotransmitters and cytokines affect their target cells through surface receptors and also by other molecular mechanisms. Cytokine receptors are present in neurons and glial cell populations in discrete brain regions. Moreover, the role of several cytokines in

hippocampal physiological processes, such as memory and learning, and their pathological involvement of cytokines in diseases like depression and epilepsy had been described. Then, interferon α; and some interleukins had effects on long-term potentiation (LTP) in the hippocampus. And some cytokines has a negative regulatory role in long-term memory acquisition. The most commonly used cytokine for the treatment of multiple sclerosis (MS) is interferon β−1a (IFNβ−1a); however, a common perception is that IFNβ−1a treatment is likely to increase memory alterations and depression during the course of MS treatment.To assess such perception we tested the effects of the chronic application of IFNβ−1a on memory, using an active avoidance test, and in inducing depression with the helplessness forced-swim. We used male Wistar rats of 100-120 g, kept according the SfN's Policies on the Use of Animals and Humans in Neuroscience Research. Rats received saline (SSI) or IFNβ−1a, 1 x 106 IU/kg subcutaneously during 7 days. In day 16 animals were trained in a standard active avoidance chamber with 2 compartments. Once animals crossed from a dark to a light compartment they receive three aversive stimuli (electric shock, 60 mV, 3s duration every min for 3 min). Animals were tested after 24, 48, 72 and 168 h the last subcutaneous administration. SSI rats significantly prevented ($P < 0.05$ Z test) aversive stimulus compared with those who received IFNβ−1a. In another experiment, rats were introduced in a water tank (20 l) at 20 °C, and its locomotor activity measured by an electronic device connected to a computer. In this experiment there were significant differences between the control and the treated subjects ($p<0.01$). Some side effects caused by the administration of IFNβ−1a were observed in rats as rhinorrhea, sneezing, loss of hair on the head region, diarrhea and weight loss. Studies in animals have demonstrated that acute activation of cytokines signaling in the brain in response to peripheral immune activation is associated with deficits in hippocampal dependent memory such as contextual fear conditioning. The present findings suggest that cytokines, such as IFNβ−1a, is able to activate neuronal circuits and neurotransmitters that organize physiological and pathological behaviors.

Disclosures: **A. Vazquez-Alvarez:** None. **D. Pineda-Vázquez:** None. **E. Leon-Cruz:** None. **I. Tiburcio-Dominguez:** None. **R. Mireles-Lopez:** None. **R. Vaquero-Garcia:** None. **C. Reyes-Vazquez:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 266.01/SS35

Topic: F.02. Animal Cognition and Behavior

Title: Multi-modal spatial learning and memory in the split-brained cockroach, Periplaneta americana

Authors: *M. POMAVILLE, D. D. LENT
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Abstract: The American cockroach, Periplaneta americana, with its large brain and demonstrated robustness to physiological manipulation, holds many advantages when exploring neural underpinnings of learning and memory. Understanding how neural circuits support various aspects and types of learning and memory is a tremendous challenge. It is often more efficient to experiment on animals such as the cockroach, to gain a basic understanding of how form follows function in neural systems. These experiments examine the interaction of multi-modal sensory acquisition, motor control, learning and memory systems and how they are organized in the brain of the cockroach. The antennal projection response (APR) is an established paradigm to explore learning and memory in the cockroach. It has been used to explore the neural basis of associations between olfactory and visual space and how this spatial encoding is integrated with motor, tactile, and proprioceptive cues. A series of experiments, each examining a different element of learning or memory in the cockroach, were performed using the APR in restrained cockroaches. These experiments were carried out on both intact brain animals, and animals which had a procedure done to divide the halves of the brain. The ability of the cockroach to learn a simple association of a light paired with an odor with sensory stimuli has been demonstrated in the split-brain cockroach, but more complex forms of learning and memory have not. Experiments here revealed multiple elements underlying complex spatial learning behavior, including, the role of unilateral and bilateral sensory acquisition and learning, memory localization within the brain hemispheres, memory transfer and interference, and the interaction of neural systems. The split-brain cockroach was developed to explore the neural basis of learning and memory. The split-brain strategy provides an obvious advantage in that it gives experimental tissue from one half of the brain and control (naive) tissue from the other half of the same brain. Neural tissue was extracted from trained cockroaches and analyzed. This analysis allowed for the examination of protein and structural changes in the brain associated with various learning and memory paradigms. The usage of split-brain animals in these experiments provided both a control and experimental specimen in one individual.

Disclosures: **M. Pomaville:** None. **D.D. Lent:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 266.02/SS36

Topic: F.02. Animal Cognition and Behavior

Support: IPI grant BB/1000313/1

Title: Effects of field relevant concentrations of imidacloprid and clothianidin on bee neuronal function

Authors: *C. MOFFAT, M. J. PALMER, C. N. CONNOLLY
Neurosci., Dundee Univ., Dundee, United Kingdom

Abstract: Neonicotinoids are nicotinic acetylcholine receptor agonists displaying high affinity in insects. Worldwide, these insecticides are used in veterinary products and as agricultural pesticides. Their high affinity and specificity to insects has underpinned their commercial success but there are increasing concerns regarding their effects on important pollinators. While there is mounting evidence that sub-lethal doses of neonicotinoids can have detrimental effects on bees; there is continuing controversy over the precise levels of pesticides to which bees are exposed in the field and stemming from this the levels that reach bee brains. This study investigated the levels of imidacloprid: the archetypal neonicotinoid reaching bee brains upon environmentally relevant levels of exposure and the effects of concentrations within this range on neuronal health and function. Feeding experiments were conducted with *Apis melliferra* and *Bombus terrestris* using food laced with environmentally relevant levels of imidacloprid. Experiments revealed low levels in the brains of both species that were sufficient to cause rapid neuronal damage in *Bombus terrestris* only. Chronic exposure of cultured *Bombus* Kenyon cells to low (nM) imidacloprid produced vulnerability to normally sub-toxic insults. Electrophysiological recordings from *Bombus* Kenyon cells revealed that sub-nanomolar concentrations of clothianidin had an apparent desensitising effect on nicotinic acetylcholine receptors. In conclusion, exposure of bees to field-relevant levels of neonicotinoids may result in brain concentrations that affect neuronal function.

Disclosures: C. Moffat: None. **M.J. Palmer:** None. **C.N. Connolly:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 266.03/SS37

Topic: F.02. Animal Cognition and Behavior

Support: WFU CMCS Pilot Grant to BVN & SEF

Title: Synaptic correlates of performance on an ecologically relevant visual discrimination task in the adult honey bee mushroom body

Authors: *B. N. VAN NEST, G. S. MARRS, S. E. FAHRBACH
Biol. Dept., Wake Forest Univ., Winston-Salem, NC

Abstract: The mushroom bodies (MBs) are paired regions of the arthropod brain associated with sensory integration and memory formation. The dendritic arborizations of the intrinsic MB neurons (the Kenyon cells) are in the calycal neuropil, where they receive sensory input from primary sensory neuropils and reward input from the subesophageal ganglion. Calycal neuropils are larger in honey bee foragers than in non-foragers, and generalist-foraging insect species tend to have larger MBs than specialist-foraging species, suggesting that larger neuropils facilitate learning of complicated foraging tasks. Prior studies have focused on the experiences that promote MB growth and the cellular processes that cause this growth. The consequences of MB growth for an individual, however, are largely unexplored. Experience clearly fine-tunes brain structure in animals, but how does this feed forward onto behavior? How does an insect benefit from enlarged MBs? We developed a challenging, field-based, visual discrimination task that mimics natural foraging behavior. Free-flying honey bee foragers from a field hive were naturally recruited by nestmates to an artificial flower patch, where they learned to associate the hue of an artificial flower with a sucrose reward. The patch contained 24 randomly arranged flowers, all identically scented with peppermint to aid in recruitment. Six flowers (25%) were painted one shade of green and contained a sucrose reward. The remaining 18 flowers (75%) were painted a similar shade of green and remained empty. The rewarding shade was alternated each day of the experiment but remained consistent throughout each bee's training. Each forager was allowed 12 visits to the flower patch, and total activity was closely monitored on all visits. Each bee was scored on how quickly the color/reward association was learned. This task revealed 3 behavioral cohorts: fast-learners (30% of those trained) learned the association in fewer than 6 visits; slow-learners (30%) took at least 6 visits to learn the association; and non-learners (40%) never learned the association. Learning scores were correlated with synaptic density in the visual region of the MB calyx (the collar), assessed by whole-brain immunolabeling for synapsin-1 (a synaptic vesicle-associated protein) and confocal microscopy.

Disclosures: B.N. Van Nest: None. **G.S. Marrs:** None. **S.E. Fahrbach:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 266.04/SS38

Topic: F.02. Animal Cognition and Behavior

Support: BMBF Grant 01GQ0941

Title: Dynamic levels of phosphorylated Apis mellifera CREB in inner compact cells of the honeybee mushroom bodies and their role in the stimulus responsiveness of an animal

Authors: *D. EISENHARDT, K. GEHRING
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Abstract: The transcription factor cAMP-response element-binding protein (CREB) plays a role in the excitability of neurons and in long-term memory (LTM) formation. Phosphorylation of CREB by protein kinase A (PKA) enables the binding of co-factors such as the CREB binding protein (CBP) to CREB, thereby inducing CREB-dependent transcription. An increase of phosphorylated CREB (pCREB) is regarded as an indicator of transcriptional activation by CREB. CREB has been isolated from several insect species and plays a crucial role in LTM formation in fruit flies. Little is known about the role of CREB in insect behavior and the localization of respective CREB-dependent processes in the insect brain. In this study, quantitative western blot analysis and immunohistochemistry were used to characterize the localization of Apis mellifera pCREB (pAmCREB) in the honeybee brain and explore its functional role in behavior. Prominent Anti-pCREB immunoreactivity (IR) was found in the inner compact cells (IC), a particular Kenyon cell (KC) population in the mushroom bodies (MBs) receiving multimodal sensory input. The amount of pAmCREB in the central brain and the IC was affected by age but was not modified upon olfactory classical conditioning or unpaired odor and sucrose presentations. However, bees of the unpaired group that showed responses to an odor upon the presentation of a reward had increased pAmCREB levels in the central brain and the IC, in contrast to bees that did not respond to the odor at all. Thus, pAmCREB levels in the IC might play a role in reward-related behaviors.

Disclosures: D. Eisenhardt: None. **K. Gehring:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 266.05/SS39

Topic: F.02. Animal Cognition and Behavior

Title: Cell polarity protein scribble is required for forgetting of olfactory memories

Authors: *I. CERVANTES-SANDOVAL, R. L. DAVIS
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Abstract: Forgetting of continuously acquired memories is crucial for proper function of the memory system. Nevertheless, little is known about the molecular, cellular and circuit basis of memory forgetting. Here we showed that Scribble, a scaffolding protein known mainly for its role as a cell polarity determinant, is required for normal forgetting of olfactory memories in *Drosophila*. Knocking down Scribble in either dopaminergic or mushroom body neurons, important constituents of the learning and forgetting circuits, impairs normal memory loss. Additionally, epistasis experiments showed that Scribble works in line with dopamine signaling, previously described to regulate active forgetting. Functional imaging experiments demonstrated that this memory loss impairment is due to miscommunication between dopaminergic and mushroom body neurons. These observations indicate that Scribble is a new molecular component required for the normal propagation of the forgetting signal.

Disclosures: I. Cervantes-Sandoval: None. **R.L. Davis:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 266.06/SS40

Topic: F.02. Animal Cognition and Behavior

Support: RFBR

Council for grants of RF President

Russian Academy of Sciences

Title: PKMzeta is necessary for maintaining the long-term facilitation of glutamatergic synaptic inputs but not of somatic glutamate responses in the snail neurons

Authors: *P. M. BALABAN, M. LEMAK, T. KORSHUNOVA, M. ROSHCHIN, A. ZUZINA, A. TIMOSHENKO, A. MALYSHEV
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Abstract: In behavioral experiments it was shown previously that selective inhibitor of PKMζ Zeta Inhibitory Peptide (ZIP) impairs aversive context memory in terrestrial snail Helix lucorum. Long-term facilitation of excitatory (presumably glutamatergic) synaptic inputs from sensory neurons to giant premotor interneurons triggering tentacle withdrawal is supposed to be a basis of aversion learning and memory in terrestrial snails. We investigated whether PKMzeta takes part in maintenance of long-term facilitation in neural circuit underlying aversive tentacle withdrawal. Long-term facilitation of excitatory synaptic inputs to premotor interneurons was induced by high-frequency stimulation combined with 5 serotonin bath applications and lasted at least four hours. We found that bath application of 2*10-6 M ZIP at 90 min after tetanization reduced EPSP amplitude almost to the non-tetanized EPSP values. Application of scrambled ZIP peptide at the same concentration didn't affect the EPSP amplitude in comparison with saline or scrambled ZIP applications. Results support the idea of PKMζ involvement in post-induction maintenance of long-term synaptic plasticity in CNS of Helix. It was shown previously that repeated (1 per min) pressure applications of glutamate on the somatic membrane ("artificial synapse") of the giant premotor interneurons triggering tentacle withdrawal in the snail elicit local potentials that can be facilitated for hours by 5 2-min serotonin applications (Balaban et al.. Eur J Neurosci. 2004 Jan; 19(2): 227-33). We repeated similar experiments under ZIP/scrZIP added 120 min after the facilitating procedure. Obtained results showed that in conditions of "artificial synapse", facilitated responses to glutamate were not influenced by ZIP or scrZIP application. These results suggest that PKMzeta is not involved in postsynaptic plasticity of somatic glutamate responses in the snail neurons.

Disclosures: **P.M. Balaban:** None. **M. Lemak:** None. **T. Korshunova:** None. **M. Roshchin:** None. **A. Zuzina:** None. **A. Timoshenko:** None. **A. Malyshev:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 266.07/SS41

Topic: F.02. Animal Cognition and Behavior

Support: NIH R37 NS19904

Title: miR-iab-8(4as)-3p is necessary during development in the αβ and α'β' mushroom body neurons for optimal learning and memory retention

Authors: *G. U. BUSTO, T. GUVEN-OZKAN, R. L. DAVIS
NEUROSCIENCE, THE SCRIPPS RESEARCH INSTITUTE, JUPITER, FL

Abstract: MicroRNAs (miRNAs) are small non-coding RNAs involved in the post-transcriptional regulation of gene expression. A compelling sum of data indicates a major role for those molecules in biological processes as diverse as development and tumorigenesis. Their pivotal role in organismal development as orchestrator of gene expression made them likely actors in neurodevelopment. We recently screened 134 individual miRNAs for functional involvement in *Drosophila* melanogaster memory using 'sponges' to inhibit their activity in the nervous system. Among the miRNAs identified, several were necessary during development for normal adult memory formation. We find that miR-iab-8(4as)-3p is necessary during development specifically in the αβ and α'β' mushroom body neurons. Inhibition of miR-iab-8(4as)-3p limits the formation of anesthesia-sensitive memory but does not alter odor or shock perception using an olfactory classical conditioning assay. Protein-synthesis dependent long-term memory is also impaired. Four potential target genes have also been investigated. These results highlight the role of a new miRNA that may be central for the development of mushroom body neurons, neurons critical for memory formation. More broadly, our results may offer new insights into the molecular mechanisms altered in the neurodevelopmental disorders that impair learning and memory.

Disclosures: G.U. Busto: None. **T. Guven-Ozkan:** None. **R.L. Davis:** None.

Poster

266. Invertebrate Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH GRANT DC005784

O'Neil Charitable Trust

Title: Neural pathways routing early memory decay in *Drosophila*

Authors: *Y. SHUAI[1], A. HIROKAWA[1], Y. AI[1], W. LI[2], Y. ZHONG[1]
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Abstract: Memory fades as time passes by. Emerging evidence suggests that memory decay is subserved by an "organized" active removal process. The *Drosophila* early olfactory aversive memory has been a leading model in studying this so-called "active forgetting", where small G-protein Rac and dopamine were found to be key regulatory signals. The existing data also suggest the mushroom body (MB) as an important brain locus for early memory forgetting. However, it is yet to be known at the MB micro-circuit level how neurons participating in forgetting segregate from and interact with those in memory. Here, we performed an extensive characterization of the MB circuits in forgetting. We first further delimited Rac function to the γ lobe subset of MB intrinsic neurons, which send parallel axons only to the MB medial lobes. To understand the involvement of MB extrinsic neurons (MBen), we then performed a behavioral screen of ~40 relatively specific MBen Gal4 drivers, which together cover the majority of MBen connected to the medial lobes. The screen uncovered two population of MBen that bi-directionally regulate early memory decay when inactivated and hyper-activated. The blockade of them does not affect learning, suggesting that the micro-circuit for forgetting are segregable from those for learning. Moreover, we provide anatomical and physiolgical evidence that they respectively intersect with the MB γ lobe and β' lobe, indicating that they interact with memory circuits of different stages. We propose that a forgetting micro-circuit embedded in the MB network guide early labile memory towards destruction.

Disclosures: Y. Shuai: None. **A. Hirokawa:** None. **Y. Ai:** None. **W. Li:** None. **Y. Zhong:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

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Title: An importin role in long-term memory consolidation

Authors: *Q. LI[1], X. ZHANG[1], X. LIANG[1], L. WANG[1], Y. ZHONG[1,2]
[1]Tsinghua Univ., Beijing, China; [2]Cold Spring Harbor Lab., New York, NY

Abstract: It is believed that importin-mediated nuclear transport is important for long-term synaptic plasticity from invertebrates to mammals. Surprisingly, little is known whether importins participate in long-term memory formation. Here we report that an importin can bi-directionally regulate aversive long-term memory strength in *Drosophila*. We found acutely down-regulation of the importin during consolidation led to LTM defect, while up-regulation led to LTM enhancement. Moreover, this change on LTM strength was confined in mushroom body neurons. Thus, our results demonstrate a critical role of importins in memory consolidation.

Disclosures: Q. Li: None. **X. Zhang:** None. **X. Liang:** None. **L. Wang:** None. **Y. Zhong:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NIH 5R25GM069621-11

USDA NIFA2010-65105-20625

Title: Octopamine's role in sexual behavior

Authors: *A. I. FERNANDEZ, J. LIM, J. JAMES, K.-A. HAN
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Abstract: Octopamine (OA) is a major monoamine neuromodulator in invertebrates. It regulates numerous physiological processes including motivation, pheromone response, olfaction, ovulation, learning and memory. These processes are likely mediated by multiple OA receptors. There are four known OA receptors in *Drosophila*: OAMB, Octβ1R, Octβ2R, and Octβ3R. The goal of our study is to understand the mechanism by which OA regulates sexual behavior. To achieve the goal, we have investigated basal and high order courtship behaviors of the flies deficient in OA biosynthesis or individual OA receptors. For basal activities, we examined a male's behavior towards a female and measured duration and latency of courtship and copulation. We also performed the conditioned courtship test, in which male flies learn to modify their courtship activity after experiencing rejection from mated females. If the rejected males learned well, they will suppress courtship towards a virgin female. When subjected to basal and conditioned courtship assays, the OA receptor mutants, oamb, octb1r and octb2r, exhibited different phenotypes. Studies are in progress to identify specific behavioral components and relevant neural sites that the OA receptors regulate sexual behavior. This study is supported by the RISE NIH 5R25GM069621-11 and USDA NIFA2010-65105-20625 grants.

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Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 266.11/SS45

Topic: F.02. Animal Cognition and Behavior

Title: Behavioral assays to study deficits in spatial cognition in *Drosophila* melanogaster

Authors: *J. APARICIO VALENZUELA, D. HWANG, D. D. LENT
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Abstract: The fruit fly, *Drosophila* melanogaster, has proven to be a useful model organism in the study of many human disease processes and they are commonly used in both genetic experiments and neurological experiments. The most advantageous reason that *Drosophila* makes for a good model organism when looking at neurodegenerative diseases is that around 70% of disease-associated human genes have a fly homolog, which makes research with this organism very feasible. However, there is yet no easy measure of complex behavior and often we can only use basic measures to view decline in behavior and any quantitative measure of cognition in fruit flies is lacking. Most insects, including *Drosophila*, have very good learning

abilities. Experiments here quantified the behavior of flies in a place learning/spatial memory assays in order to elucidate cognitive traits. Using GAL4-UAS and GAL80 temperature sensitive mutants to express ectopic disease associated proteins (e.g. Tau & ABeta42) in specific brain regions in a set of experiments and the use of ion channel insertions in another set of experiments, aspects of spatial cognition in fruit flies were examined. Training flies in a place memory assays provided insight into issues of visual perception, visual learning and short-term memory. The use of long-term probe trials provided better understanding of processes of consolidation and long-term memory. This assay demonstrates variable rates of learning, variable disruption in short-term and long-term memory. These differences were attributed to differential expression of the ectopic proteins in the mushroom bodies and central complex of the flies. These brain regions were found to be individually important in certain elements of perception and learning. However, only when both regions were functionally intact was more complex spatial cognition revealed. This is one of the first studies to focus on cognitive decline in *Drosophila*. This has significance in that it relates to in that overexpression of proteins that cause neurodegeneration and dementia. By learning more about the molecular pathways and the quantitative expression levels of disease associated proteins in the nervous tissue of fruit flies and correlating that with a quantitative measure of spatial cognition, we can further enhance our understanding of neurodegenerative diseases in humans.

Disclosures: J. Aparicio Valenzuela: None. **D. Hwang:** None. **D.D. Lent:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 266.12/SS46

Topic: F.02. Animal Cognition and Behavior

Support: PAPIIT IN204014-3

Title: Sleep pattern and hierarchical organization in Procambarus clarkii

Authors: K. MENDOZA-ÁNGELES[1], N. JIMÉNEZ-MORALES[1], G. ROLDÁN-ROLDÁN[2], *J. HERNANDEZ-FALCON[3]
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Abstract: The main outcome of agonistic interactions is the establishment of a dominance relationship that determines access to resources. In crayfish, smell is the most important sensory cue in agonistic interactions. After several social interactions, crayfish triads establish a hierarchical order with one dominant and two submissive animals (submissive 1, and submissive 2). In a given group, hierarchy is stable when measured in repeated trials. When measured on a daily basis, the intensity of positive interactions (threats, attacks and fights) diminishes, while negative interactions (retreats and avoidances) increase. In sleep-deprived triads, positive interactions raise. The main goal of this work was to investigate the role of sleep deprivation in the establishment and maintenance of hierarchical organization of crayfish triads and its effects on olfactory threshold.We used male adult Procambarus clarkii triads in intermolt. We studied: a) The sleep pattern in isolated animals and when they were submitted to social interaction; b) Agonistic interactions in control and sleep-deprived triads, and c) The behavioral response to olfactory stimulus in control and sleep deprived individuals. Our results indicate that: a) Sleep-patterns in isolated animals are highly variable and dependent on the studied animal. However, when animals are submitted to social interactions, they show a fragmentation of the previous pattern and a reduction in total sleep duration, which are clearer in submissive 2 crayfish. b) As we have previously reported, positive contacts increase in sleep-deprived triads, in amount, intensity and duration of the encounters. We also found that negative contacts increase too. These results indicate that memory recognition among a triad is not impaired but disturbed by sleep deprivation c) Olfactory stimulation with urine from a dominant crayfish produces an escape tail flip in submissive animals. The latency for this response is longer in sleep deprived individuals than in control animals, the longest latencies were for submissive 2 animals. These results imply that submissive animals identify the dominant one through urine signals, and that sleep deprivation impairs olfactory perception.

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Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 266.13/SS47

Topic: F.02. Animal Cognition and Behavior

Support: Florida State University Center for Research and Creativity Grant

Title: Defining the parameters through which sleep deprivation affects the induction of associative memory using *Aplysia* californica

Authors: *L. C. LYONS[1], R. T. WILLIAMSON[2], J. SANCHEZ-PACHECO[2], H. C. KRISHNAN[1]
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Abstract: The induction and formation of memory represents a dynamic process modulated by multiple factors including the circadian clock and sleep. Defining the mechanisms through which endogenous factors target short and long-term memory formation is necessary for identification of approaches to improve memory and to mitigate the toll that modern society exacts upon individuals who are working longer hours or shift-work. Given the high degree of conservation underlying memory formation across species, the marine mollusk *Aplysia californica* with its relatively simple neural circuitry provides an ideal model for detailing *in vivo* interactions between sleep, the circadian clock and memory formation. We recently characterized sleep in *Aplysia* using behavioral criteria for defining invertebrate sleep (Vorster et al., 2014). *Aplysia* sleep appears dually controlled by the circadian clock and homeostatic processes with *Aplysia* sleep occurring solely during the night and rebound sleep occurring after sleep deprivation. Using an associative learning paradigm, learning that food is inedible, in which the animal associates a specific netted seaweed with the inability to swallow, we previously found that 9 hours sleep deprivation starting 3 hours after lights out in 12 h:12 h light-dark cycles was sufficient to block the induction of short and long-term memory. In our current research, we extended these studies to investigate the magnitude and timing of recovery sleep necessary following 9 hours sleep deprivation to restore the induction and formation of memory as well as the duration and timing of sleep deprivation necessary for inhibiting memory. We found that 9 h sleep deprivation persistently inhibited the induction of short and long-term memory for 24 hours following sleep deprivation, whereas similarly handled daytime control animals demonstrated robust STM and LTM. While over-training via additional training sessions was not sufficient to rescue the induction of long-term memory immediately after sleep deprivation, it was sufficient to ameliorate the persistent effects of sleep deprivation rescuing the induction of memory 24 hours after sleep deprivation. The persistent effects of acute sleep deprivation naturally reversed within 48 hours for both short and long-term memory. We also found that 6 hours sleep deprivation either during the first half or last half of the night was sufficient to block the induction of long-term memory. These behavioral studies have established *Aplysia,* with its relatively simple neural circuitry, as a valid model system for studying the interactions between sleep and memory formation.

Disclosures: L.C. Lyons: None. **R.T. Williamson:** None. **J. Sanchez-Pacheco:** None. **H.C. Krishnan:** None.

Poster

266. Invertebrate Learning and Memory I

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Program#/Poster#: 266.14/SS48

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IOS-116987

Title: Robust neural and behavioral sensitization after peripheral, but not central, application of serotonin

Authors: *R. CROOK[1], P. PAREKH[3], M. LERNER[4], J. JO[2], L. KLAASSEN[5], E. T. WALTERS[1]
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Abstract: Sensitization of defensive reflexes in *Aplysia* is a useful model to study fundamental mechanisms related to learning and memory and to injury-related phenomena such as chronic pain. Serotonin (5-HT) has well-studied facilitatory effects on central synapses of nociceptive sensory neurons in *Aplysia* and can also induce hyperexcitability in the soma and peripheral axons of these sensory neurons. However, little is known about the effects of central versus peripheral 5-HT delivery on defensive reflexes or the activity of peripheral axons. We used a reduced preparation of the pleural and pedal ganglia, the p9 nerve and the tail to test the effects of peripherally- and centrally applied 5-HT on reflex sensitization and afferent activity. Intracellular recordings from nociceptors were used to assess the effect of 5-HT application on the p9 nerve. Thresholds were decreased compared to baseline both in the presence of 5-HT and five minutes after washout. During application of 5-HT to a nerve segment with the tail but not the CNS attached, the first application of 5-HT to the nerve produced rapid and sustained increases in activity of unidentified axons in the p9 nerve compared to seawater treatment. Later applications produced progressively smaller increases. In tests where the tail was reversibly blocked by isotonic MgCl2 in nerve wells distal to the 5-HT application site, there was less afferent activity in the presence of 5-HT. After washout, there was no difference in the number of spikes produced by electrical nerve stimulation between 5-HT-treated and control preparations. We tested the effect on the tail withdrawal reflex (TWR) of applying 5-HT to the pleural ganglion, p9 nerve, or tail tissue. 5-HT applied peripherally to either the tail or the nerve had a stable enhancing effect on the TWR apparent within 30 minutes of 5-HT washout, persisting in the intermediate term (2-4 h) and longer term (up to 7 h). In contrast, applying 5-HT

centrally to the pleural-pedal ganglia had no effect on the TWR at any of time points tested. The inability of central 5-HT application to elicit behavioral sensitization combined with the persistence of peripherally-induced sensitization, suggests that 5-HT has complex effects within the CNS, including strong suppressive effects on defensive reflexes. It also points to a large role in long-lasting nociceptive sensitization from 5-HT and other injury-related signals on nociceptor terminals and axons in peripheral tissues.

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Poster

266. Invertebrate Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IOS1120304

Texas Research Development Funds

Constance E. Boone Award to Malacology

Title: Single exposure to natural noxious stimuli produces short-term suppression of feeding in *Aplysia*

Authors: K. D. WOLFE, M. L. WAINWRIGHT, D. L. SMEE, *R. MOZZACHIODI
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Abstract: An increasing body of literature demonstrates that fear carries potent ecological consequences. Extensive work has detailed how prey respond with adaptive behavioral changes to various indicators of predation risk, including predator presence, predator kairomones, and alarm cues released by conspecific. Such behavioral responses include enhancing defensive behaviors like vigilance, defensive reflexes, or escape responses at the expense of non-defensive behaviors like feeding. However, most studies examined defensive behavioral changes during or immediately following cue detection, giving little attention to the persistence of modification in the intermediate or long term. Moreover, it is unclear to what degree and duration non-defensive behaviors, such as feeding, are altered following exposure to various chemosensory alarm cues. Using a well-studied neurobiological model organism, the sea hare *Aplysia californica*, we

sought to characterize the effects of single exposures to natural noxious stimuli on feeding behavior at different time points following exposure. We introduced *Aplysia* to the following stimuli: a natural predator, the lobster *Panulirus interruptus*, sub-lethal attacks and exposure to a novel predator, the blue crab *Callinectes sapidus,* and ink and opaline (alarm cues secreted by attacked *Aplysia* conspecifics). Feeding, quantified by measuring the number of ingestive radula movements (i.e., bites), was measured prior to and 15 min, 2 h and 24 h after exposure to one of the above treatments. Untreated animals served as controls. *Aplysia* attacked by blue crabs, exposed to lobsters, or presented with ink or opaline suppressed feeding between 15 min and 2 h, relative to unexposed animals. *Aplysia* did not suppress feeding following exposure to crabs. No stimulus induced intermediate or long-term changes in biting, as evidenced by the lack of feeding suppression between 2 h and 24 h following exposure. This finding suggests that single exposures to natural cues only produce short-lived behavioral changes, and that *Aplysia* differentiate the potential risk pervaded by various stimulus sources. Ongoing studies are investigating whether repeated exposures to select stimuli can extend feeding suppression for longer periods of time. Future experiments will characterize the neural mechanisms underlying feeding suppression induced by the above stimuli using *in vivo* recordings of nerve activity along the chemosensory pathway and from the motor outputs of the feeding neural circuit.

Disclosures: K.D. Wolfe: None. **M.L. Wainwright:** None. **R. Mozzachiodi:** None. **D.L. Smee:** None.

Poster

266. Invertebrate Learning and Memory I

Location: Halls A-C

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Program#/Poster#: 266.16/SS50

Topic: F.02. Animal Cognition and Behavior

Support: Israel Science Foundation Grant 1379/12

Title: Molecular correlates of long-term memory consolidation after a brief training of *Aplysia* with inedible food at night, with a protein synthesis inhibitor

Authors: R. LEVY[1], *A. J. SUSSWEIN[2,3]
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Abstract: Training *Aplysia* with inedible food for 5 min or longer during the daylight hours leads to protein-synthesis dependent memory when measured 24 hrs later. In three conditions, memory is not produced: 1) when animals are trained at night; 2) when animals are trained in the presence of the protein-synthesis inhibitor anisomycin during the day; 3) when animals are trained for only 3 min during the day. However, a 3 minutes training at night with anisomycin produces memory 12, 24, and 36 hours after training. Long-term memory after training at night is not seen only after a brief training: a longer training also produces memory, provided that animals are treated with anisomycin. Thus, combining 3 variables that each alone blocks memory (brief training, night training, anisomycin) leads to memory. Memory was blocked by treating animals with the transcription inhibitor DRB, indicating that memory formation depends on transcription, and probably also on translation of the transcribed mRNAs when the effects of anisomycin during training wear off. We have examined molecular correlates of memory formation after training for 3 min at night after treatment with anisomycin. Using real-time PCR, changes in expression of a number of mRNAs whose synthesis is associated with memory formation were measured 2 hrs after training. Controls were treated with anisomycin at night. Training caused significant increases in the expression of *Ap*CEB/P, and of *Ap*CREB-1, but not of *Ap*CREB-2. Since both trained and untrained animals were treated with anisomycin, the increased expression of *Ap*CEB/P and *Ap*CREB-1 caused by training could not be attributed to anisomycin induced superinduction. The findings are consistent with the hypothesis that training at night is ineffective in producing memory because training causes the active synthesis of one or a number of proteins blocking memory formation. Anisomycin blocks synthesis of the blocker. In addition, proteins that must be expressed as a result of training during the day are already present at night, thereby allowing the training to produce long-term memory.

Disclosures: R. Levy: None. **A.J. Susswein:** None.

Poster

266. Invertebrate Learning and Memory I

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Program#/Poster#: 266.17/SS51

Topic: F.02. Animal Cognition and Behavior

Support: NIH NS19904

Title: Solute carrier protein is required in the adult mushroom body neurons for the regulation of *Drosophila* memory acquisition and memory loss

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Abstract: Memory acquisition and loss are critical for animals to response properly to constantly changing environments. However, the underlying molecular mechanisms remain largely hidden, especially for memory loss. Until recently, few genes have been demonstrated to function in memory loss, including transcriptional repressors, cytoplasmic protein kinases and phosphatases, and trans-membrane adhesion molecules and receptors. In our current study, a *Drosophila* SLC (solute carrier protein) gene was identified by its function as a memory suppressor gene. RNAi knockdown of SLC in the CNS slowed both memory acquisition and memory loss relative to control flies. Spatial mapping studies identified the *Drosophila* mushroom bodies (MB) as the site for its memory suppressor function. Conversely, SLC overexpression in MB significantly accelerated memory loss. The memory enhancement phenotype occurring from SLC knockdown was completely rescued by a SLC overexpression transgene. Immunohistochemistry studies revealed broad expression of SLC expression in CNS but enhancement in the mushroom body dendritic area. Biochemical studies pursued by expression of *Drosophila* SLC in HEK 293 cells and fly primary neurons revealed the SLC transports L-carnitine, acetylcholine, betaine, dopamine, and histamine. Ongoing dietary supplementation experiments are establishing the relationships between different substrates and the SLC-related memory phenotype. Overall, this research provides insights into the nature of memory suppressor systems.

Disclosures: Y. Gai: None. **Z. Liu:** None. **M. Chakraborty:** None. **R. Davis:** None.

Poster

267. Motivation and Reward

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Support: NIH Grant MH63649

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NIH Training Grant DC00011

Title: Two cortical hedonic hotspots: Orbitofrontal and insular sites of sucrose ‘liking’ enhancement

Authors: *D. C. CASTRO, N. S. CHESTERMAN, M. K. H. WU, K. C. BERRIDGE
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Abstract: Over the last decade, the affective taste reactivity test, which measures the affective orofacial reactions to taste stimuli, has helped identify three subcortical "hedonic hotspots", or sites in which hedonic reactions can be neurochemically amplified, in the rat: one in nucleus accumbens, one in ventral pallidum, and one in the brainstem parabrachial nucleus. However, whether or not there are any cortical sites capable of generating a hedonic enhancement is still unknown. Neuroimaging work in humans has indicated two potential cortical sites especially code hedonic experiences of pleasant foods: orbitofrontal cortex (OFC) and insular cortex (IC). To determine whether these cortical areas contain causal hedonic hotspots, we performed targeted microinjections of the mu opioid receptor agonist DAMGO, or of the orexin receptor agonist Orexin-A in either OFC or IC before recording affective orofacial 'liking' reactions elicited by oral infusions of sucrose or quinine solutions. We found that injections of DAMGO or orexin into any mediolateral site in the rostral half of OFC enhanced positive hedonic reactions to sucrose, without altering disgust reactions to quinine. In contrast, any mediolateral microinjection site in the caudal half OFC actually suppressed hedonic reactions to sucrose (i.e., a hedonic coldspot). For motivation however, mu or opioid stimulation enhanced palatable food intake at all sites throughout OFC, both rostral and caudal. In insula, we found a hotspot in posterior IC (e.g., sensory/visceral IC) where both opioid or orexin agonist microinjections enhanced sucrose 'liking' reactions. Oppositely, we found a caudal coldspot in anterior IC (i.e., associated with disgust) where opioid or orexin microinjections suppressed hedonic reactions to sucrose. Altogether, these data support the existence of two novel hedonic hotspots in the cortex, one in rostral OFC, and one in caudal IC, and also identify suppressive cortical coldspots.

Disclosures: D.C. Castro: None. **N.S. Chesterman:** None. **M.K.H. Wu:** None. **K.C. Berridge:** None.

Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: NIH Grant DA015188

NIH Grant MH63649

Title: Optogenetic VTA dopamine stimulation supports motivated 'wanting' for a reward but does not enhance hedonic 'liking'

Authors: *M. J. ROBINSON[1], D. C. CASTRO[2], K. C. BERRIDGE[2]
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Abstract: Optogenetic stimulation (Channelrhodopsin) of ventral tegmental area (VTA) dopamine neurons in TH-Cre transgenic rats has been implicated in self-stimulation, but whether this same stimulation can enhance the rewarding properties of an external sensory reward such as sweet food remains unknown. First, we confirm that TH-Cre dopamine neuron activation supports self-stimulation, showing rats would touch a metal spout and also learn to go to a specific location to earn VTA laser stimulation. We further show that VTA stimulation causes enhancement of motivation to earn an external sucrose reward by increasing breakpoint in a progressive ratio task, and additionally amplifies and narrows motivated choice by making a rat intensely prefer to earn a VTA laser-paired sucrose pellet over an otherwise equal sucrose pellet alternative reward. The preference induction was even strong enough to cause a normally less desirable salt pellet to become preferred over sucrose. Finally, however, we show using the affective taste reactivity test, that dopamine laser stimulation in VTA DA neurons and in specific projections to the nucleus accumbens, fails to enhance hedonic 'liking' reactions to sucrose, even in the same rats that it made 'want' sucrose more. Altogether, these data implicate VTA dopamine in incentive motivation, but not hedonic impact, and help extend understanding of dopamine and reward.

Disclosures: M.J. Robinson: None. **D.C. Castro:** None. **K.C. Berridge:** None.

Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: NIDA Grant DA015188

NIH Grant MH63649

Title: Optogenetic stimulation of central amygdala magnifies motivation and controls choice for cocaine reward

Authors: *S. M. WARLOW[1], M. J. F. ROBINSON[2], K. C. BERRIDGE[1]
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Abstract: Normal motivation requires making choices among competing incentives. Pathologies such as drug addiction can be characterized by distortion of choice, and excessive desire focused inappropriately on one incentive at the expense of others. The amygdala has been implicated in assigning focused incentive motivation to a particular target on the basis of learned associations. We have previously reported that optogenetic ChR2 stimulation of the central nucleus of the amygdala (CeA) severely biases choice for a paired sucrose pellet over another identical sucrose pellet (Robinson & Berridge, 2014). Can CeA optogenetic stimulation also bias choice for earning one cocaine reward above others? Here we optogenetically stimulated CeA to create a focused pursuit of an arbitrarily designated cocaine reward. Rats were given a choice between earning two identical cocaine infusions, but only one cocaine option was accompanied by CeA laser stimulation. Rats intensely pursued the cocaine infusion accompanied by CeA laser stimulation while ignoring the otherwise identical cocaine infusion that had no laser. Furthermore, CeA stimulation amplified motivation to pursue cocaine in a progressive ratio test. Rats exhibited higher breakpoints for cocaine reward when accompanied by CeA laser stimulation than when cocaine was earned alone. Finally, this CeA amplification and narrowing of pursuit was not due to simple CeA reward effects, as rats did not self-stimulate in CeA nor did CeA laser induce a place preference. We propose that ChR2 stimulation of CeA can intensify and narrow the focus of incentive motivation for cocaine reward, just as it does for a natural sucrose reward.

Disclosures: S.M. Warlow: None. **K.C. Berridge:** None. **M.J.F. Robinson:** None.

Poster

267. Motivation and Reward

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Title: Stimulation of dopamine D3 receptors attenuates the expression of pavlovian conditioned approach responses and motivation for incentive cue presentation

Authors: *K. M. FRASER[1,2], J. L. HAIGHT[3], S. B. FLAGEL[4,1]
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Abstract: Cues in the environment can guide behavior in adaptive ways, bringing one in close proximity to valuable resources (e.g. food, water, sex). However, some cues, specifically those that attain incentive salience, can gain inordinate control over behavior and direct actions in a maladaptive manner, as is evident in addiction and other disorders of impulse control. Reward cues acquire incentive salience via Pavlovian learning processes. Although a role for dopamine has been identified in these processes, it is still unclear as to which receptors are involved. Here we investigated the role of the dopamine D3 receptor in the attribution of incentive salience to discrete reward cues. To do this, we utilized an animal model that allows us to parse the neurobiological processes underlying the attribution of incentive vs. predictive value to a discrete cue paired with food-delivery. Outbred Sprague-Dawley rats were first characterized as sign- or goal-trackers based on 7 days of Pavlovian conditioning wherein brief presentation of a lever-cue was paired with delivery of a food reward. Sign-trackers attribute incentive salience to the reward-paired cue, as measured by interaction with the cue upon its presentation. In contrast, goal-trackers use the reward-paired cue merely as a predictor of reward delivery and upon its presentation orient behavior accordingly towards the food cup (i.e. the goal). Following acquisition of these respective conditioned responses, we used a within subject design to determine the role of the D3 receptor in the expression of sign- and goal-tracking behaviors. Increasing doses (0.01-0.32 mg/kg) of 7-OH-DPAT were administered and compared to the effects of vehicle injections on alternating days. We found that stimulation of the dopamine D3 receptor attenuates the expression of both of sign- and goal-tracking behavior. There was a dose-dependent effect on sign-tracking behavior, with the largest effects in response to the two highest doses of drug. Interestingly, goal-tracking was attenuated only at the lowest drug doses. To further examine the effects of D3 stimulation on the attribution of incentive salience to a discrete reward cue, we examined the effects of 7-OH-DPAT on the conditioned reinforcing properties of the lever-cue. Administration of the D3 agonist significantly attenuated the motivation to work for presentation of the lever-cue in sign-trackers, but not goal-trackers. This work highlights a role for the D3 receptor in the expression of Pavlovian conditioned approach responses and in the attribution of incentive salience to discrete reward cues.

Disclosures: K.M. Fraser: None. **J.L. Haight:** None. **S.B. Flagel:** None.

Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: HHMI JFRC Graduate Scholar Program

Howard Hughes Medical Institute

Title: A role for negative reinforcement in AGRP neuron-mediated instrumental food seeking

Authors: *Z. HUANG CAO[1,2], J. BETLEY[1], S. M. STERNSON[1]
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Abstract: Agouti related peptide (AGRP) neurons in the arcuate nucleus of the hypothalamus are sufficient to elicit avid food seeking and consumption behaviors. Recent data indicates that these homeostatic neurons transmit a negative reinforcement teaching signal in which increased AGRP neuron activity evokes an unpleasant internal state and reduction of AGRP neuron activity in hunger is reinforcing. One implication of AGRP neuron-mediated negative reinforcement is that, over multiple sessions, elevated food-seeking performance of instrumental actions is expected to require a decline in AGRP neuron activity. To test this, we used cell-type specific optogenetic manipulations of AGRP neuron activity during and following an operant task. Under food restriction, animals maintained high levels of lever pressing for food on a 15-day progressive ratio 7 schedule of food pellet reinforcement. Mice trained under food restriction but then re-fed ad libitum initially showed similarly elevated lever pressing during AGRP neuron photostimulation. However, subsequent tests with this protocol resulted in a progressive decline in lever pressing for food. Disruption of AGRP neuron-mediated negative reinforcement of instrumental food seeking was most sensitive to high effort motivated responding. These experiments indicate that elevated AGRP neuron activity during and following instrumental responding for food reduces the level of effort expended to seek and consume food, consistent with a role for negative reinforcement in AGRP neuron-mediated food seeking behavior.

Disclosures: Z. Huang Cao: None. **J. Betley:** None. **S.M. Sternson:** None.

Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: NIDA Intramural Research Program

Title: Identifying functional alterations in neuronal ensembles activated during 1-day acquisition of operant learning in rats

Authors: *L. R. WHITAKER, K. B. MCPHERSON, J. M. BOSSERT, Y. SHAHAM, B. T. HOPE
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Learned associations between environmental stimuli and rewards drive goal-directed learning and motivated behavior. These associations are thought to be encoded by specific patterns of sparsely distributed neurons called neuronal ensembles that are determined by the cues and reward. The question remains as to how neurons in these ensembles are functionally altered during learning, and which of these changes encode learned associations. The objectives of our study were to identify ensembles of neurons strongly activated during acquisition of operant learning, and then to determine functional alterations specific to these neuronal ensembles. During two days of magazine training, the Experimental group received food pellets in the magazine paired with a 5-second light cue every 4 minutes. The Control group was placed in the same environment with no light cue or pellets and instead received the same number of pellets in their home cages after the session. On test day, rats in the Experimental group were allowed to lever press for food pellets in the self-administration chambers for two hours with the 5-second light cue paired with each lever press. Rats in the Control group had access to the lever, but lever pressing did not elicit any cues or pellet release. The Experimental group quickly formed an association between lever pressing and food reward, and maintained lever pressing throughout the duration of the session. The Control group pressed significantly less than the Experimental group, usually for a brief duration at the beginning of the session. Immunohistochemical analysis indicates induction of Fos expression in medial prefrontal cortex and nucleus accumbens—two regions critical for the acquisition of goal-directed learning and motivated behavior. In future experiments, we will use *c-fos-GFP* transgenic rats in which strong neuronal activation activates the *c-fos* promoter and drives expression of GFP. Whole cell brain slice electrophysiology will then be used to assess functional alterations in behaviorally activated GFP-labeled neuronal ensembles.

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Poster

267. Motivation and Reward

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NSFC (91132701)

Title: Specific impairments of brain activations in individuals with trait anhedonia: A preliminary study

Authors: *R. C. CHAN[1], Z. LI[1], W.-Z. XIE[2], C. YAN[3]
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Abstract: Objectives: Anticipatory and consummatory dissociation of hedonic experience may manifest as trait anhedonia in healthy and clinical populations. Most studies on trait anhedonia used monetary-based incentive delay paradigms and very few studies have focused on affect- or social-based stimuli. It is still unclear if the underlying neural mechanisms of the monetary-based and affect-based incentive delay paradigms really dissociate from each other. We aimed to examine the similarities and differences between the Affect Incentive Delayed (AID) and the Monetary Incentive Delay (MID) imaging paradigms on brain activation. Method: We administered the AID and the MID tasks to 28 healthy participants (age=18.88+1.81). A cue signaling the type of forthcoming feedback (reward or punishment) was displayed to the participants, followed by a target-hit task with corresponding reward or punishment. All participants were scanned with a 3-Tesla scanner (Siemens 3T-Trio A Tim, Erlangen, Germany). All the participants were also administered the Chapman Social Anhedonia Scale, the Chapman Physical Anhedonia Scale, and the Temporal Experience of Pleasure Scale, and were subsequently clustered into an anhedonic group (n = 8) and a non-anhedonic group (n = 20) according to these scales scores. Results: In the anticipatory phase of the MID, the left substantia nigra, the right caudate body, the right insula, the right medial frontal gyrus, the right parahippocampal gyrus, and the left insula were activated to the positive cue, while the left substantia nigra and bilateral thalami were activated to the negative cue. In the consummatory phase of the MID, the right medial frontal gyrus, the left anterior cingulate cortex, the right

insula and the right precuneus were activated when gaining monetary points, while none of activation was found when losing monetary points. In the consummatory phase of the AID, the right inferior frontal gyrus, the right anterior cingulate cortex and bilateral superior frontal gyrus were activated to the positive pictures, while the right superior temporal gyrus, bilateral medial frontal gyrus and bilateral inferior frontal gyrus were activated to the negative incentives. In the subgroup analyses, the anhedonic group exhibited significant hypoactivation than the non-anhedonic group in the left pulvinar, the left claustrum and the left insula to the positive cue during anticipation of AID. Conclusions: The present study demonstrates that both the AID and the MID tasks have unique and similar activation patterns. Our findings also suggest that the AID task is more sensitive to detect anhedonia in people with trait anhedonia.

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Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: R01MH068073

Title: Improving how we measure motivation by dissociating goal directed responding from arousal induced responding with a novel progressive hold down task

Authors: *M. BAILEY[1], G. JENSEN[2], K. TAYLOR[4], C. MEZIAS[4], C. WILLIAMSON[3], V. MAGALONG[4], R. SILVER[2,4], E. SIMPSON[5], P. BALSAM[4,3,5]
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Abstract: Motivated behavior consists of a goal directed and arousal component. Commonly used measures of motivation such as the progressive ratio schedule (PR) do not dissociate these two processes. For example, amphetamine is known to increase responding in a PR as well as to induce hyperactivity and arousal. We directly address this challenge by developing a novel behavioral assay known as the progressive hold down (PHD) which can in conjunction with a PR schedule dissociate goal directed persistence and arousal. We use methamphetamine (meth) treatment in both the PR and the PHD to show how separating behavior into these two

components of motivated behavior more precisely informs our understanding of how alterations in motivation affect responding. Adult male C57Bl/6 mice received operant lever press training. Mice were then trained to hold the lever in the depressed position to earn reward. We next tested mice on the PHD task, in which each reward required a progressively longer hold of the lever. In this PHD, mice press for longer durations until reaching a breaking point, which ranged from 54 seconds to 235 seconds between subjects. The PHD task is sensitive to motivation manipulations as mice had significantly higher breakpoints when hungry compared to sated mice. Moreover, behavioral output is increased when the reward magnitude is increased. Meth was injected IP at 1.0 mg/kg 20 minutes before behavioral sessions and mice were tested in either the standard PR or the PHD tasks. In the PR, treatment with meth lead to an increased number of lever presses, longer sessions, and more earned reinforcers - all suggesting that goal directed motivation was increased. However, on the PHD task, meth did not alter the breakpoint but did impair the efficiency of responding because of bursts of brief duration responses. Taken together these data indicate that meth acts to increases hyperactivity or arousal rather than enhanced goal directed motivation. Our novel assay allows behavior to be measured in a way which dissociates goal directed motivation from arousal and hyperactivity. This gives us a new tool for dissecting the impact of pharmacological and genetic manipulations on different specific aspects of motivation.

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Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: Bial Foundation 176/10

FCT SFRH / BDINT / 51554 / 2011

Title: Optogenetic self-stimulation of hypocretin neurons

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Abstract: Hypocretinergic (HCRT) neurons, with cell bodies located in the Lateral and Dorsomedial Hypothalamus (LH and DMH), project to the main monoaminergic nuclei in the brain, including the ventral tegmental area (VTA). Through these connections, hypocretinergic neurons closely interact with systems that modulate emotion, the sleep/wake cycle, energy homeostasis and reward. Previous research has shown that animals will self stimulate electrodes implanted in the LH, and that intrinsic LH neurons are necessary for this process. Given the well known relationship between VTA dopaminergic neurons and reward seeking behavior we hypothesized that stimulation of LH HCRT neurons is reinforcing and induces self stimulation. To address this hypothesis we used mice with cre-dependent expression of channel rhodopsin (ChR2) in HCRT neurons, and tested if these mice would engage in self-stimulation of these neurons. ChR2 was delivered to the LH of Cre-HCRT mice using an adeno-associated virus, and optic fibers were implanted above the injection site in order to allow for light-mediated stimulation of HCRT cell bodies. After recovering from surgery these mice were trained in an operant box containing two levers - an active lever that controlled delivery of light pulses through the optic fiber and inactive lever that had no such effect. Dependent on specific stimulation conditions, including the cell infection rate and the laser power, male mice acquired the operant task, with high press rates on the active lever, in order to modulate the activity of HCRT neurons. These results suggest that hypocretin neurons are part of a circuitry that is involved in motivation, and that stimulation of hypocretinergic neurons has reinforcing properties, probably relevant for reward-seeking behavior.

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Poster

267. Motivation and Reward

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nimal Phenotyping Core of the Michigan Diabetes Research Center (NIH Grant P30 DK020572)

Michigan Nutrition Obesity Research Center (DK089503)

Title: The enhancement of cue-induced motivation in obesity-prone vs. resistant rats is accompanied by sensitization to cocaine and increased CP-AMPA receptor expression in the NAc

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Abstract: While the decision to eat is shaped strongly by hunger, satiety, and energy demand, it is also greatly influenced by environmental cues associated with food (food-cues). For example, in humans exposure to food-cues, like a blinking donuts sign, can increase ratings of desire to eat, and the amount of food consumed (Fedoroff et al., 1997, Soussignan et al., 2012). Similarly, in rodents food-cues can elicit approach, reinforce operant responding, and increase food consumption (Kelley et al., 2005; Holland and Petrovich, 2005; Cardinal et al., 2002; Balleine, 2005). Obesity-prone people are more susceptible to these motivational effects of food-cues, and food-cues activate the striatum more strongly in obese vs. non-obese people (Rothemund et al., 2007; Stoeckel et al., 2008). This differential activation may be driven in part by alterations in AMPA receptor (AMPAR) expression as AMPARs provide the main source of excitation to the striatum, play a role in behavioral responses to food-cues, and are increased after exposure to sugar (Crombag et al., 2008; Peng et al., 2011; Tukey et al., 2013). We recently found that rats that are susceptible to diet-induced obesity show enhanced attraction to and motivation for food-cues compared to non-obese rodents fed the same diet. Here, we determined whether mesolimbic function differs in obesity-prone vs. resistant rats prior to and/or after exposure to the junk-food diet. In addition, alterations in striatal AMPAR expression and function were also evaluated. We found that both obese and obesity-prone rats showed a sensitized locomotor response to cocaine compared to non-obese and obesity-resistant rats, consistent with enhanced reactivity of mesolimbic systems. In outbred obese rats, sensitization was seen after "junk-food" diet was removed and rats were given ad lib access to standard lab chow only for 2 weeks, whereas sensitization was evident in selectively bred obesity prone vs. resistant rats without any diet manipulation. In addition, prolonged exposure to a “junk-food” diet followed by a return to standard lab chow for two weeks was associated with a selective increase in surface GluA1 protein expression only in obese rats. This selective increase in GluA1 in obese rats in the absence of changes in GluA2 may be indicative of calcium-permeable AMPARs (CP-AMPAR), which play a role cue-induced drug craving (Loweth et al., 2013; Wolf and Ferrario, 2010). These results are being confirmed by whole-cell patch clamp recordings and will be discussed in light of the potential contribution of enhanced glutamatergic transmission and sensitization of incentive-motivation to obesity.

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Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: NIH Grant NS23805

Title: Activation of ventral pallidum versus lateral preoptic area: double dissociation by threat discounting and locomotion

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Abstract: We have been interested in brain substrates that may subserve an adaptive balance between threat tolerance and reward acquisition. In an earlier study (Soc Neurosci Abst 393.16, 2013), we evaluated the hypothesis that threat tolerance varies directly in relation to locomotor activation elicited by disinhibition of the lateral preoptic area (LPO). That study exploited the innate aversion of rats to bright illumination and their reluctance to enter open spaces (thigmotaxis). We observed that unfasted rats readily entered the field centers of dimly illuminated activity monitors to acquire sweet chocolate pellets (TestDiet, Richmond, IN, cat. #1811256, 45 mg), but almost never took pellets in monitors fitted with overhead spotlights (GE 90W 120V 1260 lumens) aimed at the field centers. However, the willingness of rats to consume pellets in spotlighting was marginally, but significantly, increased by stimulating locomotion with LPO infusions of bicuculline (bic), a GABA A receptor antagonist. In the present study, the experiment was repeated with the infusions targeted to the ventral pallidum (VP), which activate locomotion less vigorously than the LPO infusions (Brain Struct Funct 219:511-26, 2014). In contrast to the LPO study, in which the increase in threat tolerance observed following LPO activation was slight, rats receiving bic infusions into the VP essentially disregarded the presumed threat of spotlighting, acquiring as many sweet pellets in the spotlit as in the dimly lit control condition. In contrast, the numbers of pellets ingested in dim lighting by rats receiving VP bic infusions were no greater than the numbers ingested by the vehicle-infused controls, and, insofar as neither the bic-infused rats nor the vehicle-infused controls routinely consumed all of the 20 pellets allotted per session, pellet consumption elicited by VP bic may reflect other than a

compulsive feeding response. Also, consistent with previous results cited above, VP bic infusions done in the present study increased locomotion slightly or not at all. Thus, the present data complete a double dissociation of bic infusions into the LPO and VP. LPO infusions increase locomotion robustly (3-8 fold) and pellet taking in the spotlight modestly. Infusions into the VP increase locomotion not at all or modestly (less than 2 fold), but greatly enhance pellet-taking in the spotlight. Some tolerance of threat in the pursuit of reward is essential to the survival of organisms, but a tendency to discount threat becomes maladaptive. Liberal threat discounting by humans contributes to faulty decisions and adverse or injurious consequences. The VP may contribute to overriding threat.

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Poster

267. Motivation and Reward

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Title: Lesions of the paraventricular nucleus of the thalamus differentially affect the acquisition and expression of Pavlovian-conditioned responses

Authors: *J. HAIGHT[1,2,4], K. M. FRASER[3], B. N. KUHN[2], H. AKIL[4], S. B. FLAGEL[1,4,2]
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Abstract: In recent years, evidence has emerged suggesting a role for the paraventricular nucleus of the thalamus (PVT) in the processing of reward-associated cues. However, the specific role of the PVT in these processes has yet to be elucidated since much of the previous work is confounded by the fact that Pavlovian-conditioned reward cues can act as both predictive and incentive stimuli. Using an animal model that captures individual variation in response to discrete reward-associated cues, we have been able to parse the incentive from the predictive

properties of reward cues. When rats are exposed to a Pavlovian conditioning paradigm, wherein a discrete cue predicts food reward, some rats, termed sign-trackers (STs), attribute incentive salience to the cue. Other rats, termed goal-trackers (GTs), treat the cue as a predictor. Here we investigated the role of the PVT in the expression and acquisition of these conditioned responses (CRs). First, outbred Sprague-Dawley rats were trained in a Pavlovian-conditioning task. After sign- and goal-tracking CRs were acquired, ibotenic acid was used to lesion the anterior and posterior PVT. Following recovery, rats resumed Pavlovian training and the expression of their CRs was measured. Lesions of the PVT after rats had learned their respective CRs affected GTs, but not STs. When compared to sham-operated controls, PVT lesions attenuated the expression of a goal-tracking response, and increased a sign-tracking response selectively in GTs. To determine whether PVT lesions affected the incentive motivational properties of the lever-cue, rats were subsequently tested in a conditioned reinforcement paradigm. PVT lesions did not affect responding for lever-cue presentation in STs, but increased responding in GTs. In a separate experiment, we assessed the effects of PVT lesions on the acquisition of sign- and goal-tracking CRs. This was accomplished using selectively bred rats lines in which it is known a priori whether these rats will acquire a sign- or goal-tracking CR. PVT lesions were performed prior to training and following recovery rats underwent 12 sessions of Pavlovian conditioning. Sign-tracking rats with PVT lesions showed an exaggerated sign-tracking response, compared to sham-operated controls. In addition, PVT lesions attenuated the development of a goal-tracking response in rats with an inherent tendency to goal-track. Together, these data suggest that the PVT is critically involved in both the acquisition and expression of sign- and goal-tracking behaviors, and that this structure plays a critical role in the attribution of incentive motivational properties to reward cues.

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Poster

267. Motivation and Reward

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W81XWH-11-1-0584

NIH T32 Postdoctoral Training Grant

JSPS Postdoctoral Fellowships for Research Abroad

Title: Local injection of oxytocin into the primate amygdala enhances prosocial motivation

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Abstract: The primate amygdala (AMYG) is thought to be critical for mediating social behavior. Single-unit recording studies in AMYG have found motivational signals (Paton et al., 2006) as well as signals relevant to social information, such as identity and facial expression (Gothard et al., 2007). However, the contributions of AMYG to motivations during social interactions (social motivation) remain unknown. We previously showed that when there is no cost to actor monkeys in a social reward-allocation task, the activity of AMYG neurons tracks the reward values associated with each decision similarly across rewards delivered to oneself, another individual, or both, and that these signals predict how prosocial or antisocial actor monkeys are across trials (Chang and Platt, 2013, SfN abstract). These results suggest that AMYG neurons encode the value of rewards received by others with respect to the value of rewards experienced by oneself. The neuropeptide oxytocin (OT) is thought to modulate social motivation and saliency by acting on multiple brain regions. Previous studies in humans and rodents have implicated AMYG to be one of these core sites of OT action. Furthermore, OT-induced changes in BOLD activity in AMYG seem to be critical in individuals with autism. Together, these observations suggest that OT may influence social behavior via modulation of activity in AMYG. To test this idea, we probed the impact of local injections of OT into the AMYG on social decisions in male rhesus macaques. On alternating sessions (separated by at least one day), we locally injected OT (5µg/µl in 2µl saline) or vehicle (saline, 2µl) into the AMYG sites where we recorded social reward-related activity, while the actor chose between rewarding himself and both himself and the recipient as well as when he chose between rewarding the recipient and no one (Chang et al., 2011, 2012, 2013). As a control, we injected OT or vehicle into the dorsolateral prefrontal cortex (dlPFC), an area that has not been implicated in OT regulation of social function. OT injections in AMYG, relative to vehicle, increased the frequency of prosocial choices. OT injections in AMYG also modulated gaze directed at the recipient following all decisions, relative to vehicle. Changes in social gaze varied between individual monkeys. By contrast, these injections did not affect choice reaction times. Finally, OT injections in dlPFC did not influence any of these behaviors. Our results demonstrate that locally enhancing OT levels in AMYG enhances the motivation to make prosocial decisions.

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Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

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Title: Motivation for palatable food in an animal model of diet-induced obesity

Authors: *F. CORTÉS SALAZAR[1], J. O. SUÁREZ-ORTÍZ[1], D. DÍAZ-URBINA[1], V. E. LÓPEZ-ALONSO[1], J. M. MANCILLA-DÍAZ[1], N. TORRES-TORRES[2], R. E. ESCARTÍN-PÉREZ[1]
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Abstract: Obesity is a global health problem associated with diseases that significantly increase mortality rates. Despite obesity and overweight have multifactorial causes, recurrent over-consumption of energy dense foods is frequently present in these conditions. These diets are commonly highly palatable and activate the brain reward circuit, promoting over-consumption of food. The finding that children and adults are more motivated to work for food than non-overweight peers suggests that the processing of the rewarding properties of food might be related to obesity induced by diet, however it is not clear if differences in motivation to eat among obese and non-obese is a cause or a consequence of over-consumption of food. Accordingly, the present study was aimed to evaluate in an animal model of obesity (diet-induced obesity, DiO) if the chronic (6 months) exposure to a high-fat diet induced changes in motivation to eat palatable food using a progressive ratio (PR) schedule of reinforcement and to determine if the initial motivation to eat predicts the final weight gain in rats exposed to the high-fat diet. Male Sprague Dawley rats (weighting 100-150 g at the beginning of the experiment) were feed with a high-fat diet (45% of energy from fat, n=24) or the control diet (similar flavor, 10% of energy from fat, n=12) for 6 months. Motivation for palatable food (chocolate flavored sugar pellets) was measured at three different points of time, at the beginning of the protocol (after 2-3 weeks), after 3 months, and after 6 months of exposure to the high-fat diet (independent groups of rats). We measured biochemical markers in palsma (Leptin, cholesterol, triglycerides, and insulin), weight gain (g), and the fat mass/body weight ratio to validate the

obesity model. By measuring break points under a PR schedule of reinforcement we assessed motivation for palatable food (rats were trained with fixed ratio 1 and 5 schedules before the PR schedule). We found that animals exposed to the high-fat diet gained significantly more body weight after 4 months of the protocol. Additionally, we found that the biochemical markers of obesity (high levels of Leptin, cholesterol, triglycerides, and insulin in plasma) were higher than controls after 4 months of high-fat diet consumption. According to the behavioral tests, break points of rats exposed to the high-fat diet were significantly higher than controls only in the first period of evaluation and the initial break points did not predict the body weight gain. Our results suggest that differences in motivation to eat palatable food are not directly associated to the development obesity.

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Poster

267. Motivation and Reward

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Support: NIH - P01 DA021633

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Title: The effect of glucocorticoid receptor antagonism on sign-tracking behavior

Authors: S. SEWANI[1,2], K. H. LONG[1,2], T. SHAPIRO[1,2], H. AKIL[1], *S. B. FLAGEL[1,2]
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Abstract: Cues in the environment can guide our everyday behavior leading us towards valuable resources. However, cues can also acquire the ability to guide behavior in maladaptive ways. For example, cues that have previously been associated with drug-taking behavior can instigate drug-seeking behavior and relapse. Reward cues gain inordinate control over behavior when they are attributed with incentive motivational value (i.e. incentive salience), but this only occurs for

some individuals. Using a Pavlovian conditioning paradigm, it has been shown that some rats, termed sign-trackers, approach and engage a discrete reward-associated cue, whereas other rats, goal-trackers, approach the location of reward delivery upon presentation of the cue. Thus, the cue attains incentive motivational value for sign-trackers and not goal-trackers. It has previously been shown that sign-trackers exhibit a greater increase in corticosterone in response to repeated pairings of a cue and reward in a Pavlovian conditioning paradigm. Here we examined the effects of systemic glucocorticoid receptor (GR) antagonism on the propensity to attribute incentive salience to a discrete cue associated with a food reward. We used selectively bred rats that differ in their predisposition towards sign- or goal-tracking behavior in order to examine the effects of GR antagonism on the acquisition of these conditioned responses. Rats were injected with the GR antagonist mifepristone (40mg/kg, i.p.) prior to Pavlovian autoshaping sessions, and their propensity to approach and contact the reward associated cue or the location of reward delivery was measured. There was no effect of mifepristone administration on the first day of Pavlovian training; however, sign-tracking behavior was attenuated across sessions in mifepristone-treated rats with an inherent tendency towards this behavior. These rats were less likely to contact the reward-associated cue, and when they did contact it they did so with less vigor and at a slower rate compared to their vehicle-treated counterparts. Goal-tracking behavior was not affected by mifepristone in these rats. Interestingly, when taken off of the drug after 9 days of training, the rats that were previously treated with mifepristone continued to exhibit attenuated sign-tracking behavior relative to vehicle-treated control rats. Thus, GR antagonism appears to block the development of a sign-tracking response in rats with an inherent tendency to exhibit this behavior. These findings demonstrate a role for glucocorticoids in the attribution of incentive motivational value to discrete reward-related cues.

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Poster

267. Motivation and Reward

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Title: Selective optogenetic stimulation of d1 'direct' or d2 'indirect' neurons of nucleus accumbens versus lateral septum

Authors: *S. L. COLE, M. J. F. ROBINSON, K. C. BERRIDGE
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Abstract: The nucleus accumbens (NAc) shell and lateral septum are two brain regions that are similar in neuron types and anatomical projection patterns, and are both implicated in motivation and reward. The NAc has two main output projections: a "direct" pathway to the brainstem, comprised of neurons bearing D1 dopamine receptors that projects directly to ventral tegmentum, and an "indirect" pathway containing D2 neurons, which projects first through the ventral pallidum and hypothalamus before going to midbrain regions. The lateral septum is also comprised of both D1 and D2 containing neurons, and has connectivity patterns similar to NAc medial shell . Here, we optogenetically stimulated selectively either D1 containing neurons or D2 containing neurons in NAc shell versus lateral septum as anatomical structures,using Cre mice differentially expressing channelrhodopsin (ChR2) in either D1 or D2 neuron types. We compared stimulation effects on 2 tasks: 1) an 'internal reward' self-stimulation task in which mice directly earned ChR2 laser stimulation by touching a metal empty spout and 2) an 'external sensory reward' task in which laser stimulation modulated an external food reward: mice could choose to earn either a sucrose pellet reward that was paired with laser stimulation or an alternative equally good sucrose reward without laser stimulation. Results showed that for the 'internal reward' task, NAc D1 stimulation or NAc D2 stimulation were each sufficient to support laser self-administration. However, mice never showed self-stimulation in lateral septum regions. By contrast, for the external reward task, laser stimulation of lateral septum D1 neurons (but not D2 neurons)enhanced the preference for the laser-paired sucrose reward and intensified its pursuit at the expense of the alternative sucrose-without-laser reward. However, laser stimulation of NAc shell did not enhance the external sucrose reward (neither D1 or D2). These results show differences between reward functions of neuronal populations in NAc shell and lateral septum, as well as implicating a somewhat surprising self-administration reward role for D2 neurons in NAc and potentially its indirect pathway.

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Poster

267. Motivation and Reward

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Support: Funding Program for Next Generation World-Leading Researchers (LS074) to M.M. from Cabinet Office, Government of Japan

Title: Representation of past negative outcome and subsequent behavioral shift in the primate lateral habenula and anterior cingulate cortex

Authors: *T. KAWAI[1,2,3,5], H. YAMADA[3,4], N. SATO[2], M. TAKADA[1], M. MATSUMOTO[3,4]
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Abstract: Neurons in the lateral habenula (LHb) and anterior cingulate cortex (ACC) are excited by negative experiences. However, little is known about how their signals contribute to subsequent choice behavior. To address this issue, we recorded single-unit activity from the LHb and ACC in monkeys performing a reversal learning task. While the monkey was gazing a fixation point, two saccadic targets were presented on both the left and the right sides of the point. The monkey was required to choose one of the targets with a saccade. Choosing one target was followed by reward with 50% probability, whereas choosing the other was followed by no-reward. The reward-position contingency was fixed within a block of 20 to 40 trials, and then reversed without any instruction. The monkey learned to choose the rewarded direction by trial-and-error, and changed the choice to the alternative if the choice was repeatedly followed by no-reward. Thus, the monkey decided to "stay" the current choice or "shift" to the alternative in the next trial through the experience of several past outcomes. We recorded the activity of 62 LHb neurons and 359 ACC neurons in two monkeys. Ninety-two percent (57/62) of the LHb neurons and 35% (125/359) of the ACC neurons were more strongly activated by no-reward outcome than by reward outcome. To examine how these no-reward responsive neurons contribute to subsequent choice behavior, we conducted a multiple linear regression analysis of their activity. We found that the no-reward response of ACC neurons reflected several past outcomes, and that this response further represented whether the monkey stay the current choice or shift to the alternative in the next trial. Notably, these two responses were observed in different ACC neurons. On the other hand, the response of LHb neurons mainly reflected the no-reward outcome in the current trial. The response latency was significantly shorter in LHb than in ACC neurons (LHb, 207.3ms ± 18.1ms; ACC, 580.2ms ± 38.1ms; Wilcoxon rank-sum test, $P < 0.01$). Our findings suggest that the ACC links past negative experience to subsequent adaptive choice, whereas the LHb may be involved in this process by signaling the current negative outcome via reciprocal connections between the two areas.

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Poster

267. Motivation and Reward

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Title: Impact of binge eating upon learning and performance of a sucrose discrimination task

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Abstract: Binge eating disorder (BED) is characterized by the rapid ingestion of palatable food in a brief period of time. In rodents, sucrose intermittent access produces behavioral responses similar to those observed in BED followed by neurochemical changes that affects brain reward system. However, BED impact over gustatory system remains elusive. It is not known whether BED can produce a long-term modulation over perceptual and/or oromotor abilities of rats to detect and identify sucrose and whether these changes contributes to BED maintenance. We explored the effect that BED over sucrose discrimination. During 28 days three groups of rats were assigned to one of three conditions: 1) Intermittent (INT/BINGE) 2h sucrose access, 2) Ad Libitum (AdL), 24h sucrose access, 3) Chow (CH) 2h sucrose access on day 1 and 28. All rats had 12h chow access in the dark phase. During the 2h sucrose access, INT/binge group significantly consumed 15 and 21 ml more sucrose than AdL and CH group, respectively. At day 29, sucrose access was suspended for all groups, subjects were water deprived for 22h and discrimination training began. Rats were trained to emit 2-4 dry licks in a central spout to receive a drop of water (washout) followed by 2-4 dry licks to randomly receive a single 15µl drop of a discriminative taste cue: 3% or 10% sucrose concentration. Subsequently, subjects required to stop licking and emit differential responses (left or right) according to the taste cue received. Correct responses were reinforced by three 15µl water drops. Additional Licks (AL's) after cue provides the reaction time needed to detect the sucrose drop. During the first training week,

before learning occurs, licking pattern among groups was significantly different. AL's evoked by taste cues was concentration-dependent and was significantly lesser only for the INT/BINGE group relative to AdL and CH group, indicating that BED rats can better halt the consummatory reflex evoked by a drop of sucrose, which might allow these animals to overconsume sucrose in a short period of time. Similarly, Body Movement Time (BMT, time elapsed between the last lick in the central spout and go to collect the reward in the lateral spout) was shorter for INT/BINGE than for AdL and CH groups. In sum, these data suggest that BED increases the motivation to collect a reward, by reducing the BMT and improving the fine-grain top-down control of oromotor responses. Learning and performance changes in sucrose discrimination will be evaluated; as well as BED effect over neuronal activity of the insular and orbitofrontal gustatory cortices during task.

Disclosures: **E.G. Fonseca de la Cruz:** None. **M. Villavicencio:** None. **R. Gutierrez:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; CONACYT 179484, ICYTDF-PICSA12-126, Salud2010-02-151001, Productos Medix 0001275.

Poster

267. Motivation and Reward

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 267.19/TT2

Topic: F.03. Motivation and Emotion

Support: DA033926

Title: Optogenetic stimulation of ventral tegmental area dopamine cells facilitates operant shock avoidance behavior

Authors: *J. M. WENZEL, E. B. OLESON, V. C. CHIOMA, W. N. GOVE, A. RAGANATH, L. N. SMITH, J. F. CHEER
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Abstract: The role of the mesolimbic dopamine system in positive reinforcement processes has been well documented. Utilizing a signaled operant footshock avoidance procedure our laboratory has previously shown that the mesolimbic system is also involved in negative reinforcement learning. Indeed, dopamine transients within the nucleus accumbens at the

presentation of a warning signal preceding footshock delivery were shown to predict successful shock avoidance, revealing a positive correlation between phasic mesolimbic dopamine transmission and shock avoidance. In order to assess a causal relationship between phasic dopamine transmission and avoidance behavior, the current study employed optogenetic techniques in a transgenic rat model. Briefly, rats expressing Cre-recombinase driven by the tyrosine hydroxylase promoter (TH::$Cre^{+/-}$ rats) received bilateral transfection with a Cre-inducible channelrhodopsin-2 (ChR2-EYFP) virus into the ventral tegmental area (VTA) as well as implantation of chronic bilateral optical fibers aimed at the VTA. Following recovery, animals learned a signaled operant shock avoidance task wherein illumination of cue light served as a warning signal which was presented 2s before the onset of footshock (0.6mA over 0.5ms, occurring at 2s intervals). Execution of a single lever press during this initial 2s interval resulted in the avoidance of footshock, whereas a lever press made after the initiation of shock delivery resulted in escape from footshock. Animals were trained on this task until they reached a stable level of avoidance behavior with successful avoidance on >50% trials for three consecutive days. Animals then underwent three test sessions that were identical to training sessions except that prior to each test animals' optical fiber implants were connected to a 473nm wavelength laser via sheathed fibers allowing for the delivery of optical stimulation to the VTA. Each test session consisted of a no-stimulation 30-min baseline session followed by a 30-min stimulation session during which the presentation of each warning signal coincided with VTA stimulation (20Hz, 10 pulses, 5ms duration). In congruence with our previous work, optical stimulation of VTA dopamine cells resulted in a significant increase in operant shock avoidance, illustrating a causal role for phasic mesolimbic dopamine activation in avoidance behavior. Importantly, similar stimulation-dependent enhancement of shock avoidance behavior was not observed in genotype (TH::$Cre^{-/-}$) and virus (EYFP) control groups that underwent identical training and test conditions.

Disclosures: **J.M. Wenzel:** None. **E.B. Oleson:** None. **V.C. Chioma:** None. **W.N. Gove:** None. **A. Raganath:** None. **L.N. Smith:** None. **J.F. Cheer:** None.

Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: CHDI Foundation

Title: Motivational deficits and reduced cortico-striatal communication in Huntington's Disease transgenic rat model

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Abstract: Huntington's Disease (HD) is a neurodegenerative disorder that occurs as a result of a >37 polyglutamine (CAG) repeat expansion within the huntingtin gene (htt). Initial progression of HD is characterized by cognitive and emotional impairments including depression, anxiety and decreased motivation, followed by motor deficits such as a lack of coordination and the development of chorea. Neural degeneration affects primarily the indirect pathway of the basal ganglia as well as the cortex. Prior to significant striatal degeneration, compromised cortico-striatal communication is thought to occur, however how this correlates with motivational deficits is still unclear. In order to characterize this, a behavioral test to assess motivation was carried out in combination with extracellular electrophysiological recordings from the prefrontal cortex (PFC) and the nucleus accumbens (NAc) in freely-moving animals. 3, 6, 9, and 12 month-old BACHD TG5 rats (a transgenic animal model of HD that carries a full length human mhtt gene with 97 CAG repeats) were surgically implanted with multielectrode microarrays. Rats performed a food-motivated progressive ratio lever-pressing task while local field potentials (LFPs) and single neuron firing were recorded simultaneously. Significantly lower breakpoints were observed in TG5 rats across all age groups indicating a decrease in motivation that is characteristic of the disease. Analysis of LFPs showed an overall decrease in oscillatory accumbal gamma power as well as reduced PFC-NAc gamma rhythm coherence. Analysis of single-units revealed alterations in the percentage of encoding inhibitory neurons in the NAc in TG5 rats compared to WT rats. The impairment in cortico-striatal communication present in TG5 rats may underlie pathological changes in behavior, specifically motivational deficits found in these rats.

Disclosures: **E.A. Cole:** None. **I. Gildish:** None. **R. Cachope:** None. **J.F. Cheer:** None.

Poster

267. Motivation and Reward

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Program#/Poster#: 267.21/TT4

Topic: F.03. Motivation and Emotion

Support: UIC LAS Natural Sciences Award

Title: Psychometric assessment of brain stimulation reward discriminability

Authors: *M. S. MCMURRAY, J. A. SAUREZ, J. D. ROITMAN
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Abstract: Intracranial self-stimulation (ICSS), in which rats perform motivated behaviors to earn electrical stimulation delivered to various regions of the brain, has been widely used to investigate reward neurocircuitry. Its primary implementation has been to construct a rate-frequency (R-F) curve, which describes the animals' rate of responding across a range of frequencies. The parameters that describe R-F curves are sensitive to chemicals that alter reward processing, such as drugs of abuse or peptides of hunger and satiety. Recently, brain stimulation reward (BSR) has become increasingly used as a replacement for food reward in cognitive tasks due to its stability over time, lack of food restriction, high temporal specificity, and lack of sensory cues. In many such tasks, animals choose between different frequencies of BSR; however, the discriminability of BSR frequencies and their relationship to R-F curve parameters has not been fully characterized. To parametrically determine the discriminability of BSR frequencies, we first trained rats to lever press for 100Hz stimulation to the medial forebrain bundle, followed by standard R-F curve estimation. Once stable, pairs of frequencies from each animal's R-F curve were compared in a choice task, in which presses on one lever resulted in 0.5s delivery of the higher BSR frequency, and presses on the other resulted in the lower BSR frequency. The frequencies compared varied randomly from session-to-session and included the animal's minimally reinforcing frequency (Theta), as well as 25%, 50%, 75%, 100% and 125% of the maximally reinforcing frequency. Discriminability, as indicated by subject preference, was assessed for both the absolute and ratio differences between the two frequency values. We found that regardless of ratio, frequency differences lower than 7Hz did not support a clear preference, suggesting that animals cannot effectively discriminate BSR stimulations that differ in less than 7Hz. Additionally, there was a strong correlation between an animal's discriminability threshold and both the slope and Theta of their R-F curve. These results have implications for the design of cognitive tasks relying on BSR, and suggest that numerous aspects of an animal's R-F curve may determine the reinforcing value of BSR.

Disclosures: M.S. McMurray: None. **J.A. Saurez:** None. **J.D. Roitman:** None.

Poster

267. Motivation and Reward

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Program#/Poster#: 267.22/TT5

Topic: F.03. Motivation and Emotion

Support: Lundbeck Foundation Grant R140-2013-13057

Title: Resolving the interface between glucose homeostasis and reward

Authors: *T. L. DAHLE-MORVILLE[1], M. KERAMATI[2], B. GUTKIN[3], H. R. SIEBNER[1], O. J. HULME[1]
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Abstract: Given the narrow range of physiological states compatible with survival, coupling reward maximisation to homeostatic optimisation places fundamental constraints on adaptive behaviour. Although there is converging behavioural evidence that homeostatic errors systematically amplify reward-values associated with minimising those errors, the neuroanatomical and computational basis of this homeostatic-reinforcement interface remains unresolved. Whilst acquiring high-resolution fMRI data, glucose-deprived subjects engaged in a simple probabilistic foraging task in which they chose between monetary and glucose prospects. We modelled the subjects' choice behavior as a function of blood-glucose and gut hormones using a Homeostatic Reinforcement Learning (HRL) framework, in which glucosensory states map onto putative drive states, and where rewards, defined as drive-reductions are learnt, and maximised using model-based RL-algorithms. We found that the homeostatic errors induced by glucose deprivation systematically modulated glucose-foraging behavior; that systemic-glucose correlated with slow-drift signal in the hypothalamus; and that homeostatic error systematically scaled the neurometric encoding of a diversity of reward variables.

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Poster

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Support: Consejo Nacional de Ciencia y Tecnologia de Mexico Grants 179484

Salud2010-02-151001

ICYTDF-PICSA12-126

Productos Medix 0001275

Title: Nucleus Accumbens shell neurons monitor tastant consumption independent of their palatability

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Abstract: Gustatory stimuli such as quinine or sucrose innately evoke either aversive or palatable responses such as gaping or licking (in rodents). It has been reported that neurons in the rat Nucleus Accumbens (NAc) shell selectively respond to quinine or sucrose by increasing or decreasing their firing rate (Roitman et al. Neuron, 45(4): 587-597, 2005). However, this behavioral task does not separate taste palatability from oromotor responses. The question then arises as to whether such NAc responses arise from differences in palatability and/or oromotor responses. To test this hypothesis, we recorded activity of 232 neurons from NAc shell of rats performing a palatability-detection Licking Reaction Task (LRT) (Perez et al. AJP-Regulatory, 305(3): 252-70, 2013). In this task during each trial, rats were required to lick an empty spout (dry licks) 3 to 5 times at the central port of a behavioral box before randomly receiving a 10-µl drop of a cue [water, quinine hydrochloride (QHCl), NaCl or sucrose] which served as tastants (with different palatability's) and as a signal to stop licking. Licks elicited after release of the stop signal [additional licks (ALs)] were measured. If ALs were ≤ 40 a reward of 10-µl drop of water was available in each of the two lateral spouts. We found that few neurons preferentially responded to one or more tastants (NaCl (0.4%), sucrose, (2.6%) water (4.3%) and QHCl (1.7%)). In contrast, oromotor licking responses per se evoked significantly greater neuronal modulations in that 32% of neurons were inhibited and 7% activated ~1.5 s before and during licking an empty spout. In addition, most neurons maintained the same neuronal modulation (either inhibition or activation) even after the release of the tastant stop signal. Neuronal activity then returned to baseline activity but not until ~ 0.5 s after licking was terminated. These results indicate that NAc neurons better reflect oromotor responses rather than encoding hedonic value. To support this hypothesis, we used the ensemble activity of NAc neurons and a PSTH-based classification algorithm to predict the tastant the rats received in each trial. Accordingly, the algorithm failed to predict which tastant rats received, but performance significantly improved when the algorithm classified trials as a function of short vs. long ALs, regardless of which of tastants delivered. Importantly, the ensemble of neurons accurately predicted QHCl vs. sucrose trials, since they evoked short vs. long ALs respectively, and not because NAc neurons

responded to differences on taste palatability. In summary, our data indicate that NAc neurons monitor consumption independent of tastant palatability.

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Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: NIAA INIA West Consortia

Title: Selectively bred HAD-1 rats exhibit a unique emotional phenotype predictive of excessive alcohol intake

Authors: *N. THAKORE[1], J. M. RENO[2], E. KUSEY[3], A. GONZALEZ[4], T. SCHALLERT[2], R. A. GONZALES[5], C. L. DUVAUCHELLE[6]
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Abstract: Emotionality is a well-established motivator of excessive ethanol drinking in humans. Until now, no animal studies have reported emotional response during voluntary excessive ethanol drinking. Selectively bred High Alcohol Drinking (HAD-1) rats exhibit high voluntary alcohol intake, and are proposed to be an animal model for alcoholism. It is unknown what motivational factors drive initial drinking in HAD-1 rats. To examine this, ultrasonic vocalizations (USVs), which are considered to be an accepted index of emotional status, were recorded from HAD-1 rats across 8 weeks of 7-hr "drinking-in-the-dark" (DID) sessions. Findings revealed that both alcohol-naïve and alcohol-experienced HAD-1 rats emit unprovoked 22-28 kHz USVs during DID sessions. Such spontaneous, unprovoked 22-28 kHz USVs are not commonly seen in the average lab rat. In fact, typical rat strains require mildly aversive stimuli (e.g., airpuffs) and alcohol dependency before emitting this type of call. Further analysis showed that alcohol experience enhances 22-28 kHz USVs produced by HAD-1 rats. Alternatively, frequency-modulated 50-55 kHz USVs were constant and equal across weeks in both groups, and do not appear to be affected by alcohol experience. A correlational analysis revealed a significant linear relationship between ethanol intake and 22-28 kHz, but not 50-55 kHz FM

calls. The results suggest that emotional phenotypes exhibiting spontaneous 22-28 kHz USVs and low levels of 50-55 kHz FM USVs may be predictive of vulnerability to excessive alcohol intake. Furthermore, the data indicate that increased 22-28 kHz USVs are directly linked to increased alcohol drinking. Taken together, these findings suggest that unprovoked 22-28 kHz USVs sensitized to ethanol in HAD-1 rats may convey an aspect of emotional response that was not previously associated with this call type.

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Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: NIDA grants DA022340 and DA033926

Title: Conditional deletion of CB1 receptors on cholinergic terminals and its functional consequences

Authors: *V. KASHTELYAN[1], J. M. IRVING[1], A. FITOUSSI[1], H.-L. WANG[3], M. MORALES[3], J. F. CHEER[1,2]
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Abstract: Deficits in memory and attention play an important role in schizophrenia and other neurological diseases. Cannabinoids have received increased attention as activation of CB1 receptors produce similar cognitive impairments to those associated with schizophrenia. Importantly, blocking CB1 receptors facilitates working memory. This effect is reversed by administration of rivastigmine (a cholinesterase inhibitor), suggesting an interaction between cannabinoid and cholinergic systems. The mechanism through which CB1 receptors interact with cholinergic interneurons to modulate memory, attention, and ultimately behavior remains unknown. To investigate this question, we generated mice lacking CB1 receptors on cholinergic terminals. We first hypothesized that this specific deletion would increase acetylcholine release leading to enhanced performance on cognitive tasks. Specifically, we concentrated on the dorsal

lateral striatum for its role in reward-directed locomotor behaviors and on the medium septum for its connection to the hippocampus. Using a combination of radioactive in-situ hybridization and immunohistochemistry, we initially verified that CB1 receptors co-localize with cholinergic terminals in both anatomical regions. Next, we observed that knockout animals express normal cholinergic populations, but these did not co-localize with CB1 receptors. To evaluate changes in working-memory, we used a delay-non-match-to-sample task and found that mice lacking CB1 receptors in cholinergic terminals had better retention at intermediate to long delays compared to wild-type animals. Next, we assessed motivational processes with a progressive ratio paradigm and found that knockout animals have higher breakpoints for palatable foods compared to control animals. Cholinergic neurons have also been implicated in the place conditioning properties of cocaine; therefore, we determined whether this comprised a CB1 receptor-dependent component. However, we did not observe differences in cocaine conditioned place preference in mutant animals compared to controls. These findings suggest that CB1 receptors on cholinergic terminals facilitate acquisition of information and its processing depending on the unconditioned characteristics of the reinforcer.

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Poster

267. Motivation and Reward

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Topic: F.03. Motivation and Emotion

Support: Univ Missouri System

Title: Evaluation of behavioral differences between rats selectively bred for low and high voluntary running

Authors: *D. K. MILLER[1], J. D. BROWN[2], A. I. FILIPI[2], R. K. WILDING[2], F. W. BOOTH[3]
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Abstract: Physical activity is known to promote acquired and adaptive improvements in mental health, while low levels of physical activity are associated with poor mental health outcomes. For this study, we posed the question: does genetic inheritance of low or high physical activity

motivation cause the inheritance of negative or mental health outcomes, respectively? Our approach to answering this question was to measure how selectively breeding for low (LVR) and high (HVR) voluntary running behavior affected performance in behavioral tests classically used to determine anxiety-like behavior (i.e. elevated plus maze), depressive-like behavior (i.e. forced swim test), and nociception (i.e. thermal and mechanical stimulus sensitivity). We used young (post-natal day 30-40), male and female Wistar rats generated from 10th and 11th generation LVR and HVR lines as well as wild type (WT) Wistar rats in order to provide a comparison to non-selected rats of the same strain. One group of rats was measured for inherent performance in the elevated plus maze followed 72 hours later by forced swim testing, while a separate group of rats was used to determine inherent sensitivity to thermal and mechanical stimuli. Our results demonstrate that selectively breeding for low and high voluntary running behavior does affect inherited performance in all tests. Specifically, HVR rats are more likely to venture into novel, open spaces compared to LVR rats as measured by the elevated plus maze; HVR rats exhibit more immobile behavior than WT rats in the forced swim test; and LVR rats are less sensitive to thermal stimulation than WT and HVR rats. We conclude that LVR and HVR rats are a valuable voluntary, physical activity model for examining interactions between physical activity genetics/behavior, mental health, and nociception.

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Poster

267. Motivation and Reward

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State of Connecticut

Title: Cholinergic regulation of depression like-behavior: Role of VTA nicotinic and muscarinic receptors

Authors: *E. J. NUNES, R. J. WICKHAM, N. A. ADDY
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Abstract: The ventral tegmental area (VTA) has been implicated in numerous psychiatric disorders such as schizophrenia, bipolar disorder, and depression. Moreover, dopamine (DA) neurons in the VTA have recently been demonstrated to regulate depression-like behaviors on various animal models of depression including the tail suspension task, sucrose preference test, and social defeat stress. VTA DA neurons display two *in vivo* patterns of firing, low frequency tonic firing and high frequency phasic firing. We and others have shown that VTA nicotinic and muscarinic acetylcholine receptors (AChRs) regulate DA burst activity and downstream phasic DA release in the nucleus accumbens (NAc). While phasic DA activity has been demonstrated to play a causal role in depression like-behavior, the role of VTA AChRs on these behaviors are unknown. For that reason, we sought to determine the role of VTA cholinergic manipulations on depression-like behavior. The current studies investigated the behavioral effects of increasing VTA cholinergic tone and VTA AChR blockade during the forced swim test (FST), an animal model of depression. Intra-VTA infusion of the acetylcholinesterase inhibitor physostigmine (PHYSO) (1 or 2 μg/side) increased time spent immobile in the FST. This depression-like behavioral effect was similar to that of systemic administration of PHYSO (0.125mg/kg IP) but in contrast to what we observed with PHYSO infusion into the NAc (1 or 2 μg/side), which decreased immobility time in the FST. In addition, the same doses of PHYSO used in the FST experiments produced no significant effect on locomotor activity. Blockade of VTA cholinergic receptors with either the nicotinic AChR antagonist mecamylamine(MEC, 3 or 30 μg/side) or the muscarinic AChR antagonist scopolamine (SCOP, 2.4 or 24 μg/side) decreased time spent immobile. Next, either MEC or SCOP was co-administered with PHYSO to test whether cholinergic blockade can reverse the effects of intra-VTA PHYSO on the FST. Co-administration with AChR antagonists reversed the pro-depressive effects of intra-VTA PHYSO. Taken together, these results demonstrate that increased cholinergic tone in VTA produces a pro-depressive effect on the FST that can be reversed by cholinergic receptor blockade. Future studies should examine the effects of cholinergic manipulations in VTA on other behavioral models of depression.

Disclosures: E.J. Nunes: None. **R.J. Wickham:** None. **N.A. Addy:** None.

Poster

267. Motivation and Reward

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Title: Serotonergic neurons signal reward and punishment on multiple timescales

Authors: *J. Y. COHEN[1], M. W. AMOROSO[2], N. UCHIDA[2]
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Abstract: Serotonin's function in the brain is unclear. One challenge in testing the numerous hypotheses about serotonin's function has been observing the activity of identified serotonergic neurons in animals engaged in behavioral tasks. We recorded the activity of dorsal raphe neurons while mice experienced a task in which rewards and punishments varied across blocks of trials. We "tagged" serotonergic neurons with the light-sensitive protein channelrhodopsin-2 and identified them based on their responses to light. We found three main features of serotonergic neuron activity: (1) a large fraction of serotonergic neurons modulated their tonic firing rates over the course of minutes during reward versus punishment blocks; (2) most were phasically excited by punishments; and (3) a subset was phasically excited by reward-predicting cues. By contrast, dopaminergic neurons did not show firing rate changes across blocks of trials. These results suggest that serotonergic neurons signal information about reward and punishment on multiple timescales.

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Poster

267. Motivation and Reward

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The Guillermo Puelma Foundation

Title: Role of histamine in the ventral tegmental area during a motivation dependent, and resistance to extinction tasks

Authors: *M. A. TORO, R. FALCON, J. L. VALDES
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Abstract: Goal directed behaviors are determined by probabilistic learning and motivational aspect of the process associated to them. The mesolimbic dopaminergic pathway has been deemed as the most relevant neuromodulatory system involved in the operation of these processes, by controlling the motivational state of the subject, and learning the probabilistic relationship between actions and rewards. However the interaction of the dopaminergic system with other components necessary for goal directed behavior of the ascending arousal system, such as the histaminergic system, are still poorly understood. In this work, we studied the effects of bilateral injections of histamine or saline vehicle in the Ventral Tegmental Area (VTA) of rats, previous to: i) a progressive ratio task, were the animal (n=5, adult male Sprague-Dawley rats) must press a lever in a geometrically increasing way to get rewards, to measure motivation; and ii) a resistance to extinction task (n=5, adult male Sprague-Dawley rats), consisting on a training session were rewards are given at different probabilities (25,50,75, and 100%), and then no more lever presses are rewarded, to evaluate the resistance to extinction. The animals injected with histamine into VTA, compared to saline vehicle injections, showed a significant decrease in the motivation to get rewards in the progressive ratio and a diminished capacity to extinguish non rewarded behaviors, during the probabilistic rewards paradigm,. These results are in agreement with *in vitro* studies which suggest that the histamine may have an indirect inhibitory effect over the dopaminergic system, helping to unravel the interaction between this neuromodulatory system during goal directed behaviors.

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Poster

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Topic: F.03. Motivation and Emotion

Support: NIH RO1 AA022292

Title: Selective ablation of mu opioid receptor expressing gaba neurons in the rostromedial tegmental nucleus promotes ethanol intake

Authors: ***R. FU**, X. CHEN, W. ZUO, J. LI, J.-H. YE
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Abstract: BACKGROUND AND PURPOSE The cellular mechanisms underlying the aversive effect of ethanol that limits its intake are not well understood, although recent evidence has linked aversion with synaptic inhibition of dopamine neurons in the ventral tegmental area. Emerging evidence indicates that the rostromedial tegmental nucleus (RMTg), a newly defined midbrain structure exerts a major GABAergic inhibitory control over midbrain dopamine neurons and encodes aversive stimuli. The RMTg contains mostly GABAergic neurons and with dense μ-opioid receptor (MOR) immunoreactivity. However, the role of RMTg in the regulation of ethanol intake has not been well investigated. EXPERIMENTAL APPROACH We compared voluntary ethanol intake and locomotion in rats with intra-RMTg infusion of dermorphin-saporin or blank saporin. Dermorphin-saporin is a neurotoxin, which could selectively lesion MOR-expressing neurons. We measured ethanol intake in rats given intermittent access to ethanol (20% vol/vol) using a two bottle choice paradigm. We euthanized the rats, dissected their brains and analyzed the glutamic acid decarboxylase67 (GAD67) and MOR protein expression and immunoreactivity immediately following the behavioral test. KEY RESULTS In rats that received intra-RMTg injection of dermorphin-saporin, we observed a robust increase in the intake of and the preference to ethanol, and in the locomotor activity; but a significantly reduced GAD67 and MOR protein expression, as well as a massive loss of neurons with GAD67 and MOR immunoreactivity within the RMTg. We observed no such changes in rats that received injection of blank saporin or saline. Together, These findings indicate that MOR-expressing GABA neurons in the RMTg play a crucial role in the regulation of ethanol consumption, implicating the dysfunction of these neurons likely play a critical role in the pathogenesis of alcoholism, and that these neurons should represent an appropriate target for the development of therapeutic strategies against alcohol use disorders.

Disclosures: **R. Fu:** None. **X. Chen:** None. **W. Zuo:** None. **J. Li:** None. **J. Ye:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Support: NIH Grant DA033526

Title: Sex differences in the depressive-like effects of kappa opioid receptor activation do not depend on circulating gonadal hormones in rats

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Abstract: The neuropeptide dynorphin activates kappa opioid receptors (KORs) in neural stress circuits to produce depressive-like states. There are pronounced sex differences in behavioral responses to stress. For example, females are more sensitive to the aversive effects of drugs of abuse and stress-induced relapse. Using intracranial self-stimulation (ICSS), we previously found that gonadally intact female rats are less sensitive than males to the depressive-like effects of the KOR agonist U-50,488_regardless of estrous cycle stage. U50,488 induced sex-dependent elevations in c-Fos expression in the paraventricular nucleus of the hypothalamus (PVN) and the bed nucleus of the stria terminalis (BNST), two stress-responsive regions that express corticotropin releasing factor (CRF). We hypothesized that the effects of KOR activation on reward depend on interactions between circulating gonadal hormones and CRF. To examine the activational effects of gonadal hormones on aversive responses to U50,488, we gonadectomized male and female rats that had previously been trained in ICSS. After five weeks, during which plasma sex hormones decreased (measured with ELISA), baseline ICSS responding was similar across groups (male and female, gonadectomized and sham). Rats were treated with U50,488 (0.0, 2.5, 5.0, and 10.0 mg/kg, IP) and stimulation thresholds compared. No significant differences to U50,488-induced increases in ICSS thresholds were detected between sham and gonadectomized rats. These data suggest that sex differences in KOR-mediated depressive-like states are not due to circulating gonadal hormones. Using quantitative real-time RT-PCR, we found higher basal levels of prodynorphin mRNA in the female PVN, BNST, and amygdala, and lower KOR mRNA in the BNST. Finally, levels of CRF receptor 1 (CrfR1) mRNA were lower in the amygdala and BNST of intact female compared to male rats. These findings raise the possibility that elevated dynorphin tone in females occludes the effects of KOR agonists, and that KOR-mediated activation of CRF systems is blunted in females due to decreased CrfR1. This underscores the importance of understanding KOR function in both sexes such that pharmacotherapeutics targeting mood disorders can be rationally designed.

Disclosures: D.J. Puttick: None. **E.H. Chartoff:** None. **S.E. Russell:** None. **D.N. Potter:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Support: NIH DA030517

NIH 5T32NS007375-20

Title: Estrogen suppresses negative vocalizations in female rats during methamphetamine withdrawal

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Abstract: Withdrawal is a debilitating negative state brought on by drug cessation that is characterized by depression, anxiety, and many negative physical side effects. Avoidance of withdrawal symptoms drives addicts to continue drug use or relapse to drug use despite the many negative consequences. Thus discovery of future treatments aimed at lowering the symptoms of withdrawal are crucial to large-scale recovery. Women rate higher on depression scales during drug withdrawal than men. More importantly, negative mood correlates with drug craving in women and the induction of negative mood in a laboratory setting leads to drug craving in women but not men. Taken together, these studies show that women may be at an increased risk for negative mood during drug withdrawal and that this negative mood may put women at a greater risk for craving and possibly relapse. Women show higher levels of drug craving during the late luteal phase, when both estrogen and progesterone levels decrease. In animal models, female rats show higher levels of cue-primed reinstatement during the estrous phase, again following a decrease in estrogen and progesterone. This leads to the possibility that estrogen and/or progesterone might be protective against negative mood during drug withdrawal in females. Examination of ultrasonic vocalizations (USVs) is a useful tool for studying emotional states in rats. Studies using an air-puff device as a negative stimulus have shown that rats undergoing withdrawal from stimulants show a greater number of the vocalization type indicative of negative affect (22 kHz USVs) than rats given saline. In the present study we sought to examine estrogen's effects on air-puff induced 22 kHz USVs during methamphetamine withdrawal in female rats. Animals were implanted with either oil or estradiol containing capsules. All animals were habituated to the test chamber for 15 minutes the day prior to testing.

On the test day, animals were given a 15mg/kg injection of methamphetamine or saline. Six hours following the injection each animal was put in the testing chamber and given one air-puff every 30 sec for 9 min. Following the air-puff procedure, the animals' vocalizations were recorded for an additional 5 min. We found that oil-implanted animals undergoing methamphetamine withdrawal emitted significantly more 22 kHz USVs than their estrogen-implanted counterparts. There was no difference in the number of 22 kHz USVs emitted between estrogen- and oil-implanted females given a saline injection. This preliminary study provides evidence for estrogen's suppression of negative affect during drug withdrawal.

Disclosures: J.R. Browning: None. **J.A. Mong:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Support: Capes

CNPq

Fapesp

Title: Doublecortin-positive cells in male adolescent mice brain after repeated social defeat

Authors: *L. S. RESENDE[1,2], L. ALVES-DOS-SANTOS[1,2], J. F. S. CARILLO[1], A. S. ALVES[3], L. R. G. BRITTO[3], S. CHIAVEGATTO[1,2]
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Abstract: The early years of life constitute a sensitive period during which chronic stress may compromise the neurodevelopment. Stress has been shown to inhibit cell proliferation and, ultimately, neurogenesis in the hippocampus. Worldwide, 20-30% of adolescents are regularly involved in school bullying and the adverse experience of being bullied induces a variety of potential psychological and somatic sequelae. Here, we used social defeat, a valuable animal model for bullying in humans, to evaluate the impact of social stress in adolescent mice on anxiety, social avoidance and neurogenesis. C57BL/6 male mice aged 30 days were subjected to

daily bouts of social defeat for 10 days in a 5-min session. Encounters were performed in the home cage of a CD-1 male aggressor, and after the physical interaction the defeated mouse was left isolated for 24h in an adjacent compartment separated from the aggressor by a perforated acrylic partition. Control mice were exposed to the same situation without the physical interaction. Anxiety-like behavior and social avoidance were analyzed by the elevated plus maze test (EPM) and social approach-avoidance test, respectively. Twenty-four hours after the last test, animals were anesthetized, perfused, and brains sectioned for doublecortin (DCX) immunolabeling. Defeated adolescent mice showed social avoidance by a reduction in interaction time ($p<0.05$) and higher latency to perform the first interaction with a target mouse ($p<0.05$), but no difference in anxiety-like behaviors in the EPM when compared to controls ($p>0.05$). High levels of DCX immunoreactive cells were found in the olfactory bulb, piriform cortex, hippocampal dentate gyrus (DG) and the subventricular zone. Quantification of DCX+ cells (pixels/mm^2) in the ventral or dorsal DG did not show difference between groups ($p>0.05$). Interestingly, the positive correlation of DCX+ cells between dorsal and ventral DG in the control group ($R^2= 0.99$; $p<0.05$) was not found in the adolescent defeated mice ($R^2= 0.07$; $p>0.05$). We are now analyzing additional areas. Our results in male adolescent mice confirm the social aversion after repeated social defeat and are indicating some perturbation in the neurogenesis of particular brain areas. Ongoing studies are being conducted to further explore these findings.

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Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Support: NHMRC Project Grant 1070081

Title: Association between vitamin D levels and depression-like behaviour in mice

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Abstract: Purpose Adult vitamin D deficiency is common in up to one third of the adult population and has been associated with cognitive decline and depression in some, but not all, studies. Here we examined the effect of manipulating vitamin D levels on cognitive and depression-like behaviours in an adult mouse model. Methods Adult male BALB/c mice were fed a diet that was either deficient (0 IU), insufficient (100 IU), replete (1,500 IU) or contained excess (15,000 IU) vitamin D for at least 12 weeks. Serum vitamin D levels were confirmed at the end of the experiment. Separate groups of mice were tested in either operant chambers using the 5 choice serial reaction time task or on a behavioural test battery which included the forced swim test. Results There was a significant effect of vitamin D levels on the time taken to reach criteria for the 5CSRTT but once acquired there was no effect of diet on performance.There was a significant effect of dietary vitamin D levels on both the time spent immobile and total distance moved during the forced swim test. There was a significant difference between diets in terms of time spent immobile ($F_{3,26} = 12.5$, $p < 0.001$), with the insufficient group spending significantly more time ($p = 0.01$), and the elevated group spending significantly less time ($p = 0.02$) immobile than mice on the replete diet. There was also a significant effect of diet on total distance moved during the trial ($F_{3,26} = 23.3$, $p < 0.001$), the elevated vitamin D group moved a significantly greater distance than the other 3 dietary groups ($p < 0.001$). Conclusions Adult vitamin D dietary intake was not associated with any gross behavioural or cognitive abnormalities, however there were specific and prominent changes in the forced swim test and in the acquisition of the 5CSRTT. These data support a role for vitamin D levels in depression-like behaviour. There is epidemiological research linking low vitamin D levels with brain-related outcomes and we have previously shown that adult vitamin D deficiency is associated with altered brain function and behaviour (Groves et al., 2013). The findings open up a new frontier in understanding the role of vitamin D in brain health. If these changes generalize to humans, then this could have important public health implications.

Disclosures: **T.H. Burne:** None. **C. Simpson:** None. **J. McGrath:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Support: R01MH097718-01

Title: The effects of prenatal immune activation and infantile repeated maternal separation stress on maternal behavior

Authors: *S. CHOU, C. DAVIS, M. LI
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Abstract: Maternal care encompasses a complex group of behaviors that require the coordination of various functions, including motivational and affective regulations. It has previously been shown that a wide array of hormonal and socioenvironmental factors can disrupt maternal care and induce abnormal postpartum affective behaviors such as depression and anxiety. However, current paradigms focus largely on stress triggers applied to adult pregnant or postpartum dams, including hormone simulated pregnancy, daily postpartum corticosterone treatment, chronic gestational psychosocial stress, and repeated pup separation. We have previously shown that female postpartum dams subjected to 3 hours daily repeated pup separation on postpartum (PPD) days 6-9 display acoustic startle reflex (ASR) deficits during the early postpartum period, and that male rat offspring subjected to the double insult of prenatal immune activation and repeated maternal separation during early infancy displays abnormal cognitive behaviors and sensitivities to psychoactive drugs in adulthood. Thus, it is likely that early developmental stress in female rats increases the risk of abnormal postpartum behavior in adulthood as well. The current study investigates the long term consequence of this combination of early neurodevelopmental and infantile psychosocial stress on postpartum behavior of adult female rats. Female offspring of pregnant dams exposed to polyinosinic:polycytidilic acid (PolyI:C, 4.0 mg/kg, intravenous on gestational day [G] 13 and 6.0 mg/kg on G15) were separated for 3 hours daily between postnatal days (PND) 2-14. They were then mated on PND77 and assessed for maternal behaviors - including pup licking, nursing, retrieval, nest building - as well as prepulse inhibition (PPI) on postpartum days (PPD) 2-11. Postpartum females were also assessed for depressive symptoms in forced swim tests (FST), as well as attentional and anxiety behaviors using PPI, acoustic startle response (ASR), and fear potentiated startle response (FPS). Differential behavioral effects are discussed.

Disclosures: S. Chou: None. **C. Davis:** None. **M. Li:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Title: Δ9-Tetrahydrocannabinol withdrawal alters affective-like behaviors in mice

Authors: *S. G. KINSEY, K. M. GABELLA, M. S. JONES, S. R. NASS
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Abstract: Chronic Cannabis users develop tolerance and are susceptible to withdrawal. Reflecting the increased incidence of these phenomena, the DSM-V includes Cannabis Use Disorder and Cannabis Withdrawal Syndrome, the primary symptoms of which are increased cravings, anxiety, and depressed mood. Current rodent models of cannabinoid withdrawal quantify somatic withdrawal symptoms, including of paw tremors and head twitches. One limitation of these models is that they do not measure changes in emotionality or arousal, such as anxiety-like and depressive-like behaviors, that are key withdrawal symptoms in humans. The goal of the present study was to test the hypothesis that withdrawal from Δ9-tetrahydrocannabinol (THC), the main psychoactive component of Cannabis, alters behaviors related to emotionality in mice. Naïve male C57BL/6J mice were administered vehicle or THC (10 or 50mg/kg, s.c.) for 6 days, and then withdrawal was precipitated by acute administration of the CB1 cannabinoid receptor antagonist, rimonabant (SR141716A) (3 mg/kg, i.p.). The Tail Suspension and Marble Burying tests were performed to quantify depressive-like and anxiety-like behaviors, respectively. Mice subjected to precipitated THC withdrawal exhibited a significant increase in struggling time in the Tail Suspension Test ($t(18) = 9.2$, $p<0.01$), and a significant decrease in marble burying ($t(19) = 6.1$, $p<0.01$) with no concomitant alteration in locomotor activity. These data indicate that standard models of mouse emotionality and motivation may be useful tools for investigating cannabinoid withdrawal in rodents.

Disclosures: **S.G. Kinsey:** None. **K.M. Gabella:** None. **M.S. Jones:** None. **S.R. Nass:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Support: James and Esther King Biomedical Research Program 1KG03-33968

Title: Cotinine reduced depressive-like behavior and enhanced VEGF in mice after forced swim stress

Authors: *A. GRIZZELL[1,3], M. MULLINS[3], A. IARKOV[3], S. PATEL[3], R. ZEITLIN[3], A. ROHANI[3], L. CHARRY[3], V. ECHEVERRIA MORAN[3,2,4]
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Abstract: Depressed individuals smoke tobacco more prevalently than the general population and, in doing so, report a reduction of symptoms, suggesting that a component of tobacco smoke may be alleviating impairment associated with this debilitating disease. Cotinine, a tobacco-derived compound and predominant metabolite of nicotine, may be one such component. Cotinine is nootropic and has a demonstrated positive safety profile in humans. Like other antidepressants,cotinine stimulates the protein kinase B/glycogen synthase kinase 3 (Akt/GSK3 beta) pathway and may therefore exert antidepressant effects through a mechanism involving hippocampal neurogenesis. Over a series of experiments, we investigated the effect of cotinine on depressivelike behavior induced by forced swim (FS) stress in adult, male C57BL/6J mice. Daily oral cotinine (5 mg/kg) administered throughout the stress-exposure period consistently reduced depressive-like behavior induced by acute or repetitive forced swim stress. Furthermore, this effect was seen whether treatment began prior to or at the onset of stress-exposure. Real-time polymerase chain reaction (RT-PCR) data indicate that stress induced but cotinine normalized changes in six neurogenesis factors. Cotinine also enhanced the expression of the v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 (Erbb2) and the vascular endothelial growth factor (VEGF) genes in the hippocampi of mice subjected to repetitive FS stress. Altogether, the results suggest that cotinine may be an effective antidepressant positively influencing mood through a mechanism involving the preservation of cellular homeostasis via the enhancement of genetic expression of neurogenesis factors.

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Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Support: MH097860 (to WC)

Sackler Scholar Programme in Psychobiology (to RD)

Title: Pituitary adenylate cyclase-activating polypeptide (PACAP) disrupts motivation, attention, and social interaction

Authors: *R. J. DONAHUE, A. VENKATARAMAN, A. VAN'T VEER, C. J. WEBBER, E. G. MELONI, D. A. PIZZAGALLI, W. A. CARLEZON, Jr.
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Abstract: Exposure to severe or prolonged stress can cause psychiatric illnesses including anxiety and depressive disorders. The mechanisms by which stress induces these illnesses are not fully understood. Recent work has shown that PACAP (pituitary adenylate cyclase-activating polypeptide) is released in the brain in response to stress and produces anxiety-related behaviors. For example, PACAP treatment in rats causes persistent anxiogenic responses as reflected by increases in acoustic startle. It is well established that stress can also disrupt cognition, motivation, and social interaction. The present studies were designed to investigate how PACAP (0.25-1.0 µg, administered intracerebroventricularly [ICV]) affects behaviors that reflect these core features of mood disorders in adult Male Sprague-Dawley rats. First, we confirmed that PACAP induces anhedonia (reduced ability to experience reward) in the intracranial self-stimulation (ICSS) test. Rats implanted with an ICV cannula and an ICSS electrode were trained in the rate-frequency variant of the ICSS procedure. When reward thresholds were stable (60% correct responses and <20% omissions on 3 consecutive days). Following ICV cannula implantation and re-stabilization of performance, rats were infused with PACAP and tested in 5CSRTT 1 hr later. Finally, we examined if PACAP alters social interaction and social withdrawal. One week after ICV cannula implantation, rats were infused with PACAP and placed in a 60 x 60 x 40 cm Plexiglas chamber with a weight-matched partner rat 1 hr later. Social behavior was videotaped for 10 min and scored by an observer blinded to treatment condition. PACAP produced dose-dependent disruptions in motivation, attention, and social interaction as reflected by increases in reward thresholds in ICSS, disruptions in 5CSRTT metrics (e.g. decreases in correct responses, increases in omission errors, and decreases in post-error performance), and decreases in social interaction behaviors. Interestingly, unlike previously reported effects on acoustic startle, the effects of PACAP on these behaviors were not long-lasting. A better understanding of the impact and persistence of PACAP effects on behavior may facilitate the development of improved treatments for stress-related illnesses.

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Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Title: Lidocaine infusions in the centromedial amygdala reduce successive negative contrast, without affecting anticipatory negative contrast

Authors: *K. KAWASAKI[1], A. C. GLUECK[2], I. ANNICCHIARICO[2], M. R. PAPIN[2]
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Abstract: The amygdala plays a key role in negative emotions, including frustration induced by reward devaluations. Massive corticomedial amygdala lesions have been shown to eliminate the consummatory successive negative contrast (cSNC) effect, without apparently affecting the consummatory anticipatory negative contrast (cANC) effect in rats. In cSNC, rats given daily access to 32% sucrose consume less 4% sucrose than rats always exposed to 4% sucrose. In cANC, rats consume less 4% sucrose when each session is followed by access to 32% sucrose rather than by 4% sucrose. Pharmacological manipulations suggest that cSNC induces a negative emotion, whereas cANC induces reward anticipation. To test the hypothesis that the amygdala is involved in the emotional modulation, but not in anticipatory processes, we sought to produce reversible amygdala lesions at specific times during training. Animals were implanted with cannulae and then trained in cSNC followed by cANC. A Sucrose (32-to-4 vs. 4-to-4) x Infusion (lidocaine vs. PBS) x Session analysis of downshift behavior in the cSNC situation yielded a significant triple interaction [$F(4,120)=4.398$, $p=0.001$]. Pairwise LSD comparisons derived from the main analysis indicated that transient amygdala inactivation before the first 32-to-4% sucrose downshift reduced cSNC relative to 4-to-4% sucrose controls ($p=0.120$), and attenuated consummatory suppression relative to a 32-to-4% sucrose group treated with the PBS vehicle ($p=0.028$). The cSNC effect was present in vehicle-treated groups ($p=0.000$). Unlike in the cSNC situation, transient amygdala inactivation did not affect cANC, which occurred unchanged in groups treated with lidocaine, PBS, or nothing [Infusion x Contrast interaction: $F(2,48)=1.998$, $p=0.147$]. These results are consistent with the hypothesis that output from the amygdala is required for the emotional modulation of consummatory behavior after reward devaluations, but not for anticipating the occurrence of the same sucrose solutions presented in a sequential manner. The fact that amygdala inactivation had its effects on the very first exposure to the 32-

to-4% sucrose downshift suggests this was a nonassociative effect (i.e., emotional), rather than a consequence of memory disruption.

Disclosures: K. Kawasaki: None. **A.C. Glueck:** None. **I. Annicchiarico:** None. **M.R. Papin:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

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Title: Partial excitotoxic lesions of the rostromedial tegmentum (RMTg) diminish the inhibitory effects of lateral habenula stimulation on midbrain dopamine neurons *in vivo* and reduce the incidence of learned helplessness in rats

Authors: *P. D. SHEPARD[1], P. L. BROWN[2], H. PALACOROLLA[1], D. BRADY[1], K. RIEGGER[1], C. MAYO[1], M. KLIMA[1], G. I. ELMER[1]
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Abstract: Neurons in the lateral habenula (LHb) are transiently activated by aversive events ranging from noxious stimuli to the loss of an anticipated reward and have been implicated in aversive learning. More sustained increases in LHb activity have been linked to major depressive disorder in humans and the appearance of a depressed phenotype in animals. Functional changes associated with tonic and phasic activation of the LHb are often attributed to a corresponding inhibition of midbrain dopamine and/or serotonin neurons. A growing body of evidence suggests that activation of GABAergic neurons in the rostromedial tegmentum (RMTg), a region that receives dense projections from the LHb and projects strongly to midbrain monoaminergic nuclei, underlies the physiological and behavioral effects attributed to activation of the LHb. In an effort to test this premise, the effects of axon sparing lesions of the RMTg were assessed on i) LHb-induced inhibition of substantia nigra (SN) dopamine (DA) cell activity *in vivo* and ii) the expression of learned helplessness. All experiments were conducted in adult, male, Sprague-Dawley rats. A single midline injection of quinolinic acid (0.4 M in 166 nl of artificial cerebrospinal fluid, aCSF) significantly decreased the number of NeuN positive profiles in the

RMTg compared to rats receiving an equal volume of aCSF (VEH: 1649 ± 87, n=4; QUIN: 457 ± 55, n=21; $t(23) = -8.9$, $p < 0.001$). Two weeks following injection of QUIN or VEH, rats were anesthetized with chloral hydrate and single unit recordings were taken from spontaneously active DA neurons in the SN. Consistent with previous reports, the majority of DA-containing neurons (28/30; 93%) in VEH-treated rats were transiently inhibited by single pulse stimulation of the LHb. By contrast, DA neurons in QUIN-treated rats were less likely to be inhibited by LHb stimulation (16/24, 67%) and those cells that remained responsive were inhibited for a shorter duration (49 ± 17 ms) than VEH-treated rats (108 ± 12 ms). A separate group of experiments was conducted to assess the effects of RMTg lesions on the development of learned helplessness. VEH- (n = 18) and QUIN-treated (n = 26) rats were evaluated in a two day procedure comprised of 120 trials of unpredictable, inescapable footshock followed one day later by 30 trials of signaled, escapable shock. As a group, QUIN-treated rats showed significantly fewer escape failures (7.0 ± 1.5) than VEH-treated rats (13.7 ± 2.7; $p < 0.05$). These results suggest that the RMTg is required for the expression of learned helplessness and support the notion that this structure is an important component of a pathway mediating the aversive properties of LHb activation.

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Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Title: Superior collicular neurons reveal preferential responses to snakes in primates

Authors: *Q. V. LE, J. MATSUMOTO, Q. V. LE, E. HORI, T. ONO, H. NISHIJO
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Abstract: Snakes are suggested to be predators of early primates. Under evolutional selection pressure by snakes, specific subcortical and cortical areas have evolved to detect threat relevant stimuli, especially snakes in primates (Isbell, 2006). Consistent with the theory, we recently reported that monkey pulvinar neurons responded stronger and faster to snakes than other stimuli (Le et al., 2013). The superior colliculus (SC), which receives inputs from the retina and projects directly to the pulvinar, is one of these areas and plays a crucial role in alerting mechanisms in

mammals, especially primates. However, responses of SC neurons to snakes remain unknown in primates. In the present study, we recorded monkey SC neuronal responses to photos of snakes, monkey faces, monkey hands and simple geometrical figures. The monkeys were required to discriminate these 4 categories of the visual stimuli in a delayed non-matching to sample (DMNS) task. Of 983 neurons recorded, 356 neurons were visual responsive. Of these neurons, 43 neurons had their receptive fields at the center of the visual fields, and were located in the antero-lateral part of the SC. Although there were no significant differences in response magnitudes to the 4 categories of the stimuli, they responded faster to the snake images and ratio of snake-best neurons was larger than those of other categories. Multidimentional scaling (MDS) analysis showed that snakes were separated from the other stimuli as early as the first 25 ms period after stimulus onset. These results provide new evidence of SC involvement in rapid snake detection.

Disclosures: Q.V. Le: None. **J. Matsumoto:** None. **Q.V. Le:** None. **E. Hori:** None. **T. Ono:** None. **H. Nishijo:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 268.12/TT25

Topic: F.03. Motivation and Emotion

Support: NIH Grant AA010761

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NIH Grant AA010983

NIH Grant AA019967

NIH Grant AA022836

Title: The role of prelimbic inputs to the rostromedial tegmental nucleus in aversive behavior

Authors: *E. J. BURNETT[1], D. H. LENCH[2], T. C. JHOU[2], L. J. CHANDLER[2]
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Abstract: Activity in the rostromedial tegmental nucleus (RMTg) is associated with aversive behavior measured in various assays including conditioned fear, elevated plus maze, and tasks measuring passive avoidance. Activity in the prelimbic cortex (PrL) has also been implicated in the expression of many of these same behaviors. Importantly, the PrL sends a remarkably dense projection to the RMTg, but the contribution of this particular pathway to signaling the aversive properties of environmental stimuli has yet to be elucidated. To investigate the role of the PrL-RMTg pathway in aversive signaling, we first measured the effect of optogenetic stimulation of this pathway in a real-time conditioned place task. Rats were bilaterally stereotaxically injected with AAV expressing channelrhodopsin in the PrL cortex and implanted with bilateral optic fibers in the RMTg. Six weeks following surgery, rats were tested in an unbiased two-compartment apparatus where one compartment was paired with the delivery of 10 mW of 473 nm blue light at 60 Hz and the other compartment was associated with no light delivery. Rats spent significantly less time in the light-paired compartment than the unpaired compartment ($p \leq 0.001$) indicative of real-time place aversion in response to stimulation of PrL terminals in the RMTg. In a follow up experiment, we examined cFos induction in RMTg-projecting PrL neurons following the presentation of a cue predictive of footshock. Rats were unilaterally stereotaxically injected with the retrograde tracer cholera toxin B (CtB) into the RMTg. One week following surgery, rats underwent fear conditioning to associate a tone with the delivery of a 0.5 mA footshock. Control rats were exposed to the same number of tone and shock presentations but in a randomized, unpaired manner. Rats were sacrificed 90 min following presentation of the tone alone and brains were processed for cFos and CtB visualization using standard immunohistochemical methods. In control animals, who did not associate the presentation of the tone with a shock, exhibited no cFos expression in RMTg-projecting PrL neurons. In contrast, re-exposure to the tone in conditioned rats resulted in a modest but significant enhancement of cFos expression in PrL neurons co-labeled with CtB ($p \leq 0.05$). Together, these data suggest that RMTg-projecting PrL neurons are involved in modulating the behavioral response to aversive stimuli.

Disclosures: **E.J. Burnett:** None. **D.H. Lench:** None. **T.C. Jhou:** None. **L.J. Chandler:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 268.13/TT26

Topic: F.03. Motivation and Emotion

Title: Substantia nigra pars reticulata guides gaze to reward-associated objects and punishment-associated objects

Authors: *A. GHAZIZADEH, O. HIKOSAKA
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Abstract: Visual objects that have been repeatedly associated with reward (good objects) attract gaze even in the absence of immediate reward. Recent studies from our lab showed that the basal ganglia play a crucial role in this process through the inhibitory connection from the substantia nigra reticulata (SNr) to the superior colliculus (Hikosaka et al, Ann Rev NS 2014). SNr neurons are inhibited by 'good' objects, thereby disinhibiting SC neurons (Yasuda et al, 2012). However, there may be other reasons why a visual object attracts gaze; one of them may be punishment. Here we asked two questions: 1) Do punishment-associated objects attract gaze?; 2) If so, do the basal ganglia also contribute to the punishment-induced gaze bias? Two male rhesus monkeys (Macaca mulatta) participated in this study. They experienced many visual objects (n=24 fractals) , each of which was consistently associated with juice ('good'), airpuff ('punish'), or no outcome ('neutral'). On most trials (75%) two objects were presented from different categories (good, punish or neutral) and the monkey chose one by making a saccade to it. On the other trials (25%) one object was presented, which was followed by the associated outcome regardless of the animal's action. To answer question #1, we presented 4 randomly chosen objects and let the monkey look at them freely (free viewing). Neither juice nor airpuff was presented in free viewing. The monkey's gaze was attracted more strongly to 'punish' objects compared to 'neutral' objects (i.e., more frequent saccades to and within the object, longer duration of gaze on the object), although gaze bias was strongest to 'good' objects. To answer question #2, we recorded activity of single SNr neurons and presented these objects sequentially while the monkey kept fixating. Most SNr neurons were inhibited by both 'good' objects and 'punish' objects, and excited by 'neutral' objects. These results suggest that the basal ganglia facilitate gaze orienting to both 'good' and 'punish' objects - a behavior that might reflect 'motivational salience' (Matsumoto & Hikosaka, 2009).

Disclosures: A. Ghazizadeh: None. **O. Hikosaka:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

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Program#/Poster#: 268.14/TT27

Topic: F.03. Motivation and Emotion

Support: MRC career development award to H.F.C.

C.U.W is supported by an MRC BCNI studentship

Title: Differential regulation of negative emotion by areas 25 and 32 of the marmoset ventromedial prefrontal cortex

Authors: *C. U. WALLIS[1,2], A. C. ROBERTS[1,2], H. F. CLARKE[1,2]
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Abstract: There is strong evidence that the human ventromedial prefrontal cortex (vmPFC) is important for emotion regulation. Abnormal vmPFC activity is consistently associated with disorders of negative emotion (anxiety and depression), and its reversal is associated with successful treatment. A functional subdivision regarding the role of the vmPFC in emotion regulation has been suggested, with the subgenual region, focused on area 25, associated with negative affect, and a perigenual region, including area 32ac, associated with positive affect (Myers-Schulz & Koenigs, 2012). Most experimental studies on the role of the vmPFC in negative emotion have been carried out on fear conditioning and extinction in rats. Yet findings regarding the role of infralimbic and prelimbic regions in emotion regulation do not translate to areas 25 and 32, respectively, of the human vmPFC (their putative structural homologues (Vogt et al., 2013). To bridge the gap between rodents and humans, this study investigates the functions of areas 25 and 32 in the marmoset (Callithrix jacchus); a new world primate with a highly developed PFC; cytoarchitectonically similar to that of humans. Implanted cannulae were targeted at areas 25 and 32 of the marmoset vmPFC, to enable temporary pharmacological inactivation by infusion of muscimol and baclofen (GABAA and GABAB receptor agonists) or a vehicle control. The effects of these manipulations on both cardiovascular and behavioural indices of emotion were assessed under (i) an affectively neutral condition and (ii) during an aversive Pavlovian discriminative conditioning paradigm. In the neutral condition, inactivation of area 25 vs. 32 produced contrasting effects. Compared to vehicle, inactivation of area 25 reduced both vigilant scanning behaviour and cardiovascular activity (heart-rate, blood pressure, and sympathetic activity). Inactivation of area 32 increased vigilant scanning behaviour, blood pressure and sympathetic activity. Whilst inactivation of both areas 25 and 32 disrupted discriminative, conditioned behavioural and heart rate responses, initial results suggest this discriminative loss is due to reduction and facilitation of emotional reactivity respectively. The results suggest that inactivation of areas 25 and 32 of the marmoset vmPFC differentially effect behavioural and cardiovascular indices of emotion, with inactivation of area 25 reducing, and inactivation of area 32 increasing, negative emotional reactivity. This supports the proposed vmPFC functional sub-division regarding emotion regulation in humans (Myers-Schulz & Koenigs, 2012).

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Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 268.15/TT28

Topic: F.03. Motivation and Emotion

Title: Emotion regulation preference in anger: A combined behavioral- resting state fMRI approach

Authors: J. ROEBBIG[1], D. KUMRAL[1], A. SCHAEFER[1], M. GAEBLER[1], M. ERBEY[3], J. REINELT[1], L. SCHAARE[1], A. REITER[2], A. BABAYAN[1], *A. VILLRINGER[1]
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Abstract: Successful emotion regulation (ER) of anger is vital for psychological and cardiovascular health (Suls, 2013). Most studies addressing ER, with the most adaptive ER strategy known as cognitive reappraisal (Ochsner et al. 2012), conceptualized it as a stable personality trait, while recent findings suggest that an emotional state's intensity also determines how it is regulated (Sheppes et al., 2011). Here we addressed ER preference in anger on the behavioral level, comparing the predictive value of trait reappraisal and anger intensity. Furthermore, we investigated whether trait reappraisal is represented in resting state-functional connectivity (rs-fc), the intrinsic synchronization of functionally coupled networks, a method that can serve as a measure of personality (Fulwiler et al., 2012). In 23 healthy participants (11 males; age: 23.8±2.86 years), we acquired 15 min of rs-scans in addition to their emotion regulation- (ERQ) and anger disposition (STAXI). On the following day, we asked participants to remember two anger autobiographical memories of varying intensity and to choose either reappraisal or distraction to regulate. For rs-fMRI data analysis we defined the left and right amygdala as seed regions and investigated their whole-brain rs-fc, including trait reappraisal, trait anger, and gender as covariates. Thresholds were $p < .01$ on the voxel- and cluster level (FDR corrected). Our behavioral results indicate that trait reappraisal is a stronger predictor for ER choice (Wald-$\chi^2(1) = 7.22$, $p = .007$) than emotional intensity (Wald-$\chi^2(1) = 3.71$, $p = .054$). Correlation analysis of rs-fMRI data and trait reappraisal (see Figure 1) revealed a positive association between the connectivity of amygdalae and bilateral insula and an anticorrelation of the right amygdala and areas of the mPFC, which overlap with previous fMRI findings on ER

(e.g., Ochsner et al., 2012) and anger regulation (Fulweiler et al., 2012). We show that trait reappraisal is associated with individual differences in rs-fc and influences ER preference in autobiographical anger regulation.

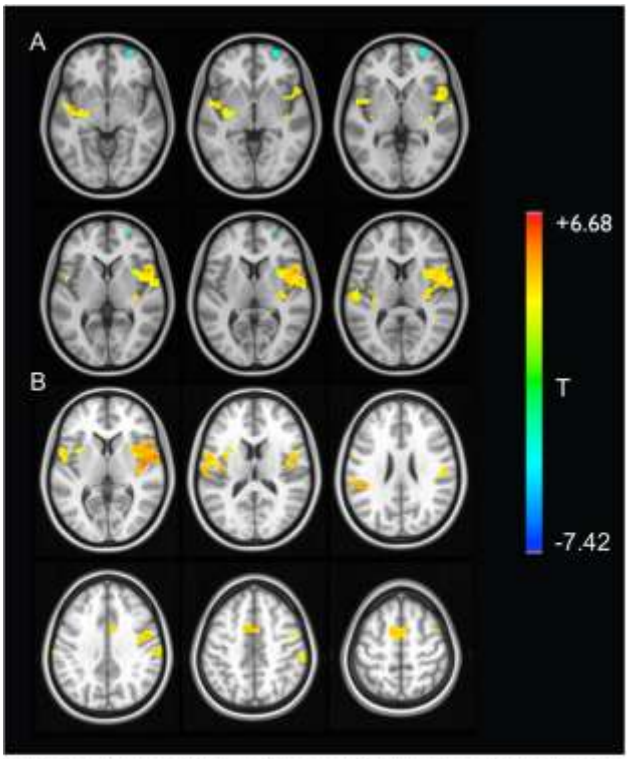

Figure 1. Association between trait reappraisal and rs-fc from the unilateral amygdalae (x = ±24, y = 0, z = -16). The analysis revealed a positive association (warm colors) between trait reappraisal and the positive coupling of right amygdala (Figure 1A) with right insula (x = 51, y = 12, z = 6; 4239 mm³; T = 3.77), left insula (x = -39, y = -15, z = 12; 1584 mm³; T = 3.25), as well as an inverse coupling (or anticorrelation; cold colors) with right medial orbitofrontal gyrus (30, 48, -12; 567 mm³; T = -2.92) and left middle frontal Gyrus (x = -30, y = 24, z = 36; 468 mm³; T = -4.09). The correlation of trait reappraisal and the rs-fc between left Amygdala (Figure 1B) revealed a positive association with the right insula (x = 45, y = -6, z = 9; 3735 mm³; T = 3.79), left insula (x = -42, y = -6, z = -9; 3375 mm³; T = 3.22) and the bilateral medial frontal gyrus (x = -9, y = 6, z = 45; 1890 mm³; T = 4.15).

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Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 268.16/TT29

Topic: F.03. Motivation and Emotion

Support: MEXT Japan 19890019

Title: Cholinergic modulation of emotion and memory in humans: a pharmacological fMRI study

Authors: *Y. NISHIO[1], N. ABE[3], Y. SHIGEMUNE[4], S. MUGIKURA[2], T. FUJII[5], E. MORI[1]
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Abstract: We utilized pharmacological functional magnetic resonance imaging to explore the impact of cholinergic blockade on emotion and cognition. Subjects underwent emotion rating and two-week-delayed recognition memory test for emotionally-negative and emotionally-neutral pictures under trihexyphenidyl, a selective M1 receptor antagonist, and under placebo. Emotion rating was unaffected by drug, whereas emotional memory bias was significantly reduced by trihexyphenidyl. BOLD responses in the prefrontal, insular and visual cortices were unmodulated by cholinergic manipulation, whereas ventral striatal and amygdalar responses were significantly reduced by trihexyphenidyl. These results suggest that subcortical cholinergic system mediates unoconscious emotional modulation of cognition.

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Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 268.17/TT30

Topic: F.03. Motivation and Emotion

Title: The invisible variable: Sleepiness and suicidality in a large national college health survey

Authors: A. TARTER, *J. R. PRICHARD
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Abstract: Introduction Insomnia is both comorbid for and a consequence of depression. Longitudinal studies in adults with PTSD and population studies with school aged children have demonstrated that sleep problems are also independent predictors of suicidal thoughts and attempts. More research is needed to evaluate the interactions between poor sleep and suicidality

in emerging adults, a vulnerable population who is at high risk for both mood disorders and profoundly disturbed sleep. Our study evaluated the relationships between sleep difficulties and self-harm behaviors and suicidal thoughts in a large national sample of college students. Methods Data from the Spring 2009 American College Health Association National College Health Assessment-II were analyzed for trends in daytime sleepiness, suicidality (self-harm behaviors, suicidal thoughts, and suicide attempts in the last year) among undergraduate students (n = 72,966) with diagnosed insomnia, with probable undiagnosed insomnia, and without major sleep initiation and maintenance problems. Results Five percent of the total population had been diagnosed with or treated for insomnia within the last year. Of these students, 71.3% had a comorbid anxiety disorder and 62.2% had a comorbid depression diagnosis. Students with diagnosed insomnia were at elevated risk for self-harm behaviors [O.R. 3.02, C.I. 2.74-3.34], suicidal ideation [O.R. 3.28, C.I. 2.99-3.60] and suicide attempts within the last year [O.R. 5.89, C.I. 5.01-6.02]. Another 8.3% of the population had probable undiagnosed insomnia (defined here as sleep initiation and maintenance problems >1.5 s.d. from the mean; trouble falling asleep approximately 6 days/week and awakening too early 4 days/week.) These students were also at elevated risk for self harm behaviors, suidicidal ideation and suicide attempts [O.R. > 2.7, for all cases]. Conclusion College health professionals can provide more effective and targeted interventions for students by understanding the elevated risk factors for self-harm, suicidal ideation, and suicidal attempts in those with insomnia.

Disclosures: A. Tarter: None. **J.R. Prichard:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

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Program#/Poster#: 268.18/TT31

Topic: F.03. Motivation and Emotion

Support: Beyondblue

Hunter Medical Research Institute

Title: Participant expectations of the Healthy Body Healthy Mind exercise program for youth with major depressive disorder

Authors: *R. CALLISTER[1], A. GILES[2], Y. NASSTASIA[3], A. BAKER[3], S. HALPIN[4], B. KELLY[3]

[2]Sch. of Biomed. Sci. and Pharm., [3]Sch. of Med. and Publ. Hlth., [4]Sch. of Psychology, [1]Univ. of Newcastle, Callaghan, Australia

Abstract: Although regular exercise may benefit young people with major depressive disorder (MDD), engaging them and attaining adherence during an exercise program are substantial challenges. Expectations may influence the success of such interventions including adherence. The aim of this analysis is to examine mental health characteristics, exercise self-efficacy and outcome expectations, as well as relationships among these variables prior to randomisation in the Healthy Body Healthy Mind (HBHM) trial of the efficacy of an exercise intervention for youth with MDD. Methods: Eligible participants were youth aged 15-25 years. Participants completed online questionnaires (satisfaction with life, self-esteem, exercise self-efficacy, exercise and HBHM program expectations), then the paper-based Beck Depression Inventory (BDI-II) and Beck Anxiety Inventory (BAI) and underwent a structured diagnostic interview (SCID) with a clinical psychologist to determine whether they met criteria for current MDD (according to DSM-IV). Relationships between variables were investigated with Spearman's correlation coefficients. Results: Data from eligible participants with MDD (n=39, Female=33, mean age 21 y, range 16-25 y) were examined. Median (interquartile range (IQR]) BDI of 31(IQR 18) was classified as severe; BAI of 17 (IQR 20) was moderate; satisfaction with life (13, IQR 5; maximum 35) was very low; self-esteem (2; IQR 2; maximum 5) was low; and self-efficacy for exercise (120; IQR 61; maximum 220) indicated moderate confidence they could exercise three times a week for the next three months. Expectations that they would obtain benefits from exercise on depression, anxiety, stress, sleep, etc, were high (29; IQR 5; maximum 35) and expectations of likely benefit from the HBHM program were high (22; IQR 5; maximum 27), however the extent of improvements expected was only 50% (IQR 20%). Significant correlations ($p<0.05$) were found between BDI and BAI ($r=0.79$), self-esteem ($r=-0.53$) and satisfaction with life ($r=-0.40$) but not with outcome expectations ($r<0.25$). Self-esteem and exercise self-efficacy were both correlated with satisfaction with life ($r=0.33$ and $r=-0.38$, respectively). Discussion: Participants in this trial have moderate-severe MDD, moderate anxiety, low self-esteem and very poor satisfaction with life. Those who volunteered for this exercise-training program expect to benefit in a number of ways including reducing their depression and anxiety symptoms as well as reducing stress and improving sleep quality and energy levels but the extent of improvement expected is modest. Supported by grants from Beyondblue and Hunter Medical Research Institute

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Poster

268. Motivation and Emotions: Negative Emotional States

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Program#/Poster#: 268.19/TT32

Topic: F.03. Motivation and Emotion

Support: NIDA Grant 431495

Title: Recalling the positive past dampens the physiological response to acute stress

Authors: *M. SPEER, H. MANGLANI, E. S. KIM, M. R. DELGADO
Psychology Dept., Rutgers Univ., Newark, NJ

Abstract: Recalling happy memories from the past can elicit positive feelings and enhance an individual's wellbeing (Young et al., 2013). Thus, one potential adaptive function in reminiscing about the positive past may be the ability to cope with stressors in daily life. The goal of this study was to examine whether positive emotion evoked by recalling the positive past can blunt the effects of a negative affective state (i.e., acute stress). That is, can such memories dampen the hypothalamic-pituitary-adrenal (HPA) axis stress response (i.e., cortisol levels)? To test this, 96 healthy participants described and gave subjective emotion ratings for specific episodic memories of positive (e.g., family vacation) and neutral (e.g., commuting to work) content. Three days later, they were either exposed to an acute stressor (i.e., socially evaluative cold pressor task) or performed a non-stressful control task. Afterwards, they were asked to retrieve a subset of the same episodic memories triggered by visual word cues (e.g., family vacation). The memories were of only positive valence or only neutral valence, creating 4 experimental groups (stress-positive, stress-neutral, control-positive, control-neutral). To measure changes in cortisol levels over time, salivary cortisol samples were collected at baseline (before stress or control task), peak (after recalling autobiographical memories, 20 min), and recovery (50 min). As expected, participants who recalled positive memories rated their memories with greater positive affect and stronger emotional intensity than participants who recalled neutral memories. In support of the idea that internally generated positive emotion evoked by memory recall can buffer the physiological response to acute stress, we observed a negative correlation between subjective ratings of positive emotion experienced during autobiographical recall and cortisol response. Critically, the stress-positive group showed a sharper decrease in cortisol rise 20 min after stress exposure compared to the stress-neutral group, even though the two groups did not differ in subjective ratings of stress or skin conductance response during acute stress exposure. Interestingly, individuals who experienced greater positive affect during recall were also individuals who reported higher levels of resiliency, highlighting how something naturally-occurring like recalling the past may be a potentially advantageous strategy for buffering negative affect in some individuals. Our findings support the idea that positive autobiographical

memories may serve an adaptive function of regulating emotion and reinforcing positive wellbeing.

Disclosures: **M. Speer:** None. **H. Manglani:** None. **E.S. Kim:** None. **M.R. Delgado:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 268.20/TT33

Topic: F.03. Motivation and Emotion

Title: Effects of prenatal music on the emotional behavior and neuronal activity in offspring

Authors: *Y. TAKANO[1], S. YANAGITA[2], N. KUBOTA[3], T. MATSUZAWA[1], K. TAKEDA[3]
[1]Dept. of Pharmaceut. Sci., [2]Dept. of Sci. and Technol., [3]Res. Inst. for Sci. and Technol., Tokyo Univ. of Sci., Chiba, Japan

Abstract: Music has overarching influences in human life. Music often attenuates anxiety and improves learning ability, and has been widely used as an additional tool for medical treatment. Recently, effect of prenatal music on offspring is thought to be one of fascinating topics as with that many pregnant women play music for their babies *in utero*. Furthermore, it is believed that music is more effective tool for fetal development since the prenatal period shows high sensitivity to environmental situations. Although some previous studies have shown that the relationship between prenatal music and developmental changes are being revealed, it remained unclear about the neuronal mechanism underlying the effects of prenatal music in offspring. In this study, we investigated the effects of prenatal music on emotional behavior in offspring. We also examined the effects of prenatal music on neuronal activity in brain regions with immunohistochemistry. In order to evaluate continuous neuronal activity, we used a long lasting marker for neuronal activity, FosB. Pregnant Wistar/ST rats were exposed to music environment during the late gestational period while control dams were exposed to a similar decibel ambient noise. Offspring at six weeks old, we measured emotional behavior using open field test (OFT), elevated plus maze (EPM), and forced swimming test (FST). In this results, there were no significant differences in anxiety-like behavior in the OFT and EPM regardless of prenatal music environment. On the other hand, prenatal music environment significantly increased the immobility time of FST in the offspring compared with ambient noise environment. There were no differences in the number of FosB positive cells in several brain regions related in emotional behavior. Additionally, we used an acute marker for neuronal activity, c-Fos, in order to evaluate

neuronal activity for stress response in FST. Prenatal music significantly increased the number of c-Fos positive cells in several brain regions related in emotional behavior compared with ambient noise environment. Therefore, our results indicated that prenatal music influenced depression-like behavior in the offspring but the continuous neuronal activity in several brain regions related depression-like behavior, suggesting that prenatal music might influence other neuronal systems related depression-like behavior.in the brain.

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Poster

268. Motivation and Emotions: Negative Emotional States

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 268.21/TT34

Topic: F.03. Motivation and Emotion

Title: Does the response to negative TV news depend on the level of emotional burnout?

Authors: *S. TUKAIEV[1], I. ZYMA[2], S. SOBISHCHANSKYI[3], Y. HAVRYLETS[4], M. MAKARCHUK[3], V. RIZUN[4], I. SOSIEDKA[2], I. BABYN[2]
[1]Dept. of Human and Animals Physiol., Natl. Taras Shevchenko Univ. of Kyiv, Inst. of Biol., Kyiv, Ukraine; [2]Dept. of Physiol. of Brain and Psychophysiology, [3]Dept. of Human and Animals Physiol., [4]Inst. of Journalism, Natl. Taras Shevchenko Univ. of Kyiv, Kyiv, Ukraine

Abstract: TV is usually focused on the negative aspects of life, which leads to stress, and, further, to burnout. However, the impact of the initial emotional state of a viewer on the perception of TV news haven't been studied previously. The purpose of this study was to investigate the response to violent TV news depending on the level of burnout. 110 healthy volunteers (18-22 y.o.) participated in the study. The first part of it (53 volunteers) involved 5 75-sec long videos depicting violence, suffering, war, and street accidents. We estimated the spectral power density (SP) and the levels of coherence of all frequencies from 0.2-35 Hz. In the second part (57 volunteers), we used the P300 component with a simple discrimination task in "oddball" paradigm to investigate P300 modulations. The participants were presented a set of 72 negative and 72 neutral frames taken from TV news plots. We used the following tests: Syndrome of emotional burnout (V.Boyko), WAM (Wellbeing, Activity, Mood), and the State Anxiety Inventory by C. Spielberger, Y. Hanin. The data of psychological tests were collected before and after the experiment. Enhancement of state anxiety and worsening of mood after

watching negative TV news were observed only for the participants without the desensitization due to burnout. After watching the negative TV-news plots, we observed adequate semantic associative emotional processes in the group without burnout (increasing alpha-3). Formation of the resistance stage led to the decrease in SP in theta, alpha1, alpha2 and beta1-subbands indicating the "removal" of negative news from the conscious processing and analysis of negative information. The decrease of interhemispheric coherence of low- and high-frequency components of EEG indicates altered reactions and the development of deterioration processes in those beginning to exhibit burnout. The amplitude of P300 showed reduced sensitivity to the affective content of frames, perhaps due to blunting: only those without burnout showed larger amplitudes of P300 after negative images than the neutral ones. Thus, burned-out people demonstrate desensitization toward the emotional content of TV news.

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Poster

268. Motivation and Emotions: Negative Emotional States

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Support: NIH Grant AA074711

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INIA West-Consortium

Waggoner Center for Alcohol and Addiction Research

Indiana Selectively Bred Rat Lines, Indiana University School of Medicine

Title: Persistent enhancement of unprovoked 22-28 kHz USVs and decreased 50-55 kHz USV counts after alcohol experience may underlie alcohol avoidance behavior in low-alcohol preferring rats

Authors: *C. L. DUVAUCHELLE[1], N. THAKORE[1], J. RENO[2], E. KUSEY[1], W. T. MADDOX[2], R. GONZALES[1], T. SCHALLERT[2]

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Abstract: Emotionality plays an important role in excessive alcohol drinking as well as alcohol avoidance behaviors. In rodents, 22-28 kHz and 50-55 kHz frequency-modulated (FM) USV calls are reliably associated with negative and positive emotional states, respectively. To study the role of emotional status associated with excessive drinking and alcohol avoidance, ultrasonic vocalizations (USVs) were recorded from selectively bred high- and low-alcohol preferring rat lines (P/NP and HAD-1/LAD-1) during 6 wks of daily 4-hr water only access sessions. Prior to USV recordings, animals in each rat line had access to water only (EtOH Naïve) or water and alcohol (EtOH Experienced: 24-hr alcohol access 3 days/wk x 6 wks) while in the home cage. For the EtOH Experienced groups, mean daily 24-hr EtOH intake (g/kg) was comparable between P and HAD-1 rats (P: 7.31 +/- 0.34 SEM; HAD-1: 7.46 +/-0.30 SEM) and NP and LAD-1 rats (NP:2.26 +/-0.3; LAD-1: 3.36 +/-0.58 SEM). Data analyses revealed significant differences across all USV measures (e.g., 22-28 kHz and 50-55 kHz USV counts, mean frequencies and durations) in comparisons between EtOH Naive and EtOH Experienced animals of both bi-directional lines (e.g., HAD-1 vs LAD-1 and P vs NP). Of particular interest are USV data revealing that increased 22-28 kHz and decreased 50-55 kHz USV counts emitted from low-alcohol preferring rats (NPs and LAD-1) endured for weeks after alcohol experience, even though prior alcohol intake was relatively low. Our overall findings suggest that alcohol experience results in persistent, negative emotional status that may underlie alcohol avoidance in rats selectively bred for low alcohol preference.

Disclosures: C.L. Duvauchelle: None. **N. Thakore:** None. **J. Reno:** None. **E. Kusey:** None. **W.T. Maddox:** None. **R. Gonzales:** None. **T. Schallert:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 268.24/TT37

Topic: F.03. Motivation and Emotion

Title: Changes in effective connectivity between dorsal and ventral prefrontal regions moderate emotion regulation

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Abstract: Introduction Reappraisal, the cognitive reevaluation of a potentially emotionally arousing event, has been proposed to be based upon top-down appraisal systems within the prefrontal cortex. It still remains unclear, how different prefrontal regions interact to control and regulate emotional responses. We used functional magnetic resonance imaging (fMRI) and dynamic causal modeling (DCM) to characterize the effective connectivity between the inferior frontal gyrus (IFG), dorsolateral PFC (DLPFC), and other reappraisal-related regions during emotion regulation (ER). Methods 23 right-handed subjects (mean age=22.95±3.57 years) performed specific reappraisal strategies (Increase, Decrease) while viewing 60 highly arousing extreme sports film clips and 42 neutral film clips in the scanner (3T, Siemens). The experimental design involved four different conditions: 'Increase' (engage with the depicted situation), 'Decrease' (distance from it), 'Look Neutral' and 'Look Sports' (no reappraisal). We used an event-related design (2s instruction, 8s picture/film clip, 4s "How do you feel?" rating, 4-8s fixation). Based on previous findings, we used 5 ROIs (IFG, DLPFC, SMA, SMG, V1) as sources and systematically constructed a model space, divided into two model families with two different central nodes (family1: DLPFC; family2: IFG). All competing models within one family had the same architecture with six endogenous connections that modeled the forward and backward connections between regions. Within each of the two families the modulatory effects of ER were modeled. Results The DCM results revealed that family1, with DLPFC as central node, represented the best explanation of the data with a total exceedance probability of 0.997. Within family1, the winning model had an exceedance probability of 0.557. The analysis of the intrinsic connection strength of the winning model showed that most endogenous connectivity parameters were significant. One intrinsic connection (IFG to DLPFC) was negative. The analysis of the modulatory effects of ER revealed significant modulations of connectivity from the DLPFC to the IFG and from the IFG to the DLPFC. Reappraisal reduced the effective connectivity between the DLPFC and IFG in both directions. Conclusion The results of our DCM analyses support the hypothesis that ER is mediated by bidirectional changes of connection strength between the IFG and DLPFC. The findings indicate a feedback mechanism between the DLPFC and IFG during ER, in which the selection process of a reappraisal, most likely involving the IFG, modulates the connectivity between IFG and DLPFC.

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Poster

268. Motivation and Emotions: Negative Emotional States

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Program#/Poster#: 268.25/TT38

Topic: F.03. Motivation and Emotion

Support: The Polish National Science Center Grant 3608/B/H03/2011/40

Title: Impact of frustration on brain activation pattern of subjects with different temperamental traits during a tactile discrimination task

Authors: M. BIERZYNSKA[1,2], M. BIELECKI[2], A. MARCHEWKA[1], W. ZAJKOWSKI[2], P. SOBCZAK[2], W. DEBOWSKA[1], *M. M. KOSSUT[1]
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Abstract: Frustration is a complex psychological construct, most clearly defined as an emotional state that occurs when an expected gratification is not fulfilled (Dollard et al, 1939) or an expected goal not attained (Anderson & Bushman, 2002). Laboratory experiments on effects of frustration on cognitive abilities were narrowed to its affective subcomponents, mostly acute stress, a crucial affective component of frustration. To examine effects of frustration on people with different temperamental traits we designed a new stress inducing procedure allowing for comparison of performance on the same task prior to and after the stress induction in an fMRI setting. The effectiveness of the procedure was verified with GSR. The subjects were screened with the Formal Characteristics of Behavior - Temperament Questionnaire by Zawadzki and Strelau (1997), high emotionally reactive (n=15) and low emotionally reactive (n=14) were selected. They were examined with a 3-Tesla MRI scanner (Siemens Magnetom Trio TIM,) equipped with 32-channel phased array head coil. Prior to the scan, they attended two weeks of Braille reading training. To induce frustration, we gave misleading negative feedback information to subjects performing in the scanner sensory tactile discrimination based on a well learnt task of detecting raised dots. fMRI procedure consisted of three parts: two runs of tactile discrimination divided by Frustration Induction Procedure. During the Braille runs subjects were asked to compare two (same-different) simultaneously applied Braille characters. During the Frustration run the subjects were asked to touch a new set of stimuli and discriminate if the signs were symmetrical or unsymmetrical. New stimuli were not previously learne and meaningless. Despite correct responses, negative feedback was entered. We contrasted the second and first Braille run. Second level contrasts from single subjects were entered into paired t-test design placing the task on two levels: after and before stress induction. The results revealed increased activity in superior frontal gyrus, cingulate cortex, thalamus and precuneus during the run following frustration induction, confirming the effectiveness of the proposed stress manipulation and identifying neural substrates of frustration. The results were different in the two

temperamentally different groups. Low emotionally reactive group showed increased activity in the posterior cingulate gyrus, middle temporal gyrus and precuneus. This result suggests that temperamental traits may have significant impact on reactions under frustration.

Disclosures: **M. Bierzynska:** None. **M. Bielecki:** None. **A. Marchewka:** None. **W. Zajkowski:** None. **P. Sobczak:** None. **W. Debowska:** None. **M.M. Kossut:** None.

Poster

268. Motivation and Emotions: Negative Emotional States

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Topic: F.03. Motivation and Emotion

Support: GTC - 34064/2013

Title: Differential effects of neuroticism on the entropy of spontaneous brain activity at rest

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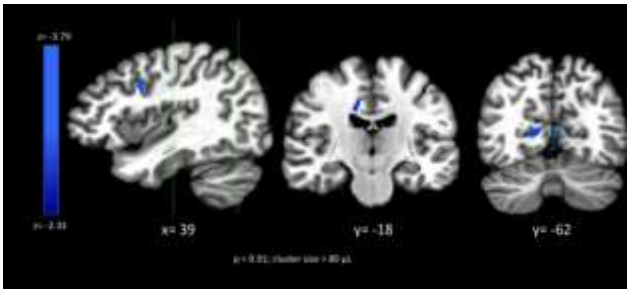
Abstract: Neuroticism is a stable personality trait, characterized by a tendency to experience higher anxiety, depression, and other negative emotions and has been consistently linked to an increased risk for psychopathology. We questioned if neuroticism would modulate resting brain activity. We designed an fMRI study to assess this relationship in regards to the Hurst Exponent (HE), an index of time series predictability. In fMRI time series, HE typically ranges between 0.5 (lowest predictability) to 1 (highest predictability). Methods: Thirty-one healthy volunteers (25 F; mean age±s.d.: 25±3 yrs) were enrolled. All subjects were medication free and did not refer history or presence of any disorder that could affect brain function. A single resting state time series of 512 volumes was acquired for each subject on a 3T Siemens SKYRA (32-channel coil - 16 axial slices; TR/TE 1560/30 ms; matrix 94x94). Subjects were instructed to lie in the scanner with eyes closed. To assess neuroticism we used the Anxiety-Neuroticism factor of the Zuckerman Kuhlman Personality Questionnaire (ZKPQ), a 99-item self-administered scale measuring five independent personality traits. ZKPQ was administered one week prior the scan. We used CAMBA for time and spatial registration and for HE calculation. We used AFNI for Talairach transformation and to compute a multiple regression in which neuroticism was used as a criterion to predict HE. Clusters with a volume > 80 mm3 at a p-value threshold of 0.01 were

considered significant. Results: Neuroticism negatively predicted the HE in the precuneus (PCUN), cingulate cortex (CC), and right inferior frontal gyrus (IFG) (fig. 1). Discussion: Higher neuroticism seems to be associated with increased predictability, time redundancy and most likely rigidity of activity in brain areas involved in emotion regulation (CC; IFG), introspection, rumination and self-referential processes (PCUN). We note these areas showed altered resting state activity in anxiety and mood disorders. Our results offer neurobiological support for the link between neuroticism and psychopathology.



Disclosures: I.A. Cristea: None. **C. Gentili:** None. **E. Ricciardi:** None. **D. David:** None. **P. Pietrini:** None.

Poster

269. Optical Methods I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 269.01/TT40

Topic: G.04. Physiological Methods

Title: Optimum optical wavelength pair for high-CNR multi-distance NIRS system

Authors: *J.-K. CHOI[1], M. CHOI[2], J. KIM[1], G.-P. HWANG[1], H.-M. BAE[1]
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Abstract: Near-infrared spectroscopy (NIRS) has been widely used to monitor local changes in cerebral hemodynamics, which are caused by blood oxygenation and deoxygenation due to functional brain activities. Modified Beer-Lambert law (MBLL) is the main governing equation for NIRS, which extracts the concentrations of oxy- and deoxyhemoglobin by using light signals with two wavelengths. The fidelity of the extracted oxy and deoxy hemoglobin can be quantified by using concentration-to-noise ratio (CNR). The CNR is strongly coupled with electrical signal-to-noise ratio (SNR), power contrast in multiple-wavelength measurements, and the propagation

trajectory of the light signal. The light reaching 2~4cm separated photo detectors propagate in a banana shape through the brain channel. Typically the wavelengths are selected to maximize the contrast between two measurements assuming that the electrical signal-to-noise ratio (SNR) is sufficiently large and the incident light penetrates deep into the cerebral cortex. However, the electrical SNR degrades and eventually limits the CNR when the separation between the source and detector pair increases. In the case of limited source and detector separation, only a small fraction of emitted photons reach the cerebral cortex of the brain. Thus the CNR is low despite high electrical SNR. Consequently, the wavelengths should be chosen carefully depending on the separation between source and detector pair under the given incident light power because the sensitivity of the photo detector, the absorption and scattering parameters (μ_a and μ_s) and the differential path-length factor (DPF) of light are dependent on a single parameter, which is the wavelength of the light. In this paper, the optimum wavelength pair for maximum CNR under various circumstances is investigated. Simulated and measured results validate our investigation.

Disclosures: **J. Choi:** None. **M. Choi:** None. **J. Kim:** None. **G. Hwang:** None. **H. Bae:** None.

Poster

269. Optical Methods I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 269.02/TT41

Topic: G.04. Physiological Methods

Support: NIH R01 EY015514

Title: Fine spatiotemporal control of voltage-gated ion channels in neuronal dendrites

Authors: *A. V. FEDORCHAK, R. H. KRAMER
UC Berkeley, Berkeley, CA

Abstract: Voltage-gated ion channels are widely implicated in active dendritic signal propagation and intrinsic excitability. Not much is known about how the precise dendritic distribution of voltage-gated ion channels contributes to dendritic function. Here, we introduce a novel method to control voltage-gated ion channels in acute rat (Sprague-Dawley) brain slices with spatiotemporal resolution comparable to that of neurotransmitter uncaging. To achieve such control we use the small molecule Quarternary-ammonium Azobenzene Quarternary-ammonium (QAQ), which blocks voltage-gated ion channels under 488 nm light, in conjunction with 488 nm laser photoisomerization and 390 nm wide-field photoisomerization. Next, we determine the

dendritic length in which blocking voltage-gated channels significantly dampens signal propagation. We then block voltage-gated ion channels either along the main apical dendrite or at the main apical branch-point, and compare the corresponding effects on signal propagation. This method allows one to investigate voltage-gated ion channels and active dendritic properties in finer detail than has previously been achieved, and this study reveals the extent to which the spatial distribution of voltage-gated ion channels affects active signal propagation.

Disclosures: A.V. Fedorchak: None. **R.H. Kramer:** None.

Poster

269. Optical Methods I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 269.03/TT42

Topic: G.04. Physiological Methods

Support: Sloan Foundation

Title: Centering device for high content imaging of neuronal screens using micro culture platforms

Authors: *K. GORDON[1,2], A. M. TAYLOR[1,2,3]
[1]UNC/NCSU Joint Dept. of Biomed. Engin., [2]Neurosci. Ctr., [3]Carolina Inst. of Developmental Disabilities, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Objective: High-throughput screening (HTS) has been difficult to utilize for primary neurons because they are post mitotic, require high plating densities, and are harvested directly from brain tissue from a finite number of animals. These limitations therefore create tremendous costs for screens with neurons. Whereas traditional screening methods use at least ten thousand neurons per well of standard 384-well plates, here we use 'microraft' culturing platforms to grow and isolate significantly smaller (500-700 cells) populations of neurons and then place them into 384-well plates for screening. This method offers a unique advantage because it decreases the number of cells necessary for screening, but still allows enough culturing density for proper viability. Furthermore the evaporative issues that have plagued small neuron cultures are also avoided because of the larger volume of 384-well plates. These microraft platforms, which are flat 500 um square magnetic polystyrene particles, must be centered in order to utilize automated high content imaging systems in cell based screens. Therefore, the objective of this work is to create a device to achieve the maximum centering efficiency of these microrafts and validate its

use with primary neurons. **Methods:** The magnet array plate was first conceived and modeled in 3D using SolidWorks. Finite element models were then created using COMSOL to analyze the magnetic field, force of attraction, and interaction between neighboring magnets. A test prop was created from poly(dimethylsiloxane) (PDMS) with a 3 x 3 embedded array of magnets, and the final device was fabricated out of a translucent polymer using an automated CNC milling machine. **Results and Conclusions:** The results of this study include finite element analysis, physical centering tests and cell viability measurements using the magnet array plate. The finite element analysis shows trends in the attractive force on the microrafts at various distances from the magnet as well as the field interaction between neighboring magnets. We proved that microrafts can be attracted and centered with no interference from neighboring magnets. The physical centering tests consisted of two different techniques and the mean centering efficiency as well as the overall technique efficiency is reported and compared. Additionally, cell viability assays were performed measuring neuron viability before and after the centering process. The results from these tests conclude that this device displays a remarkable ability to center and secure the microrafts, with no loss in neuron viability, when loaded before or after placement of the multiwell plate over the magnet array plate.

Disclosures: K. Gordon: None. **A.M. Taylor:** None.

Poster

269. Optical Methods I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 269.04/TT43

Topic: G.04. Physiological Methods

Title: Chronic cranial window with access port allowing repeated cellular manipulations and electrophysiology

Authors: *C. J. ROOME, B. KUHN
Optical Neuroimaging Unit, OIST, Okinawa, Japan

Abstract: Chronic cranial windows have been instrumental in advancing *in vivo* imaging studies, permitting long term high resolution imaging in various brain regions (Holtmaat, A. et al. 2009). Despite their utility, a major limitation remains in the window design: Access to the brain beneath the window for cellular manipulations such as drug delivery or dye loading but also electrophysiology is presently not achievable but indispensable for many projects. To overcome this limitation we have designed a chronic cranial window that allows direct brain

access via glass or quartz pipettes and metal electrodes. This device comprises of a regular cranial window glass with drilled access hole which is sealed with biocompatible silicone before mounting. This chronic cranial window with access port is cheap, easy to manufacture, and can be mounted just as the regular glass coverslip window described by Holtmaat et al., 2009. Multiple injections or recordings can be performed through the silicone seal by beveled or skillfully broken glass or quartz pipettes, patch pipettes, or metal electrodes. Following each retraction of the pipette or electrode, the silicone re-seals and by this maintains the sterile cranial window necessary for long term, high resolution *in vivo* imaging. As an example, we demonstrate how the chronic cranial window with access port can be used to combine *in vivo* imaging with cellular manipulation by repetitively bolus loading calcium sensitive dye (oregon green bapta-1 AM) into cortical layer 2/3 of barrel cortex and recording their spontaneous activity in lightly anaesthetized mice for up to 8 times over a period of 6 weeks. During this period we do not find any signs of infection or change in neuronal activity. However, care has to be taken to avoid blood vessel puncture. As for the regular chronic cranial window, regrowth of bone can hamper the imaging quality and prevent simple access to the brain. As an example for electrophysiology, multiple recordings with metal electrodes can be easily performed through the access port of the window. Additionally, in a terminal experiment, the silicone plug can be removed exposing the dura which allows electrophysiology experiments as in acute preparations. This includes *in vivo* whole-cell patch clamp or sharp recordings. Reference: Holtmaat, A. et al. (2009) Nature protocols 4, 1128 - 1144

Disclosures: **C.J. Roome:** None. **B. Kuhn:** None.

Poster

269. Optical Methods I

Location: Halls A-C

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Topic: G.04. Physiological Methods

Support: NIMH Grant MH084961

NIH Grant RR025786

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Title: Intrinsic connectivity network strength modulated by working memory load: An fNIRS study

Authors: *F. A. FISHBURN[1,2], M. E. NORR[3], A. V. MEDVEDEV[4], C. J. VAIDYA[3,5]
[2]Interdisciplinary Program in Neurosci., [3]Dept. of Psychology, [1]Georgetown Univ., Washington, DC; [4]Ctr. for Functional and Mol. Imaging, Georgetown Univ. Med. Ctr., Washington, DC; [5]Children's Res. Inst., Children's Natl. Med. Ctr., Washington, DC

Abstract: Background: In light of findings showing similar resting-state and task-evoked functional network architecture in fMRI, we examined whether intrinsic connectivity networks (ICNs) identified with fNIRS (functional Near-Infrared Spectroscopy) during the resting-state were sensitive to cognitive load demands. We identified ICNs in the resting state and used regression to examine working memory (WM) load-dependent activation and functional connectivity (FC) of individual networks. **Methods:** Sixteen subjects (6 male) were subjected to a 10-minute resting scan followed by a 6.5-minute letter n-back task with loads of 1-, 2-, and 3-back. Optical data were recorded on a two-wavelength (690 and 830 nm) continuous-wave CW5 imaging system (TechEn, Inc., Milford, MA). The 40 optical channels covered: ventrolateral prefrontal cortex (vlPFC), dorsolateral prefrontal cortex (dlPFC), frontopolar cortex (FP), and parietal cortex (Par). Raw signals were converted to oxygenated hemoglobin concentration. Resting-state signals were then filtered to .009-.09 Hz, downsampled to 2 Hz, and trimmed to the middle 6 minutes. Each channel was then normalized such that its root-mean-square (i.e., quadratic mean) was equal to 1. Data from each subject was concatenated to produce a group timecourse for each channel. Independent component analysis (ICA) was performed using FastICA. The positive and negative portions of each component were separated, doubling the number of components. The task signals were filtered to .009 - 2 Hz and downsampled to 10 Hz. The unmixing matrix from the resting-state ICA was used to project the task data into network space for each subject. The task-related components were then analyzed for WM load-dependent activation using NIRS-SPM. To assess FC, the outer product of the network channel weights was taken to produce an FC matrix for each network. These matrices were used to compute mean within-network correlation for each n-back load. The correlation values were then regressed against load for each network. **Results:** ICA yielded 6 networks: 1) parietal, 2) frontopolar, 3) anterior PFC, 4) vlPFC and frontal pole, 5) lateral PFC, 6) dlPFC, parietal, and frontal pole. Network activation increased with increasing n-back load for IC #5 ($t=2.18$, $p<.05$). FC increased with increasing n-back load for ICs #1 ($t=3.51$, $p<.005$) and #6 ($t=3.08$, $p<.005$). **Conclusions:** These results show that ICNs identified from resting-state fNIRS signals exhibit cognitive-load sensitive activation and FC. Thus, cognitive load effects can be expressed as a modulation of engagement of specific ICNs. Network-level analyses may facilitate probing cognitive phenomena using fNIRS.

Disclosures: F.A. Fishburn: None. **M.E. Norr:** None. **A.V. Medvedev:** None. **C.J. Vaidya:** None.

Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: National Research Foundation of Korea Grant (NRF-2010-H1C6A1)

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Title: Development of a transmembrane fusion protein with tagging peptide for optical sensing of membrane potential during neuronal activity

Authors: *S. LEE[1], Y. BANG[1,2], J. LEE[1], J. JANG[1], Y.-K. SONG[1,2]
[1]Dept. of Transdisciplinary Studies, Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Advanced Inst. of Convergence Technol., Suwon, Korea, Republic of

Abstract: Since the late 1970s when Erwin Neher and Bert Sakmann invented a method called patch clamp, it has been extensively used for studying electrical properties of neurons. The technique has many advantages but also some limitations such as invasiveness, poor spatial resolution, difficulty to target specific population of neurons and inability to study multiple cells simultaneously1. In an attempt to overcome these limitations, several types of genetically encoded voltage indicators(GEVIs) that optically image membrane potential change have been developed since the late 1990s2. Many researchers developed GEVIs so far utilizing one or two fluorescent proteins to report voltage change of neurons and majority of them placed the fluorescent proteins in intracellular side2. So we came up with an idea to place a fluorophore in the outside of a cell to improve ability of a voltage sensor in reporting membrane potential change. To realize the idea, we have been working on to devise a voltage sensor combined with a fusion protein domain3 that connects the intracellular terminus of voltage sensing part to the extracellular side. Also, we have incorporated a tagging peptide4, Avitag, to connect the voltage indicator with an extrinsic fluorophore such as quantum dots or a nanoparticle working as fluorescence quencher. Several gene constructs containing the components above have been designed and prepared. The gene constructs were expressed in HEK293 cells and in cultured

primary neurons. The expression level of the protein based voltage sensors were verified with fluorescence microscopy and western blot analysis. Future plans of this study include toxicity tests such as phototoxicity and effects to membrane capacitance, and verification of photophysical properties using electrophysiology and optical techniques in several cell types such as HEK293 cells, cultured primary neurons, and acute brain slices of mouse. References [1]Scanziani M. & Häusser M. Electrophysiology in the age of light. Nature, 461(7266), 930-939 (2009) [2]Motoh H. & Knöpfel T. Probing neuronal activities with genetically encoded optical indicators: from a historical to a forward-looking perspective. Pflugers Arch - Eur J Physiol,465:361-371 (2013) [3]Kleinlogel S.,Terpitz U., Legrum B.,Gökbuget D.,Boyden E.S., Bamann C., Wood P.D. & Bamberg E. A gene-fusion strategy for stoichiometric and co-localized expression of light-gated membrane proteins. Nature Methods, 8, 1083-1088 (2011) [4]Chen, I., Howarth, M., Lin, W. Y., & Ting, A. Y. Site-specific labeling of cell surface proteins with biophysical probes using biotin ligase. Nature Methods, 2(2), 99-104 (2005)

Disclosures: **S. Lee:** None. **Y. Bang:** None. **J. Lee:** None. **J. jang:** None. **Y. song:** None.

Poster

269. Optical Methods I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 269.07/TT46

Topic: G.04. Physiological Methods

Support: Teleton Grant GGP10138D

Title: *In vivo* measurement of intracellular Chloride and pH during neuronal development by means of 2-photon spectroscopy

Authors: S. SULIS SATO[1], P. ARTONI[1], A. I. IDILLI[2], S. LANDI[3], S. LUIN[1], R. PARRA[1], J. SZCZURKOWSKA[4], F. TROVATO[1], D. AROSIO[2], F. BELTRAM[1], L. CANCEDDA[4], *G. RATTO[3]
[1]NEST, Scuola Normale Superiore, Pisa, Italy; [2]Inst. di Biofisica, Consiglio Nazionale delle Ricerche & Fondazione Bruno Kessler, Trento, Italy; [3]Inst. Nanoscience, CNR, Pisa, Italy; [4]Inst. Italiano di Tecnologia, Genova, Italy

Abstract: The regulation of the intracellular concentration of Chloride ($[Cl^-]_i$) is an important modulator of inhibitory neurotransmission in the brain. In adult neurons, the Cl^- Nerst equilibrium is close to the resting membrane potential, and GABAergic activity through

ionotropic Cl^--permeable $GABA_A$ receptors plays its inhibitory role by inducing inward chloride fluxes that hyperpolarize the membrane potential. In the immature brain, where GABA is the predominant neurotransmitter, GABA behaves as a depolarizing and mostly excitatory neurotransmitter. By increasing network excitability and promoting the maintenance of spontaneous activity, GABA in early development exerts a key role in the maturation of neuronal networks. $[Cl^-]_i$ is regulated by the expression of two cotransporters, NKCC1 and KCC2, which move Cl^- in opposite directions. Interestingly, their expression is strongly regulated during development: NKCC1 is highly expressed in the pre and neonatal life, whereas KCC2 is mostly expressed in the adult; this should cause a shift in $[Cl^-]_i$ between neonatal and adult brains. Although several studies in slice and cell cultures have suggested that Cl^- is depolarizing in the perinatal period in immature neurons, the *in vivo* absolute measurement of the $[Cl^-]_i$ shift is still missing. Here, we have devised a method for absolute measurement of $[Cl^-]_i$ (and pH) *in vivo* in rodents by using a modified genetically-encoded sensor (ClopHensor). This sensor is formed by the fusion between a pH and Cl^- sensitive GFP (E^2GFP) and an insensitive RFP (LSS-mKate). First, we characterized the 2-photon spectral properties of the sensor, showing that increasing $[Cl^-]_i$ causes a decrease of the green/red fluorescence. A shift in pH causes a strong alteration of the 2-photon excitation spectrum of E^2GFP. Then, the sensor was targeted to the visual or somatosensory cortex by means of *in utero* electroporation at E15.5 (mouse) or E17.5 (rat). We demonstrated the integrity and stoichiometry of the sensor by *in situ* fluorescence correlation spectroscopy and by measuring trafficking across the nuclear membrane. We found that brain tissue has a sizable wavelength-dependent effect on scattering and extinction of excitation and emission and we devised a method to compensate for these effects. Finally, we determined that $[Cl^-]_i$ is very high until P8 (50.6±21.5 mM) and then strongly decreases (12.9±8.2 mM) after P18 suggesting a switch of GABAergic transmission from depolarizing to hyperpolarizing *in vivo*. Surprisingly, we have also found a shift in pH during development from more acidic values at P8 (median 6.85) to more basic values at P18 (median 7.2).

Disclosures: **S. Sulis Sato:** None. **P. Artoni:** None. **A.I. Idilli:** None. **S. Landi:** None. **S. Luin:** None. **R. Parra:** None. **J. Szczurkowska:** None. **F. Trovato:** None. **D. Arosio:** None. **F. Beltram:** None. **L. Cancedda:** None. **G. Ratto:** None.

Poster

269. Optical Methods I

Location: Halls A-C

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Topic: G.04. Physiological Methods

Support: Stanford University Bio-X Interdisciplinary Initiatives Project

National Science Foundation grant 1134416

Walter V. and Idun Berry Postdoctoral Fellowship

The Rita Allen Foundation

DARPA

Title: Genetically encoded voltage sensors for optical monitoring of rapid neuronal activity

Authors: *F. ST-PIERRE[1], Y. YANG[1], X. DING[2], J. D. MARSHALL[1], Y. GONG[1], M. J. SCHNITZER[1], M. Z. LIN[1]
[1]Stanford Univ., Stanford, CA; [2]Univ. of California at San Diego, San Diego, CA

Abstract: The ability to monitor electrical activity in genetically-defined populations of individual neurons would empower the functional dissection of the brain. In particular, being able to follow rapid trains of action potentials (APs) would help understand how neuronal networks encode and process information as patterns of electrical activity. To enable optical detection of neuronal activity, we recently developed a novel genetically encoded voltage indicator (GEVI) called Accelerated Sensor of Action Potentials 1 (ASAP1) [St-Pierre et al., Nature Neuroscience (2014)]. While ASAP1 enables optical detection of single and trains of APs *in vitro* and *in vivo*, we sought to improve its response amplitude and brightness to facilitate detection of smaller voltage transients. We also wanted to increase ASAP1 kinetics to further improve its accuracy in representing the underlying voltage transients. From our rational screening approach, we obtained new ASAP variants with improved performance. Our work also highlights how key amino acids within ASAP can tune the its fluorescence response, presumably by affecting the voltage-dependent movement of ASAP's voltage sensitive domain.

Disclosures: F. St-Pierre: None. **Y. Yang:** None. **X. Ding:** None. **J.D. Marshall:** None. **Y. Gong:** None. **M.J. Schnitzer:** None. **M.Z. Lin:** None.

Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: NIH DA029706

NIH EB003832

Hoffman-La Rouche 88610A

Title: New optical CNiFERs to detect neuropeptides

Authors: J. ZHANG[1], A. MULLER[2], L. MELLANDER[2], E. LACIN[1], D. KLEINFELD[2], M. FERNANDO[1], *P. A. SLESINGER[1]
[1]Mount Sinai, Friedman Brain Inst., New York, NY; [2]UCSD, La Jolla, CA

Abstract: Neuropeptides are essential participants in the regulation of neural activity and the control of vascular tone. Although neuropeptides are widely expressed, the mechanisms, dynamics and consequences of neuropeptide release *in vivo* remain largely unexplored, largely due to a lack of analytical approaches for neuropeptide detection We are developing new biophotonic tools to monitor the release of neuropeptides in real-time in awake animals. We have previously created a cell-based neurotransmitter fluorescent engineered reporter (CNiFER) for detecting acetylcholine (Nguyen, Schroeder et al. 2010, *Nature Neuroscience* 13:127). CNiFERs are HEK293 cells engineered to express a specific G-protein coupled receptor and a genetically encoded FRET-based Ca2+ sensor, TN-XXL. Activation of GPCRs that couple to endogenous Gq G-proteins trigger an increase in cytosolic [Ca2+] through the PLC/IP3 pathway, leading to an increase in FRET. Here, we report on the development of CNiFERs for detecting three neuropeptides; orexin, somatostatin and vasoactive intestinal peptide (VIP). For the orexin CNiFER, we used the OX1 and OX2 receptors, which both respond to orexin A and B peptides. The OX1 receptor couples to Gq while the OX2 receptor couples to Gi G-proteins. The OX2 CNiFER therefore required creating a HEK293 cell expressing a G protein chimera Gqi5, which enables the OX2 to stimulate the Gq pathway. The same approach was used for the somatostatin CNiFER, where we used the Gi G-protein coupled receptor SST2. For the VIP CNiFER, we used VPAC2, which couples to Gs G-proteins and hence required coexpression of the chimeric Gqs5 G-protein. We have created CNiFER clones for OX1 and OX2, which have EC50's of ~5 nM and ~35 nM respectively, which are comparable to the EC50s for native OX1/2 receptors in the brain (10-50 nM). The somatostatin CNiFER has an EC50 of ~475 nM and the VIP CNiFER has an EC50 of ~10 nM. Importantly, each neuropeptide CNiFER line has little or no non-specific response to a panel of other neuromodulators. These new CNiFERs provide a unique means to measure neuropeptide release *in vivo*, which in addition can be combined with complementary imaging techniques, such as genetically encoded Ca2+ indicators.

Disclosures: J. Zhang: None. **A. Muller:** None. **L. Mellander:** None. **E. Lacin:** None. **D. Kleinfeld:** None. **M. Fernando:** None. **P.A. Slesinger:** None.

Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: Burroughs Wellcome Fund

Michael J. Fox Foundation

DARPA

Searle Scholar Program

Title: Transcriptomic analysis of CLARITY-processed tissues

Authors: *J. H. CHO[1], E. MURRAY[1], X. ADICONIS[2], J. LEVIN[2], K. CHUNG[1]
[1]MIT, Cambridge, MA; [2]Broad Inst., Cambridge, MA

Abstract: Understanding the molecular basis of the pathological changes in the diseased brain is vital for developing improved therapeutics. Various technologies have been developed to deduce and quantify transcriptomic details from distinct population of cells. However, the destructive nature of existing methods makes it difficult to correlate these molecular signatures to many crucial properties (e.g. connectivity, morphology, activity). Here, we will present a novel technique capable of selectively cataloging desired transcripts from subpopulation of cells with known functional, spatial, connectomic, and morphological details. This technique exploits the imaging capability and the biomolecule-retention efficiency of CLARITY-treated brains to synthesize and capture cDNA *in situ*. First, we will evenly disperse reverse-transcriptases throughout the CLARITY-processed brain and synthesize cDNA *in situ*. Next, target cells will be optically identified and precisely heat-treated to release the bound cDNA. Freed cDNA molecules will be then extracted by electrophoresis through the porous CLARITY-treated brain into an anodic gel-loaded capillary. The gels in the capillary captures cDNA molecules as they migrate through. Once the capture is complete, the gels are melted and released from the capillary, and the cDNA bound to the gels will be then eluted and analyzed using RNA-Seq. This approach may enable gene profiling of distinct population of cells with known connectivity, morphology, and molecular properties in human brain tissues as the method does not require any genetic manipulation *in vivo*.

Disclosures: **J.H. Cho:** None. **E. Murray:** None. **X. Adiconis:** None. **J. Levin:** None. **K. Chung:** None.

Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: Simons Postdoctoral Fellowship

Burroughs Wellcome Fund Career Awards at the Scientific Interface

Michael J. Fox Foundation

Searle Scholar Program and DARPA

Title: Rapid and quantitative phenotyping of intact biological systems

Authors: *S.-Y. KIM[1], E. MURRAY[1], J. H. CHO[2], N. BAKH[2], K. OHN[2], K. CHUNG[1]
[1]Inst. of Med. Engin. and Sci., [2]Dept. of Chem. Engin., MIT, Cambridge, MA

Abstract: For decades, sectioning-based two-dimensional molecular phenotyping techniques have been extensively used for investigation of tissue samples across biology and medicine. These techniques ensure that molecular targets in ultrathin tissue slices experience relatively similar reaction conditions (e.g. probe concentration and reaction time) to achieve complete and uniform labeling of given samples. However, three-dimensional information is lost unless a sophisticated and laborious 3D reconstruction is employed. CLARITY has succeeded in proving that an intact brain could be labeled, but penetration was poor because passive diffusion of molecular probes in the dense nanoporous mesh was slow (Chung et al., Nature 2013). Even near the surface, labeling was highly non-uniform due to large variations in probe concentration and in the probe-target interaction time across the superficial layers. Moreover, labeling of the crucial inner structures remained incomplete even after months. These critical limitations of the current techniques have prevented us from obtaining system-wide quantitative molecular information from large scale intact tissues. Here we introduce a new technology (termed eTANGO) that addresses these challenges by integrating two novel concepts: stochastic electrotransport and dynamic affinity shift. The penetration problem is solved by stochastic electrotransport, which selectively and rapidly drives only charged molecules (e.g. antibodies and RNA probes) without disrupting surrounding charged matrix (e.g. brain). Furthermore, probe-target binding affinities are dynamically modulated to synchronize reaction times brain-wide. Integration of these two concepts enables all the endogenous molecular targets in billions of cells to experience the same reaction condition (time and concentration). This yields complete and uniform staining of the

intact brain within hours. We applied eTANGO to immunostain various cellular, molecular and structural markers and further demonstrate quantitative molecular phenotyping over a large volume. The unique strength of eTANGO_complete homogeneous immunostaining_also opens the possibility of high-throughput brain-wide proteomic profiling at single cell resolution. Together with CLARITY, we anticipate eTANGO to facilitate integrated understanding of large-scale intact biological systems.

Disclosures: S. Kim: None. **E. Murray:** None. **J.H. Cho:** None. **N. Bakh:** None. **K. Ohn:** None. **K. Chung:** None.

Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: Brain Korea 21 Plus Project, the Department of Electrical and Computer Engineering, Seoul National University

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Technology Innovation Program (10033657) of the Ministry of Knowledge Economy (MKE)

Global Frontier Progect(CISS-2012M3A6A6054204)

Title: Enhanced infrared peripheral nerve stimulation using local heating of gold nanorods

Authors: K. EOM[1], *S. JUN[2,3], S. HWANG[4], Y. LEE[4], K. BYUN[5], S. KIM[1]
[1]Dept. of Electrical and Computer Engin., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Electronics, [3]Dept. of Brain and Cognitive Sci., [4]Dept. of Electronics Engin., Ewha Womans

Univ., Seoul, Korea, Republic of; [5]Dept. of Biomed. Engin., Kyung Hee Univ., Yongin, Korea, Republic of

Abstract: Infrared neural stimulation (INS) is gaining increasing attention due to the large penetration depth, the contact-free and the electrical artifact-free stimulation as well as no genetic modification. However, the conventional INS suffers from tissue damage due to the high dosage of laser exposure and the poor light confinement. Hence, a safe and effective INS technique is required. Here, we propose an enhanced INS method using localized surface plasmon resonance (LSPR) of gold nanorods. To verify the photothermal effect of plasmonic gold nanorods on neural stimulation, *in vivo* experiments were performed with sciatic nerves of Sprague-Dawely rats. Pulsed infrared laser (wavelength: 980 nm, duration: 1 msec) was irradiated on to the sciatic nerves after injecting gold nanorods. From a location of 50 mm away from the optical stimulation site, compound nerve action potentials (CNAPs) were extracellularly recorded. The evoked CNAPs were compared when the optical stimulations were applied in the presence and the absence of the gold nanorods. When stimulating the nerve with gold nanorods, 0.159 J/cm2 was the stimulation threshold, while 0.480 J/cm2 was obtained in the absence of gold nanorods. Moreover, the responsivity of CNAP was more than five times higher in the presence of the gold nanorods. It is likely that the heat generated from the LSPR phenomena could effectively accelerate the depolarization of the neuronal cells with a lower optical power. In summary, we successfully showed the remote and safe activation of neural tissue via the LSPR effect of gold nanorods with the increased neural responsivity and the lower threshold level.

Disclosures: K. Eom: None. **S. Jun:** None. **S. Hwang:** None. **Y. Lee:** None. **K. Byun:** None. **S. Kim:** None.

Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: NIH Grant 5R01GM098089-02

Title: Single-molecule patch-clamp fret microscopy studies of nmda receptor dynamics in living cells

Authors: *H. LU, D. K. SASMAL
Dept. of Chem., Bowling Green State Univ., Bowling Green, OH

Abstract: Stochastic and inhomogeneous conformational changes regulate the function and dynamics of ion channels that are crucial for cell functions, neuronal signaling, and brain functions.We have developed a new combined approaches of using single ion channel patch-clamp electrical recording and single-molecule fluorescence imaging for probing ion channel conformational changes simultaneously with the electrical single channel recording. By combining real-time single-molecule fluorescence imaging measurements with real-time single-channel electric current measurements in artificail lipid bilayers and in living cell membranes, we were able to probe single ion-channel-protein conformational changes simultaneously, and thus providing an understanding the dynamics and mechanism of ion-channel proteins at the molecular level.(1-4) We will focus our discussion on the new development and results of real-time imaging of the dynamics of gramicidin, colicin, and NMDA receptor ion channels in lipid bilayers and living cells. Our results shed light on new perspectives of the intrinsic interplay of lipid membrane dynamics, solvation dynamics, and the ion channel functions. Reference: 1.Suneth P. Rajapaksha, Xuefei Wang, H. Peter Lu, “Suspended Lipid Bilayer for Optical and Electrical measurements of Single Ion Channel Proteins,” *Anal. Chem.*, **85**, 8951-8955 (2013). 2. H. Peter Lu, "Combined Single-Molecule Electrical Recording and Single-Molecule Spectroscopy Studies of Ion Channel Conformational Dynamics," an invited book chapter in Methods in Nano Cell Biology, edited by Bhanu Jena, ELSEVIER (2009). 3.G. Harms, G. Orr, H. Peter Lu, "Probing ion channel conformational dynamics using simultaneous single-molecule ultrafast spectroscopy and patch-clamp electric recording," *Appl. Phys. Lett.*, **84**, 1792-1794 (2004). 4.Greg S. Harms, Galya Orr, Mauricio Montal, Brian D. Thrall, Steve D. Colson, H. Peter Lu, "Probing Conformational Changes of Gramicidin Ion Channels by Single-Molecule Patch-Clamp Fluorescence Microscopy," Biophys. J., **85**, 1826 (2003).

Disclosures: H. Lu: None. **D.K. Sasmal:** None.

Poster

269. Optical Methods I

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 269.14/TT53

Topic: G.04. Physiological Methods

Title: Development of wearable monitoring system for acquisition of physiological information from diverse portions of the whole body using near infrared lights (NIRS)

Authors: *H. EDA[1], R. SHIMOKITA[2], T. YAMAUCHI[2], N. KAZETANI[2], H. WADA[2], T. SAWADA[2], K. UJIIE[2], K. SOETA[3], Y. OHTAKI[3], M. SAKURAI[3], M. HIRAYAMA[3], S. IJUIN[3]
[1]Grad. school of GPI, Hamamatsu, Japan; [2]Genial Light. co.,LTD, Hamamatsu, Japan; [3]ALPS ELECTRIC CO., LTD, Tokyo, Japan

Abstract: Near infrared spectroscopy (NIRS) calculates hemoglobin parameters, such as change in oxygenated hemoglobin and deoxygenated hemoglobin (deoxyHb), using the near infrared lights around the wavelength of 800 nm. We developed portable NIRS (Eda et al., SfN2007). Other compact NIRSs were reported after that. Since the portable system can measure a brain with a free posture and can evaluate a brain also outside a laboratory, it sustains the possibility of the new application of the brain measurement technique. However, some reports seem to be not brain activation but a skin blood flow, because the skin blood flow of a forehead is changed by a posture. Some reports are also showing deoxyHb increases, this is contradictory to the blood oxygenation level dependent (BOLD) theory of fMRI. The BOLD theory is founded on the decreasing of the deoxyHb. The purpose of this research is to examine the technique of measuring brain activation correctly with the free posture measurement system. It is hard to demonstrate that the data mainly reflects the brain activation. One of the proving methods is to measure parts of whole body other than a brain. We developed the wearable system, which can measure each part of the whole body. One probe of the system has two light sources and one detector. Two light sources are near-infrared LEDs of two wavelengths. Now optical measurement is used for not only NIRS (2 or more lights, near infrared, some include visible) but the pulse oximeter (2 LEDs, one is visible and the other is near infrared), the plethysmography (1 LED, visible), etc. A pulse rate can be calculated as a periodic change of hemoglobin, and it is strongly influenced of autonomic nerves and of a motion of the whole body. This system was induced into a higher sampling rate than conventional NIRS, so that a pulse waveform could be exposed. Several pieces of the whole body, such as both hands, the head, and a breast, were evaluated using this system. The analog output of a pulse waveform measured by this system and that by the pulse oximeter (Nihon Kohden Corp., Japan) were compared. We put the probe on the fingertip, the wrist, the forehead, the temple, the cheek, the top of the head, the back of the head, and the neck, the heel of a leg, and several places of the left breast. The pulse waveform has been measured, respectively. Measurement of various parts showed that pulse waveforms differed, respectively. The pulse waveform of the fingertip measured by this system and that by the pulse oximeter were in agreement. This wearable system enables it to acquire physiological information and to discuss about the reliability of the brain activation data in a free posture.

Disclosures: H. Eda: None. **R. Shimokita:** None. **T. Yamauchi:** None. **N. Kazetani:** None. **H. Wada:** None. **T. Sawada:** None. **K. Ujiie:** None. **K. Soeta:** None. **Y. Ohtaki:** None. **M. Sakurai:** None. **M. Hirayama:** None. **S. Ijuin:** None.

Poster

269. Optical Methods I

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Program#/Poster#: 269.15/TT54

Topic: G.04. Physiological Methods

Support: NSF CIF-BCSP-1212778 to R.J.G.

Title: Fruit fly functional imaging

Authors: *S. AIMON[1], T. KATSUKI[1], L. GROSENICK[2], M. BROXTON[2], K. DEISSEROTH[2], R. J. GREENSPAN[1]
[1]Kavli Inst. For Brain and Mind UCSD, La Jolla, CA; [2]Departments of Computer Sci. and Bioengineering, Stanford Univ., Stanford, CA

Abstract: We seek to understand neuronal dynamics at the level of a complete network. For this purpose we use light field microscopy to observe neuronal activity in the whole brain of behaving flies. A calcium sensor -GCamp6- or voltage sensor -Arclight- are expressed, either pan-neuronally, or in subsets of neurons broadly distributed in the brain (including dopamine, octopamine, NPF, and FruM neurons). We then observe the whole brain's fluorescence through light field microscopy, and record at 100Hz with a high-speed CMOS camera. We are currently exploring the response of the network in various situations: while the fly is behaving freely (typically walking or grooming) and in response to a range of stimuli (including an air puff, an odor, a flash of light, sound...). We have also developed a paradigm to induce panic-like and relief-like behaviors while recording brain activity. 3D stacks are then reconstructed from the light field images using wave optics to model point spread functions, before applying 3D-deconvolution. We then use techniques taken from fMRI data analysis (including seed-based correlation and independent component analysis) to extract the activity of some single neurons, brain regions, and networks. This study is a first step towards outlining brain-wide functional networks for either spontaneous or event related activity in *Drosophila*.

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Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: NIH GM053395

NIH NS069720

NIH DA035612

Title: Multichromic optical interrogation of neural systems using caged compounds

Authors: *J. M. AMATRUDO, J. P. OLSON, H. K. AGARWAL, G. C. R. ELLIS-DAVIES
Neurosci., Mount Sinai Sch. of Med., New York, NY

Abstract: Caged compounds have been widely used by neurophysiologists to study many aspects of cellular signaling in glia and neurons. Most of these studies have been conducted with near-UV light in the 330-410 nm range. Recently, two-photon (2P) excitation at about twice this wavelength range has proved very useful for the optical interrogation of visually designated spine heads, principally because two-photon uncaging can be fine scaled in terms of space, time and amplitude so as to mimic quantal release. We show that recently developed caging chromophores (RuBi and DEAC450) that are photolyzed with blue light (ca. 430-480 nm range) can be combined with traditional nitroaromatic caged compounds (e.g. MNI and CDNI) to enable two-color optical probing of neuronal function. For example, one-photon uncaging of RuBi-GABA (10 uM) with a 473-nm laser is facile, and can block action potentials evoked by 2P uncaging of CDNI-Glu (1 mM) at 720 nm. The success of these two-color experiments rests upon both the optical transparency of CDNI at 473 nm and the low concentration of RuBi required for effective GABA uncaging with blue light. The power used to uncage glutamate at 720 nm evoked no response from RuBi due to this low concentration (RuBi has 70% activity at 720nm compared its maximum at 800nm). We also show that 2P uncaging of DEAC450-Glu (0.4 mM) and CDNI-GABA (1 mM) at 900 and 720 nm, respectively, can be used to fire and block action potentials. Since 2P uncaging required that the DEAC450 cage was applied at 40x higher concentration than that required for blue light, the success of this experiment rests on the significant 2P absorption minimum of this cage at 720 nm (60-fold lower than 900 nm). Note, CDNI is optically transparent at 900 nm. To date almost no 2-color actuation experiments have been published, largely because all previous long wavelength chromophores are effectively excited at short wavelengths. Our experiments illustrate that our recently developed

chromophore (DEAC450) has taken uncaging out of the "monochrome era" in which it existed since 1978, so as to enable multichromic interrogation of neuronal function with single synapse precision. Since the uncaging technique has been applied to the widest possible array of molecules (natural products such neurotransmitters, peptides, DNA, enzymes, miRNA, calcium and cAMP, and non-natural products such as fluorophores, drugs and antibodies), we believe that bimodal, wavelength-selective examination of many cellular processes is now possible.

Disclosures: J.M. Amatrudo: None. **J.P. Olson:** None. **H.K. Agarwal:** None. **G.C.R. Ellis-Davies:** None.

Poster

269. Optical Methods I

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Support: NIH grant 5-R01MH083686-05

NIH grant 5-R37NS081242-02

Title: Imaging medial prefrontal and entorhinal cortex at cellular resolution in behaving mice using chronically implanted microprisms

Authors: *R. J. LOW, Y. GU, D. W. TANK
Princeton Univ., Princeton, NJ

Abstract: *In vivo* two-photon microscopy provides the foundation for an increasingly powerful array of techniques for measuring the structure and function of neural circuits. However, its use in deep brain tissue is limited by scattering, absorption, and out of plane fluorescence excitation[1]. Several approaches have been developed for deep imaging, but challenging tissue properties and geometry continue to prevent optical access to a key set of brain regions: those located along the walls of deep fissures. These regions include widely studied structures such as medial prefrontal and medial entorhinal cortex (mPFC and MEC), which are of central importance to learning, memory, and decision making. We present a general method for *in vivo* two-photon imaging of brain regions situated within fissures. Our approach involves inserting a right angle microprism into the fissure, which bends the optical path within the brain by 90 degrees and provides optical access to the fissure wall with subcellular resolution. We designed an implantable microprism

assembly for long-term, chronic imaging, and characterized its optical properties. We also developed novel surgical procedures for implantation amidst the unique anatomical features that surround fissures, including overlying venous sinuses (superior sagittal, transverse), associated vasculature (e.g. bridging and emissary veins), and dural folds and attachments. Our approach allows imaging during head-fixed behavior, such as navigation and decision making tasks in virtual reality. We demonstrate fissure imaging in mPFC and MEC, which lie within the longitudinal and transverse fissures, respectively (between cerebral hemispheres, and between the cortex and cerebellum). We imaged mPFC and MEC in head fixed mice running on a spherical treadmill, and performed chronic recordings over multiple weeks. We recorded structural images using fluorescent marker proteins, and measured network activity at cellular resolution using the GCaMP family of genetically encoded calcium indicators. By providing optical access to fissures, our approach opens up these regions for study at cellular resolution in behaving animals using a rapidly expanding palette of optical tools for perturbing and measuring network structure and function. References: 1. Theer P, Denk WJ. Opt Soc Am A Opt Image Sci Vis 23, 3139-3149 (2006)

Disclosures: **R.J. Low:** None. **Y. Gu:** None. **D.W. Tank:** None.

Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: CIHR MOP-86762

Title: Optimizing CLARITY for the analysis of brain wide activity mapping

Authors: ***J. R. EPP**[1], Y. NIIBORI[2], H.-L. L. HSIANG[2], K. DEISSEROTH[3], S. A. JOSSELYN[2], P. W. FRANKLAND[2]
[1]Program in Neurosciences and Mental Hlth., Hosp. For Sick Children, Toronto, ON, Canada; [2]Hosptial For Sick Children, Toronto, ON, Canada; [3]Stanford Univ., Palo Alto, CA

Abstract: Understanding the coordinated activity of neural circuits throughout the entire brain is a central goal in neurobiology. Such a goal requires techniques that allow for high throughput imaging of whole brains at cellular resolution. A recently described technique known as CLARITY can be used to produce structurally intact yet optically transparent tissue that can be

labeled and imaged without the need for sectioning. The CLARITY technique involves replacement of opaque lipids with a hydrogel matrix. By exchanging the membrane lipids with a clear hydrogel matrix, CLARITY transforms the opaque and impermeable brain into a transparent and porous structure. The hydrogel matrix maintains the structure and localization of proteins but is permeable such that immuno-labeling or *in situ* hybridization of intact rodent brains is possible. CLARITY is capable of generating incredibly detailed tissue for 3D analysis but the success of this procedure is dependent on a large number of factors such as, temperature, hydrogel composition, and polymerization conditions. Here we have investigated the effects of systematically varying these factors in order to provide an optimized and highly replicable procedure. This protocol is suitable for high throughput imaging with confocal and single plane illumination microscopy. In combination with cellular activity markers and an analytical approach for producing functional connectomes, CLARITY offers a strong approach for whole brain activity mapping in the mouse brain.

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Poster

269. Optical Methods I

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Topic: G.04. Physiological Methods

Support: NS078301

Title: Imaging action potentials and subthreshold depolarizations in single trials with single cell resolution using the FRET based hybrid voltage sensor hVOS

Authors: *N. GHITANI, P. BAYGUINOV, Y. MA, M. B. JACKSON
Univ. of Wisconsin, Madison, WI

Abstract: Genetically-encoded voltage sensors provide researchers with optical tools for imaging electrical activity in targeted populations of neurons. This approach has the potential to reveal the dynamic activity of neuronal circuits, and enable researchers to elucidate the complex mechanisms by which the nervous system processes information. The hybrid Voltage Sensor (hVOS) technique employs Förster resonance energy transfer between a fluorescent protein and small charged molecule to generate optical signals arising from voltage-induced changes in

donor-acceptor distance. We expressed hVOS in sparse subsets of layer 2/3 neurons of the mouse somatosensory cortex by in-utero electroporation. Electrical stimulation of cortical slices from these animals evoked fluorescence changes in single cells in single trials. We also recorded hVOS signals in single trials with single cell resolution in brain slices from the hippocampus and entorhinal cortex of a transgenic mouse line expressing this probe in sparse neuronal populations. These fluorescence signals tracked subthreshold depolarizations and action potentials with excellent temporal fidelity as confirmed by simultaneous whole cell patch clamp and fluorescence recordings. Action potentials triggered by current injection via the patch pipette in the entorhinal cortex produced a mean fluorescence response of 2.37 ± 0.38% and a signal to noise ratio of 11.32 ± 1.79. Signals were distinguished in as many as 9 different cells within one field of view, as well as in dendrites and cell bodies from the same neuron. The sequence of activation of cells provided insight into the propagation of electrical activity through the slice circuitry. Thus, hVOS provides a tool for studying a variety of forms of electrical signaling and circuit activity in genetically targeted cells in intact tissue. Supported by NIH grant R21 NS078301.

Disclosures: **N. Ghitani:** None. **P. Bayguinov:** None. **Y. Ma:** None. **M.B. Jackson:** None.

Poster

270. Optical Methods II

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Program#/Poster#: 270.01/TT59

Topic: G.04. Physiological Methods

Support: NIH Grant R21 NS078301

Title: Evaluating conduction and action potential broadening with an axonally targeted genetically-encoded voltage sensor

Authors: *Y. MA[1,2], M. B. JACKSON[2]
[1]Physiol. Grad. Training Program, [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Axonal conduction plays important roles in the transmission and processing of information throughout the nervous system, but the small size of axons often makes direct electrical recording of their voltage changes very difficult. We have addressed this problem by utilizing a genetically-encoded optical hybrid Voltage Sensor (hVOS), that preferentially targets axonal membranes. hVOS combines a fluorescent protein (FP) probe with a small negatively

charged molecule, dipicrylamine (DPA). DPA moves when membrane potential changes, to alter the donor-acceptor distance and generate a fluorescence change as the result of a change in Förster resonance energy transfer. The probe hVOS 2.0 was created from cerulean FP, tagged at the C-terminus with a truncated h-ras motif and at the N-terminus with a GAP-43 motif. Since GAP-43 is an axonal protein hVOS 2.0 preferentially targets axons when expressed under the control of thy-1 promoter in transgenic mice. Hippocampal slices from hVOS 2.0 transgenic mice show highly concentrated labeling in the stratum lucidum, due to dense probe expression in mossy fiber axons from granule cells. Strong labeling is also seen in the inner molecular layer of the dentate gyrus, due to probe expression in the axons of hilar mossy cells. hVOS imaging in these regions provides signals predominantly reflecting these axons, and because DPA crosses the membrane in less than 0.5 msec, hVOS signals can track action potentials with high temporal fidelity. Imaging experiments in mossy fibers revealed rapid action potentials which propagated with a conduction velocity of 0.23 ± 0.02 m/s. Furthermore, action potentials broadened during repetitive firing, with spike half-width increasing by up to 40% during high frequency stimulation (25 Hz). Spikes broadened somewhat less at lower frequencies. Frequency-dependent spike broadening in axons from hilar mossy cells was also detected in the inner molecular layer of the dentate gyrus. Conduction velocity of these axons was 0.18 ± 0.04 m/s. Thus, hVOS imaging with this axonally targeted probe provides evidence of activity-dependent action potential broadening in the axons of two distinct classes of neurons. Voltage imaging with hVOS 2.0 offers an opportunity to investigate action potential conduction and broadening in very fine axons that are inaccessible to electrical recording techniques. Genetically targeted hVOS 2.0 can be used to investigate signals in the axons of specific neuron subtypes. Supported by NIH grant R21 NS078301.

Disclosures: Y. Ma: None. **M.B. Jackson:** None.

Poster

270. Optical Methods II

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Program#/Poster#: 270.02/TT60

Topic: G.04. Physiological Methods

Support: NRF-2012R1A2A1A01007327

Title: Surface modification of micro electrode array with gold nanorods for photo-thermal stimulation of cultured neuronal networks

Authors: S. YOO[1], J. PARK[2], *Y. NAM[1]
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Abstract: Nanotechnology for controlling the molecular or cellular events in biological study has a significant impact, which is continuously extended to clinical purposes. However, design of the nanotechnologies to exploit the nerve system is still in early stage. Previously, we reported that photothermal stimulation using membrane bound gold nanorods (GNRs) can modulate electrical activity of neurons. Based on our finding, we hypothesized that nerve device that was integrated with GNR array could be a noble platform for modulation of neural network activity. Here, we developed a neural-interfacing nanoplatform by integrating GNR array with a multielectrode array (MEA). This nanoplatform provides bifunctional modality to electrically excite or optically inhibit the neural activity, which would be a useful tool for studying the dynamics of neural network. Gold nanorods were synthesized using seed-mediated method, and surface of the GNRs were coated with amine terminated poly ethylene glycol (PEG). Then, PEGylated GNRs were directly assembled on plasma treated MEA surfaces. Change of surface temperature and impedance of electrode were analyzed before and after GNR deposition. E18 rat hippocampal neurons were plated on the nanoplatform and early development and long-term survival rate were investigated. Near infrared (NIR, 785nm) laser was irradiated with 0~21mW/mm2 power density to matured neural networks, and change of spike rate was recorded. Concentration of the GNR on MEA surfaces was determined by changing the incubation time with GNRs. The number of deposited GNRs was increased by increasing the incubation time, but overlapping of the GNRs was not observed in our fabrication method. GNR array showed heat conversion of NIR laser, and its temperature was controllable depending on laser power density. Integrated MEA with GNR array did not show significant toxicity for long-term survival as well as early development. In contrast, surface photothermal stimulation (sPS) onto the nanoplatform showed significant changes on neural activity. Spontaneous activity of neurons was completely inhibited by 6mW/mm2 power density or gradually suppressed by increasing the sPS power up to 21mW/mm2. Our results indicated that GNR array integrated MEA system can provide a nontoxic and simple platform for studying the neural network and it has a great potential in implantable electrode applications.

Disclosures: S. Yoo: None. **Y. Nam:** None. **J. Park:** None.

Poster

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Topic: G.04. Physiological Methods

Support: DARPA W911NF-14-1-0013

T32 MH02001616

Stanford BioX-IIP

NAKFI

Title: Imaging neural spiking in brain tissue using FRET-opsin protein voltage sensors

Authors: *Y. GONG, M. WAGNER, J. LI, M. J. SCHNITZER
Stanford Univ., Stanford, CA

Abstract: Genetically encoded fluorescence voltage sensors offer the possibility of directly visualizing neural spiking dynamics in cells targeted by their genetic class or connectivity. Sensors of this class have generally suffered performance-limiting tradeoffs between modest brightness, sluggish kinetics, and limited signaling dynamic range in response to action potentials. Here we describe sensors that use fluorescence resonance energy transfer (FRET) to combine the rapid and substantial voltage-sensitivity of the rhodopsin voltage-sensing domain derived from L. Maculans with the brightness of engineered protein fluorophores. These FRET-opsin sensors significantly improve upon the spike detection fidelity offered by the genetically encoded voltage sensor, Arclight, by offering approximately four-fold faster kinetics and four-fold higher brightness. Using FRET-opsin sensors we imaged neural spiking and sub-threshold membrane voltage dynamics in cultured neurons and in pyramidal cells within neocortical tissue slices. In live mice, rates and optical waveforms of cerebellar Purkinje neurons' dendritic voltage transients matched expectations for these cells' dendritic spikes.

Disclosures: Y. Gong: None. **M. Wagner:** None. **J. Li:** None. **M.J. Schnitzer:** None.

Poster

270. Optical Methods II

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Topic: G.04. Physiological Methods

Support: ARC DP 120102191

Title: Analyzing morphology-dependent neuronal function using laser dendrotomy

Authors: ***V. DARIA**[1,2], M. GO[2], A. COLIBABA[3], S. REDMAN[2], S. REDMAN[2], H. BACHOR[4], C. STRICKER[2,3]
[2]John Curtin Sch. of Med. Res., [3]ANU Med. Sch., [4]Res. Sch. of Physics and Engin., [1]The Australian Natl. Univ., Canberra, Australia

Abstract: Objective: Dendrotomy can experimentally modify the neuron's dendritic structure and can be used to verify morphology-dependent neuronal firing. Several techniques have been used to sever processes from neurons in brain slices but these are invasive and may incur collateral damage to the cell and the surrounding tissue. Here, we report the use of a focused femtosecond pulsed laser as an ultra-sharp scalpel to cut dendrites and analyze the neuron's firing properties. The nonlinear nature of the multi-photon light - tissue interaction localizes dendrotomy to a diffraction-limited focal volume with minimal damage to surrounding tissue. We use a custom-built two-photon laser-scanning microscope to image as well as prune the dendritic tree of layer II/III, layer V and CA1 pyramidal neurons and assess how this affects firing. Methods and Results: We imaged neurons filled with 100 μM of Alexa-488 via a patch-pipette in 300 μm thick slices of somatosensory cortex and hippocampus from 15-19 day-old rats using 12-22 mW of 800 nm laser light. Dendrotomy sites were chosen from such images and performed with 100 ms pulses of 30-150 mW either at 720 or 800 nm. The holding current was monitored and firing patterns before and after dendrotomy were compared. Success of a cut was verified by generating EPSPs via 2P uncaging of MNI-glutamate at identified spines proximal and distal to the cut and via biocytin staining post hoc. Dendrotomy was characterized by a sudden increase in holding current regardless of the location of the cut in the dendritic tree after which it returned close to the initial value within 20 min. Successful dendrotomy was verified histologically in biocytin stainings proximal to the point of cut and by functionally checking that proximal uncaging responses remained but distal ones disappeared. Small-scale dendrotomy of 3rd- or 4th order dendritic segments did not change neuronal firing rate significantly. However, large-scale dendrotomy of 1st- and 2nd-order segments resulted in an increase in firing rate for the same current injected. Conclusion: We have demonstrated the use of laser surgery to dynamically prune the dendritic arbor of a neuron. This approach can be used to experimentally investigate the relationship between dendritic structure and neuronal function.

Disclosures: V. Daria: None. **A. Colibaba:** None. **S. Redman:** None. **S. Redman:** None. **H. Bachor:** None. **C. Stricker:** None. **M. Go:** None.

Poster

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HHMI

Title: Measuring voltage in dendrites and dendritic spines using quantitative all-optical electrophysiology

Authors: *S. L. FARHI[1], E. N. WEINSTEIN[1], A. E. COHEN[1,2]
[1]Harvard Univ., Cambridge, MA; [2]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: The dendrites of hippocampal pyramidal neurons perform computations by filtering and integrating voltage responses from multiple synaptic inputs. Dendritic integration has been investigated by dual dendritic patch clamp recordings, but these do not give the full spatial structure of the membrane voltage, and are difficult to perform on structures with small radii. Few direct measurements have been reported of the voltage responses of distal dendrites or dendritic spines. Further, the degree to which spines serve as electrical compartments remains uncertain. Voltage-sensitive dye recordings suffer from phototoxicity and require intracellular injection of the dye, which is slow and technically demanding. To study voltage in small dendrites and spines, we developed a system for quantitative and spatially resolved all-optical electrophysiology. The system consists of QuasAr2, a fast and sensitive genetically encoded voltage sensor, and CheRiff, a highly sensitive and spectrally orthogonal channelrhodopsin actuator. We applied this system to dissociated rat hippocampal excitatory neurons and made quantitative measurements of membrane voltage in dendrites and spines following activation with CheRiff or in response to synaptic activation with caged glutamate.

Disclosures: S.L. Farhi: None. **E.N. Weinstein:** None. **A.E. Cohen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Q-State Biosciences.

Poster

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Topic: G.04. Physiological Methods

Support: ANR LabEx ICST

Title: Imaging Fast Calcium Currents: a pilot study of native T-type Ca2+ channels in CA1 hippocampal pyramidal neurons

Authors: *M. CANEPARI[1], N. JAAFARI[2]
[1]Grenoble Inst. of Neurosci., Grenoble Cedex 9, France; [2]Grenoble Inst. of Neurosci., Inserm, Grenoble, France

Abstract: The measurement of fast cellular Ca2+ currents is routinely achieved using the patch clamp technique in voltage-clamp mode. This experimental approach, however, is not applicable to the study of local native Ca2+ channels during physiological changes of membrane potential in complex cells since the voltage clamp configuration constrains the membrane potential to a given value. Here, we report how Ca2+ currents from individual cells can be measured beyond the limitations of voltage-clamp using fast Ca2+ imaging with low-affinity indicators. Individual CA1 hippocampal pyramidal neuron of the mouse were filled with 1 mM of the low-affinity indicator Oregon Green 488 BAPTA-5N and fluorescence from the apical dendrite was acquired at 20,000 frames/s. The Ca2+ current was obtained by conversion of the fractional change of fluorescence into Ca2+ influx and by its differentiation. The optical measurement of the Ca2+ current was correlated with the membrane potential simultaneously measured with a voltage-sensitive dye to investigate the activation of Ca2+ channels along the apical dendrite of the neuron during the back-propagation of an action potential. By using this innovative approach, we analyzed the voltage-dependence of high- and low-voltage gated Ca2+ channels. In particular, we measured the Ca2+ current component mediated by T-type channels by using pharmacological agents and we investigated the mechanisms of recovery from inactivation of these channels. We found that T-type Ca2+ channels are inactivated when the initial membrane potential is -60 mV and that recovery from inactivation occurs if the initial membrane potential is -80 mV. When the cell is firing 2-4 action potentials at high frequency (>50 Hz), a T-type channel component independent of the initial membrane potential is observed after the first spike. Using our novel approach we investigated the mechanisms underlying this form of activity-dependent recovery from inactivation of T-type Ca2+ channel. In conclusion, the

method of imaging fast Ca2+ currents presented here is expected to become a reference approach to investigate Ca2+ channels in their native physiological environment.

Disclosures: **M. Canepari:** None. **N. Jaafari:** None.

Poster

270. Optical Methods II

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Topic: G.04. Physiological Methods

Title: Development of an integrated platform for standardized, high-throughput *in vivo* functional imaging

Authors: ***T. M. KEENAN**, K. ROLL, J. PERKINS, E. MOUNT, M. GARRETT, C. WHITE, N. ORLOVA, S. DE VRIES, C. FARRELL, C. LAU, L. NG, S. OLSEN, D. RIZZUTO, C. SLAUGHTERBECK, W. WAKEMAN, J. WATERS, D. WILLIAMS, C. REID, A. JONES, J. PHILLIPS, A. BERNARD
Allen Inst. for Brain Sci., Seattle, WA

Abstract: Information processing in the mammalian brain is achieved in large part via the physical connectivity between relevant processing centers, and the encoding and routing of information carried on those connections. Understanding how the brain receives, sorts, and interprets information to engender higher order function requires: 1) a fundamental ability to identify areas of enhanced neural activity due to changes in behavioral state, and 2) the ability to assess single cell responses within those areas. The Allen Institute is interested in a variety of questions relating to brain function and information processing and accordingly has developed a novel platform for functional imaging of mouse cortex. The platform utilizes highly-standardized procedures, controls, and analytical tools to identify functionally-defined processing centers using reflectance-based intrinsic signal imaging (ISI)and subsequently quantifies single neuron responses using 2-photon optical physiology. All experimental and tracking data is stored within an integrated and searchable database that will ultimately be developed into a publicly-available resource.

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Poster

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NSERC

Title: Two-photon optogenetic control of cAMP dynamics in dendritic spines for studying synaptic plasticity

Authors: *T. LUYBEN[1,2], M. KHAN[1], K. OKAMOTO[1,2]
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Abstract: Understanding how synaptic structure and function are modulated by neural activity is essential for elucidating the mechanisms of learning and memory. Here we study the role of cyclic adenosine monophosphate (cAMP) in the structural plasticity of dendritic spines by developing two-photon optogenetic and live imaging techniques in cultured hippocampal slices. cAMP is a ubiquitous second messenger involved in synaptic plasticity, such as the late phase of long-term potentiation (L-LTP). However, its precise dynamics and role in dendritic spines remains elusive. Dendritic spines are thought to change their shape and properties through a process called structural plasticity. Structural plasticity occurs during synaptic activities such as LTP and long-term depression (LTD). Since cAMP is involved in LTP, we hypothesize that postsynaptic cAMP may therefore play a role in structural plasticity as well. To understand how cAMP regulates structural plasticity, at first we visualized cAMP dynamics during synaptic plasticity in living neurons by preparing genetically-encoded cAMP sensors utilizing Förster resonance energy transfer (FRET) and two-photon microscopy. We fused the cAMP binding domain of Epac1 (exchange protein directly activated by cAMP) with CFP (Cyan Fluorescence

Protein) and YFP (Yellow Fluorescence Protein) to make a cAMP FRET sensor, resulting in a probe with a high sensitivity to cAMP *in vitro* (EC50 = 1.68 μM). Time-lapse imaging of two-photon FRET in living neurons showed a transient increase in cAMP after tetanic stimulation (L-LTP induction) but did not detect a change in cAMP levels after caged-glutamate uncaging (early LTP induction). In order to examine the effect of cAMP on structural plasticity, we have established a two-photon optogenetic approach to non-invasively manipulate cAMP levels by light, using a combination of two-photon microscopy and photoactivatable adenylyl cyclase (PAC). These tools were biolistically transfected into CA1 pyramidal neurons and confirmed to not interfere with the induction of structural plasticity in dendritic spines by caged-glutamate uncaging. We found that light-dependent postsynaptic PAC activation enhanced dendritic spine enlargement during the plasticity after LTP induction by caged-glutamate uncaging. This may suggest a role for postsynaptic cAMP in the maintenance of the dendritic spine structure reorganization during structural plasticity. Thus, two-photon optogenetic approach provides a powerful tool to elucidate intracellular signaling mechanisms, such as synaptic plasticity, in living neurons.

Disclosures: **T. Luyben:** None. **M. Khan:** None. **K. Okamoto:** None.

Poster

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FP7, DESIRE

Title: Scanless two-photon fluorescence imaging in the intact mouse brain

Authors: S. BOVETTI[1], C. MORETTI[1], M. DAL MASCHIO[1], S. ZUCCA[1], P. BONIFAZI[2], *T. FELLIN[1]
[1]Inst. Italiano di Tecnologia, Genova, Italy; [2]Sch. of Physics and Astronomy, Tel Aviv University, Israel

Abstract: Two-photon laser scanning fluorescence microscopy allows imaging the activity of extended cellular networks at high spatial resolution within largely scattering tissue, such as the brain. However, scanning microscopy is intrinsically limited in its time resolution by the sequential illumination scheme. Indeed, in both raster and random-access modalities, acquisition speed decreases as the number of imaged points increases. Here, we present a scanless, two-photon microscope for fast fluorescence imaging in the intact mouse brain *in vivo* based on structured light illumination by phase modulation. The microscope is based on a liquid crystal spatial light modulator (SLM), which is placed in a plane optically conjugated to the objective back-focal plane, and a galvanometric mirror-based laser scanhead. Emitted fluorescence is collected either in non-descanned mode by photomultiplier tubes or with a fast camera. In the scanless configuration, we report fluorescence calcium measurements from multiple layer II/III principal neurons in anesthetized mice at high acquisition frequency while maintaining subcellular spatial resolution and sufficient signal-to-noise ratio. Importantly, in the scanless approach, acquisition speed is independent on the dimensions of the imaged region. By monitoring the activity of neuronal populations on a fast time scale, this technique will help to elucidate the cellular mechanisms underlying information processing within brain networks.

Disclosures: S. Bovetti: None. **T. Fellin:** None. **C. Moretti:** None. **S. Zucca:** None. **P. Bonifazi:** None. **M. Dal Maschio:** None.

Poster

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Topic: G.04. Physiological Methods

Support: PON R&C PON01_00110

Title: Gabaergic transmission and its long-term potentiation revealed by voltage sensitive dye imaging at mesoscale level

Authors: *M. COLAVITA[1,2,3], C. LEMERCIER[1,2], G. TERRAL[1,2], F. DRAGO[3], G. MARSICANO[1,2], F. MASSA[1,2]
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Abstract: In cortical regions GABAergic interneurons form a heterogeneous population of cells with their intricate arborisations of dendrites and axons spreading along wide areas. This network of interneurons is critical in providing a temporal framework for principal cells activity thus regulating cortical computations. Considering the crucial role of GABAergic interneurons in the CNS and their large spatial extent, new tools to monitor their transmission in wide areas would permit us a better understanding of interneuronal networks functioning. For this aim we used Voltage Sensitive Dye Imaging (VSDI) and we took advantage of its possibility to record coincident changes in membrane potential at mesoscale level (i.e. at the level of local populations of neurons) with very high temporal resolution (milliseconds). Using acute hippocampal slices from adult mice we found that after the blockade of ionotropic glutamatergic transmission we were able to record evoked homosynaptic GABAA-mediated signal along the different layers of the CA1 region. This VSDI signal was abolished by the GABAA receptor antagonist Picrotoxin and it was increased by the benzodiazepine Chlordiazepoxide. Moreover, it could be modulated in amplitude proportionally to the applied stimulation intensity. Finally, we found that brief application of the selective group I metabotropic glutamate receptor (mGluR) agonist (S)-3,5-Dihydroxyphenylglycine (DHPG; for 10 minutes) induced a persistent enhancement of GABAergic VSDI-mediated signal lasting at least 80 minutes after washout. These results indicate that VSDI allows studying GABAergic transmission and plasticity in the hippocampus at a large spatial scale. In addition, we highlight a novel long-term potentiation specifically at GABAergic synapses. This work has been supported by grants from “Programma Operativo Nazionale Ricerca e Competitività 2007-2013 (PON R&C)”

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Poster

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Topic: G.04. Physiological Methods

Support: Burroughs wellcome fund

Michael J. Fox Foundation

DARPA

Searle Scholar Program

Title: High throughput optical imaging of the CLARITY-processed tissue

Authors: *H. CHOI, K. CHUNG
MIT, Cambridge, MA

Abstract: Understanding brain function and dysfunction requires integrative knowledge of the brain's architecture−how neuronal connectivity and molecular machinery orchestrate mental function. Recent advent of tissue processing technologies, such as CLARITY and other optical clearing methods, open the possibility of investigating anatomical and molecular architecture of intact brain without laborious mechanical sectioning and reconstruction. Conventional confocal and two photon microscopies, however, significantly limits their utility due to the slow scanning nature of the imaging methods. Here, we introduce a temporally focused line scanning two photon microscope which can provide a resolution equivalent to point scanning two photon microscopy while the image acquisition rate is limited only by the readout speed of the sCMOS camera. Further increase in depth resolution is achieved by using a rolling shutter of the sCMOS as a dynamic confocal pinhole. This new imaging system may be particularly useful for human brain mapping because the unique optical configuration and open design of the sample chamber permits samples with any shape and size (e.g. whole coronal block of the human brain, rat brain, monkey brain, and other organs) can be readily imaged. The imaging depth will be limited by the working distance of the objective, but when combined with sectioning by vibrating blade microtome, this imaging modality may even enable imaging of whole mounted primate/rat brains in an automated manner.

Disclosures: H. Choi: None. **K. Chung:** None.

Poster

270. Optical Methods II

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Michael J. Fox Foundation

DARPA

Searle Scholars Program

Title: Scalable CLARITY

Authors: *E. MURRAY[1,2], N. BAKH[3], J. H. CHO[3], S.-Y. KIM[2], K. OHN[3], K. CHUNG[1,2,3]
[1]Picower Inst. for Learning and Memory, [2]Inst. for Med. Engin. and Sci., [3]Chem. Engin., MIT, Cambridge, MA

Abstract: The recent development of the CLARITY technique has opened the doors for structural and molecular interrogation of large, intact blocks of tissues. However, there are many issues with the technique in its original form: lengthy processing time, expensive reagents, hard-to-reproduce procedures, accumulation of "black gunk" on tissue surfaces, and fundamental limitations in the size of clearable samples. Applying principles from chemical engineering, we have re-visited each stage of the CLARITY procedure in order to improve the technique as a whole, making it scalable to larger brains and more available to a wider base of users. We have (1) optimized the conditions of fixation to maximize retention of structural and molecular information, (2) increased the reproducibility of the de-gassing and tissue-hydrogel hybridization procedure through development of a customized apparatus, (3) developed a novel stochastic electrotransport device to improve the reliability and overall speed of the tissue-clearing step while decreasing the cost by ten-fold, and (4) developed a replacement immersion medium for FocusClear that can be made at a small fraction of the cost. This next generation CLARITY technique is scalable to larger samples, such as whole rat brain or large blocks of human/non-human primate tissues.

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Poster

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NSF IDBR 1353757

NIH P30 DC004657

Title: Development of a portable optical system for *in vivo* deep-brain neuronal imaging

Authors: *B. OZBAY[1], J. T. LOSACCO[2], E. A. GIBSON[1], D. RESTREPO[3]
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Abstract: Many chronic diseases of the central nervous system, such as epilepsy and depression, are thought to have important cellular and sub-cellular dynamics that may be probed to understand disease progression and to identify possible therapeutic targets. Furthermore, continuous *in vivo* monitoring of neuron activity in the brain can be used to provide feedback to better tune therapies. Currently, *in vivo* monitoring of deep-brain neurons is accomplished primarily by electrophysiological methods, such as intracranial deep-brain electrodes. While these methods can allow for single unit action potential recordings, they lack cell-type specificity, sub-cellular spatial resolution, and are limited to recordings from small populations of neurons. In this work, we utilized innovations in genetically encoded optical sensors combined with custom-designed optical tools to be used as an *in vivo* deep-brain neuronal imaging system with high spatial resolution, large imaging volume, and cell-type specificity. We have designed a portable fiber-coupled miniature imaging system that can be coupled to most commercial inverted laser scanning microscopes. The distal optics are 2 mm in diameter and can be implanted to acutely record from deep-brain regions. Lateral imaging is accomplished by the use of a high-density fiber-bundle that can achieve a resolution of ~2 μm and a lateral field of view of ~200 μm. The system is designed for multiphoton microscopy, which can be used to obtain large imaging volumes to capture extensive neuronal processes and populations. The optical system was tested *in vivo* to image neuronal GFP expressing oligodendrocyte cell bodies in an anesthetized mouse olfactory bulb. The distal optics were also implanted in anesthetized mice for acute deep-brain recording sessions. Furthermore, the recently developed genetically-encoded calcium sensor, GCaMP6s, was expressed in piriform cortex pyramidal neurons via viral delivery into mice with Ntsr1-driven Cre expression with the purpose of performing functional high resolution deep-brain imaging in an anesthetized mouse. Further development of this system offers the promise of a new type of functional imaging in an awake behaving animal allowing for the observation of morphological and physiological changes over time which will clarify both normal and pathological network dynamics.

Disclosures: **B. Ozbay:** None. **J.T. Losacco:** None. **E.A. Gibson:** None. **D. Restrepo:** None.

Poster

270. Optical Methods II

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Topic: G.04. Physiological Methods

Support: Burroughs wellcome fund

Michael J. Fox Foundation

DARPA

Searle Scholar Program

Title: Rapid *in situ* three dimensional gene profiling

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Abstract: Elucidating spatial heterogeneity and complexity in gene expression is crucial for understanding brain function and dysfunction. *In situ* hybridization is an essential tool that enables imaging of mRNA expression in a morphological context. Its power, however, has been limited to small-scale samples due to the opaque and impermeable nature of biological tissues. The advent of the CLARITY technique to render intact tissue transparent and permeable to molecules, while preserving structural and molecular information, opens the possibility of obtaining 3D transcription information from a large-scale intact tissue using fluorescent *in situ* hybridization (FISH). However, the slow nature of passive diffusion significantly limits the penetration depth of the probes and the throughput of this method. To overcome this limitation, we developed an electrophoretically assisted FISH technique to boost transport of highly charged oligonucleotide probes for rapid labeling and mapping of mRNAs in CLARITY-processed tissues. In addition, we developed a new immersion medium that preserves RNA integrity during the imaging step to enable consecutive rounds of FISH on the same tissue. After imaging, fluorescent dyes are optically inactivated to allow iterative staining and imaging cycles. We envision that this high-throughput *in situ* gene profiling technique will greatly help us navigate the transcriptional landscape of the brain in health and disease.

Disclosures: **N. Bakh:** None. **K. Chung:** None.

Poster

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Support: Alzheimer Society of Canada 1242

CIHR MOP49489123514

NSERC CREATE

Title: A comparative study of the two-photon performances of GCaMPs and GECOs

Authors: *B. PODOR[1], Y. ZHAO[2], J. WU[2], Y.-L. HU[1], M. OHKURA[3], J. NAKAI[3], R. CAMPBELL[2], R. CROLL[1], A. FINE[1]
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Abstract: Genetically encoded calcium indicators (GECI) are increasingly being used to monitor neuronal activity. With appropriate targeting strategies they permit non-invasive imaging of activity of neuronal networks, individual cells, or even subcellular compartments. Development of the first GECI, GCaMP (Nakai *et al*., 2001), has been followed by substantial progress in both the variety and utility of these indicators; new GECIs have been reported with increased sensitivity (ΔF/F, the fractional change in fluorescence upon Ca^{2+} binding), and in a range of colours ("GECOs", Zhao et al., 2011). However, newly developed sensors have mainly been characterized *in vitro* or in model cells, and their performance may be significantly different in particular neurons under experimental conditions. We therefore compared the ability of members of the GCaMP and GECO families to report proximal dendritic calcium transients imaged by two-photon laser scanning microscopy. Specifically, these were: GCaMP-3; GCaMP-7; Green-GECO1.0, 1.1, 1.2 (G-GECO); Blue-GECO (B-GECO); Red-GECO (R-GECO); Rex-1A-GECO; Rex-1B-GECO; Carmine-GECO (CAR-GECO); Orange-GECO (O-GECO); and Yellow-GECO (Y-GECO-1s). After first determining the optimal 2P excitation wavelength for each indicator, we evoked and recorded increasing numbers of action potentials in CA1 pyramidal cells in hippocampal slice cultures by intracellular current injection and monitored the achieved ΔF/F values by 2P microscopy. By far the most sensitive indicator for reporting single action potentials was GCaMP-7. Kinetics of all tested indicators proved too slow to resolve individual action potentials beyond 10 Hz, but GECO1.2, Rex-1A and Y-GECO-1s exhibited a linear relationship between number of evoked action potentials (APs) and fractional change in fluorescence up to at least 8 APs @ 100Hz.

Disclosures: **B. Podor:** None. **Y. Zhao:** None. **J. Wu:** None. **Y. Hu:** None. **M. Ohkura:** None. **J. Nakai:** None. **R. Campbell:** None. **R. Croll:** None. **A. Fine:** None.

Poster

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Topic: G.04. Physiological Methods

Support: HHMI

Stanford BioX Interdisciplinary Initiatives Program

National Academies Keck Futures Initiative

Title: Genetically targeted, cell-type specific optical recording of membrane voltage dynamics in freely moving mice

Authors: *J. D. MARSHALL[1], P. JONES[2], Y. GONG[2], J. LI[2], F. ST-PIERRE[2], M. LIN[2], M. SCHNITZER[2]
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Abstract: Electrical field recordings of aggregate neural activity such as electroencephalogram (EEG) and local field potential (LFP) recordings are widely used research and clinical tools for observing evoked potentials, distinguishing brain states such as sleep or wakefulness, detecting oscillatory electrophysiological rhythms, and tracking coherent activity across brain areas. However, field recordings are generally difficult to interpret from a mechanistic standpoint and often reflect the combined action of multiple electrophysiological mechanisms and cell types. Here we describe an optical measurement technique that, like LFP or EEG recordings, reports on the collective activation of neural populations, but unlike these methods does so in manner that is selective for a genetically specified class of neurons. To accomplish this, we combined newly developed genetically encoded optical voltage sensors [St-Pierre *et al.*, *Nature Neuroscience* (2014); Gong *et al*., *Nature Communications* (2014)] and a multi-color fiber-optic recording apparatus, which together enabled a fast optical readout of neuronal membrane potential dynamics in specific neuron types of freely behaving mice. Using this approach, we recorded oscillatory and evoked optical transients in neocortical pyramidal cells, neocortical interneurons, and in D1- and D2-dopamine receptor expressing medium spiny neurons of the striatum. The high sensitivity of our measurement approach enabled the use of sufficiently weak illumination to permit extended recordings (>1 hour/day) with minimal photobleaching. Comparisons of recordings acquired during spatial exploration versus during sleep revealed an enhancement of

delta oscillations during sleep that occurred more sparsely in time than those seen in EEG recordings. This difference between the optical and EEG measurements may reflect a superior spatial resolution for the optical approach. In optical recordings of D1- and D2-receptor expressing medium spiny neurons, we found that haloperidol-evoked high-voltage spindles were phase-locked to the cortical EEG with indistinguishable phase offsets for the two cell types. This suggests these prominent cortico-striatal oscillations are synchronized between the direct and indirect pathways of the basal ganglia. Overall, our methodology provides a versatile new approach to examine aggregated neural activity in a cell-type specific manner.

Disclosures: J.D. Marshall: None. **P. Jones:** None. **Y. Gong:** None. **J. Li:** None. **F. St-Pierre:** None. **M. Lin:** None. **M. Schnitzer:** None.

Poster

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Topic: G.04. Physiological Methods

Support: Burroughs Wellcome Fund

Michael J. Fox Foundation

DARPA

Searle Scholar Program

Title: A high-throughput technology for fixation optimization and antibody screening of CLARITY-processed tissue

Authors: *A. C. HOUSTON[1,2,3,6], E. MURRAY[1,3,4], J. CHO[1,4,5], K. CHUNG[1,3,4,5]
[2]Brain and Cognitive Sci., [3]Picower Inst. for Learning and Memory, [4]Inst. for Med. Engin. and Sci., [5]Chem. Engin., [1]MIT, Cambridge, MA; [6]F.M Kirby Neurobio. Ctr., Children's Hosp. Boston, Boston, MA

Abstract: CLARITY is a technology that renders intact biological tissue optically transparent and chemically accessible while maintaining structural and molecular information. Molecular phenotyping techniques (e.g. immunohistochemistry) can be employed to probe biochemical components (e.g. protein, DNA, RNA, small molecules) that are fixed and tethered to a

polyacrylamide hydrogel. The introduction of the hydrogel tethering, however, alters the biochemical environment within the tissue from that found within standard fixation protocols. Therefore, optimization of fixation conditions (i.e. paraformaldehyde, glutaraldehyde, acrylamide, bis-acrylamide concentrations) and immunolabel screens are critical to ensuring the validity of molecular phenotyping of a CLARITY-processed tissue. It is notable that fixation optimization and antibody screening are labor and resource intensive when CLARITY-processing a whole tissue. Thus, a high-throughput method of processing small tissue samples is needed. Here, we describe a novel method for rapid CLARITY processing and immunolabeling of thin sections. This technology platform utilizes a pressure drop to generate convective flow through the sample to rapidly deliver molecules while a membrane support is used to stabilize tissue sections. Large numbers of samples can be processed in parallel on a significantly smaller time scale allowing efficient testing of fixation conditions and screening of antibodies. By utilizing this methodology we are able to rapidly clear small tissue sections from a wide variety of host species (e.g. mouse, rat, monkey, human) and screen hundreds of antibodies under various fixation and staining conditions. Importantly, we have found the incorporation of glutaraldehyde fixation to maximize structural stability and better preserve small-molecules, such as neurotransmitters and neuropeptides, for immunolabeling. This novel high-throughput technique will extend the utility of CLARITY by greatly reducing the resources and time required in optimizing conditions for biochemically probing CLARITY-processed tissue.

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Poster

271. Optogenetics: Tool Development

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Topic: G.04. Physiological Methods

Support: KAKENHI 25430008

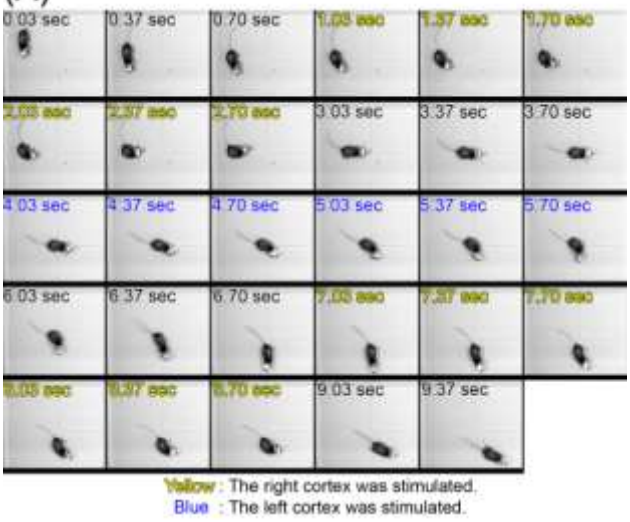
FIRST Program

Title: Programmable wireless LED stimulator for chronic stimulation of optogenetic molecules in freely moving mice

Authors: *M. HASHIMOTO[1], T. MIYATA[1], H. HIRASE[2]
[1]Dept. Cell Biol., Nagoya Univ. Med., Nagoya, Japan; [2]Lab. for Neuro-Glia Circuitry, RIKEN Brain Sci. Inst., Wako, Japan

Abstract: The cloning and subsequent bioengineering of light-driven channels (e.g., channelrhodopsins) or pumps (e.g., halorhodopsin and archaerhodopsins) has opened a new avenue in the field of optogenetics. Optogenetics is a powerful method in neuroscience because these opsins can be expressed in specific subsets of neurons by genetic manipulation, allowing dynamic modulation of the discharge of these cells by light. In animal experiments, the effectiveness of brain manipulation is ultimately assessed by an animal's behavior. Because most rodent behavioral experiments assume free movement of the animals, a light delivery method that does not compromise their movement is desired. We produced a miniaturized, multicode, multiband, and programmable light-emitting diode (LED) stimulator for wireless control of optogenetic experiments. The LED stimulator is capable of driving three independent LEDs upon reception of an infrared (IR) signal generated by a custom-made IR transmitter. Individual LED photopulse patterns are assigned to different codes of the IR signals (up to 256 codes). The photopulse patterns can be programmed in the on-board microcontroller by specifying the parameters of duration (>1 ms), frequency (<500 Hz), and pulse width (>1 ms). The IR signals were modulated at multiple carrier frequencies to establish multiband IR transmission. Using these devices, we could remotely control the moving direction of a Thy1-ChR2-YFP transgenic mouse by transcranially illuminating the corresponding hemisphere of the primary motor cortex. The photopulses (frequency, 10 Hz; duration, 50 ms; interval, 2 s) presented to the right and left motor cortex changed the moving direction of a freely moving Thy1-ChR2-YFP mouse the left and right, respectively (Fig. A). IR transmitter and LED stimulator will be particularly useful in experiments where free movement or patterned concurrent stimulation is desired.

(A)



Disclosures: **M. Hashimoto:** None. **T. Miyata:** None. **H. Hirase:** None.

Poster

271. Optogenetics: Tool Development

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Topic: G.04. Physiological Methods

Support: American Heart Association postdoctoral fellowship (AY)

Defense Advanced Research Projects Agency (DARPA) Reorganization and Plasticity to Accelerate Injury Recovery (REPAIR; N66001-10-C-2010).

Title: A large bi-directional interface for optogenetic stimulation and recording in non-human primates

Authors: *A. YAZDAN-SHAHMORAD[1], T. HANSOS[1], C. DIAZ-BOTIA[1,3], P. LEDOCHOWITSCH[3], V. KHARAZIA[2], M. MAHARABIZ[3], P. N. SABES[1]
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Abstract: Optogenetics offers the promise of manipulating large-scale neural circuits with both high temporal and spatial precision. While optogenetics has been used successfully in non-human primates (NHPs), reliable techniques have not yet been reported for large-scale, bi-directional study of neural circuits in NHP. Here we describe such a large-scale interface that combines optogenetics with high-density micro-electrocorticography (μECoG). To obtain expression across large areas of cortex, we infused AAV5-CamKIIa-C1V1-EYFP viral vector using a novel infusion technique based on convection-enhanced delivery in primary somatosensory (S1) and motor (M1) cortices. Following viral injection, a soft, transparent artificial dura (AD) was implanted to cover the injection sites. It provided an optical window to monitor the spread of viral expression using epifluorescent imaging and a platform for targeted, non-invasive optical stimulation. Based on this imaging, we estimated high levels of expression across at least 110 mm2 of cortex spanning S1 and M1. In order to record large-scale activity across this region, we incorporated a 192-channel μECoG array spanning 96 mm2 into the AD for simultaneous electrophysiological recording during optical stimulation. Our array was designed to include 300 μm diameter perforations to enable unattenuated optical access and was implanted to cover the opsin-expressing areas in M1 and S1. We implanted the μECoG both acutely (within one session) and chronically (for the span of several weeks). In both cases, we observed reliable light evoked neural responses in M1 and S1. The temporal and spatial

distribution of activity near the stimulation site varied with the stimulation parameters. Spatio-temporal analysis of the activity across the array showed reliable evoked activity not only close to the stimulation site but also in neighboring areas. For example, stimulating a particular location in S1, we were able to record reliable activity in an area of M1 at a distance of about 2 mm from the site of stimulation. These results show the feasibility of implementing a large bi-directional interface using optogentics, AD and μECoG array technologies in NHPs. This interface is a powerful tool to study circuits and connectivity across extended cortical areas, improve the specificity of neuromodulation and provide real-time dynamic titration of stimulation on the basis of the brain state.

Disclosures: A. Yazdan-Shahmorad: None. **T. Hansos:** None. **C. Diaz-Botia:** None. **P. Ledochowitsch:** None. **V. Kharazia:** None. **M. Maharabiz:** None. **P.N. Sabes:** None.

Poster

271. Optogenetics: Tool Development

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Program#/Poster#: 271.03/TT78

Topic: G.04. Physiological Methods

Title: Aramid nanofiber-epoxy coated intracortical LEDs for optogenetic stimulation

Authors: *K. E. SCHROEDER[1], C. CHENG[2], A. W. WELLNER[5], I. DIESTER[5], N. A. KOTOV[2], C. A. CHESTEK[1,3,4]
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Abstract: Most optogenetics studies in nonhuman primates currently require inserting an optical fiber on a daily basis. The cortical damage caused by this process makes chronic multichannel stimulation undesirable or unfeasible. Unlike in rodents, primate anatomy prohibits the use of any cannulas or LED probes that are fixed to the skull. Tiny, injectable light sources have been fabricated by other groups (i.e. Kim et al., Nature 2013). While these devices have been successful in rodents, we wanted to develop an implant optimized for a primate study spanning 6 to 12 months, and allowing for the arbitrary configuration of light sources as the experiment requires. We wire bonded commercially available bare-die Cree blue LEDs (220x270x50 μm, 460nm wavelength) onto a glass wafer (100 μm thickness) and diced them to get individual devices. We used conductive epoxy to attach 75 μm wire to the devices. We then dip coated the devices to provide strength and a watertight encapsulation. Recent results show that our aramid

nanofiber (ANF)/epoxy film provides superior metal adhesion performance and better neural cell biocompatibility than the more commonly used parylene-C (in preparation). 1 g of purchased Kevlar® thread was dissolved in 100 ml of DMSO with 4 g of KOH for a week to prepare a 10 mg/ml ANF dispersion. Each device was dipped into ANF nanofiber dispersion first, and then rinsed with water to remove the excess DMSO. Then, the device was dipped into 3% epoxy resin in acetone and dried in the oven at 80 degrees Celsius. Repeating this process for six dip cycles results in a pinhole-free film that is 3 μm thick. We have demonstrated implantation of the device into rat cortex using a standard stereotax micromanipulator, with no bleeding. We are now in the process of testing the devices for hermeticity and effectiveness of stimulation. In previous experiments we have stimulated with a laser (Omicron Laserage) of a comparable wavelength (473nm) with a power of 6mW out of a 200um optical fiber (Doric Lenses). During application of 50 Hz stimulation (2ms pulse width) in motor cortex (AP 1.7, ML 2.5, DV 1.0mm), we observed robust increases in locomotion and neural activity. We will take these results as baseline for comparisons with the new LED device. These LEDs are ideal for use in primates for several reasons. They can be implanted individually in any configuration, such as surrounding any electrode array, or laid on the surface of cortex. The wires can run along the surface of the brain and emerge through the bone as a bundle, reducing strain and making the surgical close easier. Finally, they are fabricated relatively simply, using commercially available materials.

Disclosures: K.E. Schroeder: None. **C. Cheng:** None. **A.W. Wellner:** None. **I. Diester:** None. **N.A. Kotov:** None. **C.A. Chestek:** None.

Poster

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Topic: G.04. Physiological Methods

Support: Hans Sigrist Foundation

NSF CBET-1403660

Title: Effect of spectrally-shaped light stimulation on Channelrhodopsin-2 currents and spike response in cortical neurons

Authors: *K. PAUL, H. TU, E. D. ARK, Y. ZHAO, S. A. BOPPART
Beckman Inst., Univ. Illinois, Urbana-Champaign, URBANA, IL

Abstract: Optogenetics has traditionally been regarded as the integration of genetic targeting/manipulation with optical stimulation. Since the initial demonstrations of neural control using the ChR2, genetic manipulation has given rise to a number of variants with different properties that either improve upon those of ChR2 or are geared towards a specific desirable response. However the possibility of controlling neuronal output by modifying the optical properties of the light stimulus has so far been neglected. Given the known state changes accompanied by varying wavelength absorbances in the ChR2 photocycle, we hypothesized that independent control of phase and amplitude at different wavelengths of pulse shaped light would alter the neuronal output properties. In this study, we have investigated the effects of this "tailored" light stimulus on ChR2 expressing excitatory pyramidal neurons in neocortical slice tissue. For our investigations, we used a light pulse shaping system (FemtoJock, BiophotonicsSolutions, Inc.) for precisely controlling the spectral and temporal properties of femtosecond pulsed light from a laser source. A Ytterbium laser source (High-Q) was used to generate ~250 fs pulses at a center wavelength of 1040 nm. Pulses were coupled into a photonic crystal fiber to spectrally broaden the light, and then sent to an optical grating to spectrally separate the light wavelengths. The light was then passed through a spatial light modulator (SLM) which enabled us to independently control the phase and amplitude of the spectrally and spatially separated light at known wavelengths. A second grating then recombined the shaped light, which was then passed through the microscope objective for ChR2 excitation in slice tissue. Whole-cell patch-clamp recordings were obtained from pyramidal neurons from slices obtained from the prefrontal and somatosensory neocortex of mice (P12-45). We measured light evoked ChR2 currents in the voltage clamp configuration and in the presence of TTX (0.5 μM). For each neuron, we obtained current responses from 3 light sources: full field continuous wave (CW) light from an LED light source (470 nm), direct 2-photon laser excitation (1040 nm) and tailored 2-photon laser excitation via pulse shaping. For the "tailored light", various configurations of phase and amplitude were independently varied and the corresponding output was recorded. We measured peak and steady state current response, "ON" and "OFF" time constants as well as the recovery time between successive pulses. In the current clamp mode, we independently varied the light amplitude or phase at known wavelengths in order to observe alterations in spike responses.

Disclosures: K. Paul: None. **H. Tu:** None. **E.D. Ark:** None. **Y. Zhao:** None. **S.A. Boppart:** None.

Poster

271. Optogenetics: Tool Development

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Program#/Poster#: 271.05/TT80

Topic: G.04. Physiological Methods

Title: Optically-integrated multiwell microelectrode arrays for high-throughput drug discovery and disease modeling with optogenetics

Authors: I. P. CLEMENTS[1], D. C. MILLARD[1], A. M. NICOLINI[1], *M. BROCK[2,1], A. J. PREYER[1], J. D. ROSS[1]
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Abstract: *In vitro* microelectrode arrays (MEAs) provide extraordinary insight into neuronal network interactions because they can actively monitor and manipulate electrical activity at both the single neuron and tissue level. Recently-developed multiwell MEA systems enable high-throughput experimentation with significantly reduced experimental time and cost. For example, 768 microelectrodes can be distributed among 48 or 96 distinct culture wells on a single microplate for highly parallel experiments. Although MEA recording electrodes can also be used to deliver electrical stimuli, optogenetic stimulation provides several advantages, including spatially uniform stimulus delivery, minimal stimulus artifacts, the ability to suppress activity, and cell-specific targeting through directed expression of light-gated ion channels. This precise control of excitation level enables *in vitro* modeling and modulation of electrical phenotypes for diseases such as epilepsy, autism, and Parkinson's disease. Here we describe the development of an LED array based optical stimulation device, specialized for integration into a multiwell MEA system. This device features independently controllable light delivery within each MEA culture well, and incorporates algorithms for automation and closed-loop control, which are essential in high well count experiments.Using both rodent primary neurons and human induced pluripotent stem cell (hiPSC) derived neurons, we demonstrated multiwell light-based excitation through expression of Channelrhodopsin-2 (ChR2) and suppression of activity through expression of Archaerhodopsin (ArchT). The system's multiwell architecture enabled rapid parallel screening of the effects of various ion channel and receptor modulators on light-evoked activity. These findings demonstrate the potential of optically-integrated multiwell MEA systems to enable high-throughput drug screening and phenotypic modeling of neurological diseases.

Disclosures: **I.P. Clements:** A. Employment/Salary (full or part-time):; Axion Biosystems. **M. Brock:** A. Employment/Salary (full or part-time):; Axion Biosystems. **D.C. Millard:** A. Employment/Salary (full or part-time):; Axion Biosystems. **A.J. Preyer:** A. Employment/Salary (full or part-time):; Axion Biosystems. **J.D. Ross:** A. Employment/Salary (full or part-time):; Axion Biosystems. **A.M. Nicolini:** A. Employment/Salary (full or part-time):; Axion Biosystems.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

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Program#/Poster#: 271.06/TT81

Topic: G.04. Physiological Methods

Title: Multi-focal photostimulations with light emitting diodes on a multi-channel electrocorticographic array in non-human primates

Authors: *M. KOMATSU[1], E. SUGANO[2], H. TOMITA[2], N. FUJII[1]
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Abstract: Optogenetics has potential applications in the study of epilepsy and neuroprostheses, as well as neural circuit dynamics. However, to achieve translation to clinical usages, optogenetic interfaces capable of chronic multisite stimulations and recordings with minimal brain trauma are required. In this study, we developed a multi-channel electrocorticography (ECoG) incorporates light emitting diode (LED) chips to perform simultaneously electrophysiological recordings and light delivery in non-human primates. This technique enables chronic optical stimulation and simultaneous monitoring of neural response at the adjacent area and the distant area of the stimulation site. A device consists of 24 ECoG electrodes and 12 blue-light LED chips. We performed chronic implantations of the devices in two macaque monkeys, which are virally transduced with channelrhodopsin-2. One of the devices was implanted in subdural frontal motor areas of a monkey and another was in subdural primary sensorimotor areas of another monkey. With one of the monkeys, we examined progress of ECoG responses over 5 months after the viral injection. The ECoG responses occurred a week after the injection at some stimulation sites, and six weeks after at all stimulation sites. Furthermore, we applied photostimuli in different locations and various intensity to the cortical surface of the monkeys. Optical stimulations evoked spatially localized ECoG potentials, and the amplitude of the ECoG responses increased with light intensity. For simultaneously applied multisite stimuli, ECoG responses were spatially separated. These results demonstrate that our device is an effective application of optogenetics.

Disclosures: **M. Komatsu:** None. **H. Tomita:** None. **N. Fujii:** None. **E. Sugano:** None.

Poster

271. Optogenetics: Tool Development

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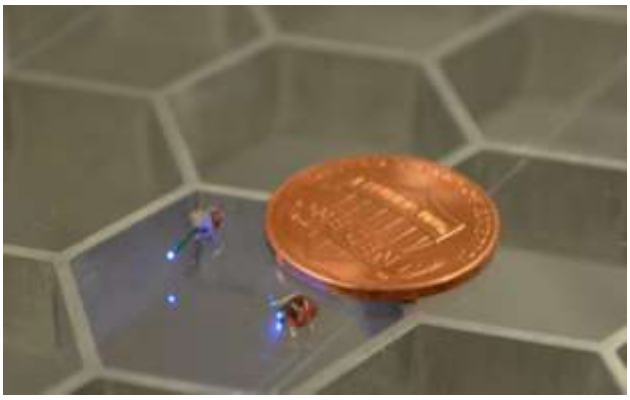
Program#/Poster#: 271.07/TT82

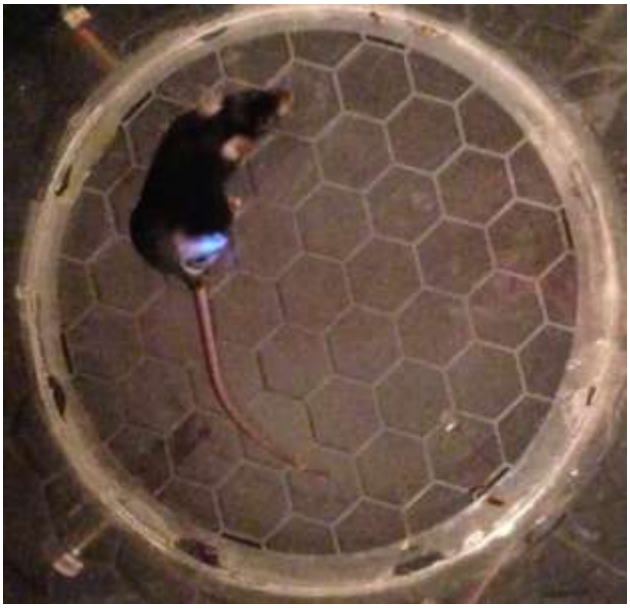
Topic: G.04. Physiological Methods

Title: Fully internal wireless optogenetics for truly untethered stimulation

Authors: *A. J. YEH, K. L. MONTGOMERY, J. S. HO, V. TSAO, E. A. FERENCZI, S. M. IYER, L. GROSENICK, Y. TANABE, K. DEISSEROTH, S. L. DELP, A. S. Y. POON
Stanford Univ., Stanford, CA



Abstract: Current techniques for untethered optogenetic stimulation require fiber optic cables or bulky head-mounted prostheses. We have demonstrated a system for optogenetic stimulation in which the entire stimulator - energy harvesting component, electronics, and light source - is less than 0.008 cm^3 in volume, weighs 20 mg, and is fully implanted within a mouse. A new form of wireless powering, in which a microwave resonant cavity is placed underneath an animal's home cage, powers the device. Evanescent waves at the surface of the resonator electromagnetically couple to high dielectric tissue, inducing energy transfer to the device through tissue. Autofocusing of electromagnetic energy to a moving mouse over an area 20cm in diameter is provided through the physical structure of the microwave resonant cavity; therefore, no electronic tracking is required. This approach has been applied to both the central and peripheral nervous system. We have successfully applied this fully internal system for truly untethered optogenetic stimulation of the motor cortex of Thy1-ChR2-YFP transgenic mice. Furthermore, we have implanted the stimulator directly at the sciatic nerve to study the pain response of AAV6-ChR2 injected mice. The system described here can be further applied to study social behavior of mice since there are little to no alteration to the appearance of mice.



Disclosures: **A.J. Yeh:** None. **K.L. Montgomery:** None. **J.S. Ho:** None. **V. Tsao:** None. **E.A. Ferenczi:** None. **S.M. Iyer:** None. **L. Grosenick:** None. **Y. Tanabe:** None. **K. Deisseroth:** None. **S.L. Delp:** None. **A.S.Y. Poon:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.08/TT83

Topic: G.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research (A)(26249051)

Title: An implantable optogenetics device based on CMOS integrated circuit technology for a freely moving animal

Authors: *M. HARUTA, S. NAKAJIMA, N. KAMIYAMA, H. TAKEHARA, H. TAKEHARA, T. NODA, K. SASAGAWA, T. TOKUDA, J. OHTA
Nara Inst. of Sci. and Technol., Ikoma / Nara, Japan

Abstract: In optogenetics study, it is important to measure and control brain activities in a freely moving animal. However, conventional imaging systems is not suitable for such condition, because invasiveness and size of these devices do not meet freely moving experiments of a small

animal. In this study, we develop a small implantable optogenetics device. This device has two functions which are brain functional image sensor and optical stimulation system (Fig. 1). For the device, we designed a custom sensor using CMOS integrated circuit technology. This enables to integrate multi functions in the device and reduce the size and weight of the device [1]. In our previous work, we developed a neural interface device for optogenetics [2]. Our novel device is developed for implanting into animal's head. A CMOS chip which is integrated with a CMOS image sensor and electrical circuits for LED light-stimulation is bonded on a flexible polyimide substrate. A LED array is integrated on the CMOS chip. The LED array has 10 × 8 blue LEDs (λ= 469 nm). This wavelength is excitation wavelength of Channelrhodopsin-2 in the neuron [3]. This device has a very compact shape with the dimension of 3.0 mm × 3.5 mm in a sensor head. We demonstrated optical stimulation with the device for adult transgenic mice expressing Channelrhodopsin-2. We placed the device directly on the brain surface. In this experiment, we performed neural recording using single-unit recording at the same point as the optical stimulation. We have successfully evoked neural activities by using the device. In the future work, we will measure intrinsic signals with a red light source (λ= 630 nm) and perform an interactive communication with the implantable device. At this wavelength, intrinsic signals arise from oxyhemoglobin in the brain. It is expected that this greatly contributes to understanding brain functions in animal behavior. [1]M. Haruta et al., Jpn. J. Appl. Phys. 53 (2014) 04EL05. [2]T. Tokuda et al., NER2013 (2013) paper FrDT8.3. [3]F. Zhang et al., Nat. Rev. Neurosci. 8 (2007) 577.

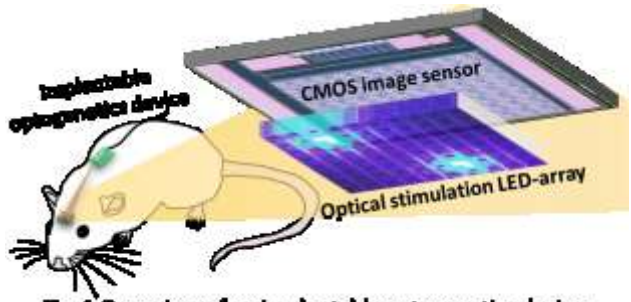


Fig. 1 Overview of an implantable optogenetics device.

Disclosures: **M. Haruta:** A. Employment/Salary (full or part-time):; full-time. **S. Nakajima:** None. **N. Kamiyama:** None. **H. Takehara:** None. **H. Takehara:** None. **T. Noda:** None. **K. Sasagawa:** None. **T. Tokuda:** None. **J. Ohta:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.09/TT84

Topic: G.04. Physiological Methods

Support: IWT Grant 110068

Title: Silicon multi electrode-optrode arrays for optogenetics *in vitro* and *in vivo*

Authors: ***L. HOFFMAN**[1,2], A. ANDREI[1], R. PUERS[1,2], G. GIELEN[1,2], D. BRAEKEN[1]
[1]Imec, Leuven, Belgium; [2]ESAT, KULeuven, Leuven, Belgium

Abstract: The technologies developed for optogenetics are important new tools to study neuronal circuits in the brain. With these fast developing tools, also other technological advances, such as devices to monitor electrical activity in the brain, are driven towards improved capabilities. To serve the fast growing and diverse field of optogenetics, ideally, these devices should incorporate multiple, independent optical outputs of different wavelengths and combine these optical outputs with electrical recording in the same plane. Tools that incorporate this set of advantages would ensure an increase in the degrees of freedom of the experimental design aiding the progress in neuroscientific research. This work presents a collection of novel electrode-optrode arrays for *in vivo* and *in vitro* optogenetic applications. In order to fabricate these tools we have integrated state-of-the-art titanium nitride electrode fabrication with silicon nitride waveguide technology. Both technologies are part of imec's CMOS compatible technology portfolio for neuronal biosensors and telecommunications and are highly reproducible, reliable and scalable. In all the presented tools, these waveguides are used to channel light of two different wavelengths (470 nm and 590 nm) into the optrode array site. The light from external sources (light emitting diodes or lasers) is coupled into these waveguides and subsequently out-coupled orthogonally at the array site by means of optical grating couplers. The *in vitro* device is composed of an array of 8 by 8 titanium nitride electrodes and 8 by 8 optrodes. Each electrode has a corresponding optrode close to it in order to register the response of optically stimulated cells. The optical outputs are approximately 6 by 20 µm thereby capable of single cell stimulation; the electrodes have a diameter of 30 µm. Both arrays have a pitch of 100 by 100 µm. For *in vivo* applications, several devices were designed. Some comprise only of optrodes and others have optrodes and electrodes. The optical-only devices are 60 µm wide and the electro-optical 100 µm. All of them contain 24 electrodes and/or 12 optical outputs (6 of each color). Additionally, the electrodes are 15 by 15 µm, while the optical outputs have the same size as their *in vitro* counterpart. For each type of probe there are three different lengths: 3, 5 and 10 µm with electrode/optrode pitches of 400, 100 and 100 µm respectively. Each option is available with thicknesses of 50 and 30 µm. Finally, the optical outputs were tested using a miniature laser diode (470 nm). The smallest optical power density measured on the grating out-couplers was 70 mW/mm², which is more than sufficient to activate opsins.

Disclosures: **L. Hoffman:** None. **A. Andrei:** None. **R. Puers:** None. **G. Gielen:** None. **D. Braeken:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.10/TT85

Topic: G.04. Physiological Methods

Support: AHA Grant

MU Research Board Grant

MU LS UROP Fellowship

NIH-IMSD Fellowship

Title: Virtual reality environment for patching and imaging in brain slices

Authors: *J. V. HIBBARD, M. A. NAVARRO, L. S. MILESCU
Biol. Sci., Univ. of Missouri, Columbia, MO

Abstract: We are developing a program that constructs a 3D representation of a virtual workspace that visualizes the brain slice and the perfusion chamber, the patch-clamp and other recording electrodes, and the imaging objectives. The user can control the operation and 3D positioning of the instruments relative to the sample, with real-time visual feedback. Specific cells can be bookmarked and linked to optical and electrical recordings, and the 3D workspace can be saved for later viewing and data analysis. We are using this software platform to explore cellular and network interactions in the respiratory pacemaker, with an experimental rig based on a Scientifica two-photon microscope and motorized positioners. This unified interface provides a more streamlined approach for combining electrophysiology, structural and functional imaging, and optogenetics experiments.

Disclosures: J.V. Hibbard: None. **M.A. Navarro:** None. **L.S. Milescu:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.11/TT86

Topic: G.04. Physiological Methods

Support: NIH Grant EY22122

NSF IOS-1120938

Title: Spatiotemporally-controlled optogenetic activation of developing ferret visual cortex

Authors: *J. OSIK[1], A. ROY[1], N. J. RITTER[1], J. R. HURVITZ WOLFF[1], J. MILLER[2], Y. WANG[1], J. FISER[3], S. D. VAN HOOSER[1]
[1]Biol., Brandeis Univ., Waltham, MA; [2]MIT, Cambridge, MA; [3]Central European Univ., Budapest, Hungary

Abstract: Realizing the full potential of optogenetic techniques will require concurrent development of light delivery technologies that improve spatial and temporal control of neuronal stimulation to facilitate increasingly informative electrophysiology recordings. Excitation (or inhibition) by light-activation of channelrhodopsin (ChR) and its many variants has quickly emerged as a preferred methodology in the neurosciences by reason of the cellular specificity achievable through conditional genetic expression. However, despite the variety of cell types targetable through the use of promoter-driven constructs, the spatial distribution of cells of any given classification can still be quite homogeneous, a fact that complicates the targeting of neural circuits that exhibit a very specific structure-function relationship, as in the circuits underlying functional maps. In studying the emergence of cortical maps *in vivo* where response variations are closely tied to spatial locations on the order of a few hundred microns in width, new light delivery techniques are needed to improve on the dispersive, on/off light control of implanted fiber optics that are commonly used to drive ChR stimulation. Two specific advances would enhance the utility of optogenetic stimulation in such applications: 1.) use of a controllable light source capable of projecting both stationary and dynamic light patterns; and 2.) improved spatial and temporal resolution while sustaining power sufficient to drive ChR activation at depth in the intact living brain. We have developed an optogenetic stimulator based on epi-illumination microscope design and equipped with a high numerical aperture objective to stimulate and collect images through the same optical axis. The stimulator is coupled to an LCD-MLA projector light source to produce high-resolution spatiotemporally varying patterns in a very confined area. We evaluate the performance of this stimulator with respect to power, optical resolution and the quality of spatial and temporal control of single and multi-unit responses at a multichannel NeuroNexus electrode. We further assay the stimulator's suitability for experimental paradigms calling for fine sequential control of horizontal cortical connections *in vivo*. Preliminary results indicate that retinotopically-constrained horizontal activation through

ChR-mediated surface training in ferret V1 is sufficient to drive asymmetry in the orientation-tuning response to drifting gratings.

Disclosures: J. Osik: None. **A. Roy:** None. **N.J. Ritter:** None. **J.R. Hurvitz Wolff:** None. **J. Miller:** None. **Y. Wang:** None. **J. Fiser:** None. **S.D. Van Hooser:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.12/TT87

Topic: G.04. Physiological Methods

Title: neuroPG: Open source software for optical pattern generation, experimentation, and data analysis

Authors: *D. MURPHY[1], B. W. AVANTS[2], J. A. DAPELLO[4], J. T. ROBINSON[2,3]
[1]Physics and Astronomy, [2]Electrical and Computer Engin., [3]Bioengineering Dept., Rice Univ., Houston, TX; [4]Hampshire Col., Amherst, MA

Abstract: Patterned illumination using a digital micromirror device (DMD) is a powerful tool for optogenetics. Compared to a scanning laser, DMDs allow for simultaneous illumination of any number of regions up to the number of pixels in the DMD. Furthermore, the intensity of illumination can be easily adjusted by modulating the light source or the DMD. Altogether, DMD illumination combined with optogenetics provides a powerful tool to investigate neural circuit behavior by allowing scientists to manipulate the complex spatiotemporal patterns of neural activity. To fully utilize DMD illumination with optogenetics, specialized software is necessary to coordinate optical stimulation patterns with the acquisition of electrophysiological and fluorescence data. To meet this need we have developed neuroPG, an open-source software package combining pattern generation and DMD control, sample visualization, and data acquisition in one application. Built on a MATLAB platform, neuroPG is also capable of post-processing, analysis, and visualization of data. The neuroPG platform greatly improves the speed and accuracy of optogenetic experiments using DMD stimulation. It enables experiments such as mapping neural responses, tracing circuit connectivity, and investigating synaptic integration. It also facilitates the task of data analysis, saving researchers a great deal of time and effort. By changing microscope objectives, it is possible to greatly increase the available field of view and allow simultaneous stimulation of tens or hundreds of neurons. Even at this scale, neuroPG

allows researchers to conduct complex experiments within the limited time window of patched cell viability.

Disclosures: D. Murphy: None. **J.A. Dapello:** None. **B.W. Avants:** None. **J.T. Robinson:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.13/TT88

Topic: G.04. Physiological Methods

Support: NIH Grant 1R43 AG046030

Title: A turn-key system for optogenetic light delivery and simultaneous electrophysiological recording

Authors: *D. A. JOHNSON[1], S. GABBERT[1], H. P. HARMON[1], E. NAYLOR[1], D. A. JOHNSON[1], P. G. HAYDON[2], R. DOYLE[2], D. J. HINES[2], P. A. PETILLO[1]
[1]Pinnacle Technol, Inc., LAWRENCE, KS; [2]Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Optogenetics harnesses a combination of genetic and optical methods to directly control neuronal events in specific cells of the central nervous system. These methods can be used to provide an unprecedented understanding of neuronal activity. Numerous commercial sources for optogenetic components exist, but turn-key systems that combine optogenetics with other neurophysiological measurement modalities do not. We successfully combined an optogenetic light delivery system and electrophysiological recording into a single turn-key, modular system designed for use with rodents. The system is capable of delivering a select wavelength of light to a specific brain region while simultaneously recording electrical signals. All synchronization between the electrophysiological, mechanical and visual inputs, and optical and stimulus outputs are controlled via a master timing, digital input/output platform as well as sophisticated software timing techniques. The optogenetics light source and coupling fiber is implemented on a standardized platform that can be easily, and accurately, implanted using existing stereotaxic techniques. Since the light source is at the head, fiber optic commutators are not required. The approach will be compatible with wireless implementations in the future. Cultured astrocytomas transfected with YFP-ChR2 were used to verify that sufficient power was delivered to the fiber optic tip to trigger relevant opsin responses. A two-photon fluorescence

microscope was used to monitor and image a light-evoked response, with RHOD 2/AM used as the fluorescent reporter of Ca^{2+} activity. For measurement of evoked Ca^{2+}, cells were incubated with RHOD 2/AM for 30 min prior to imaging. Baseline cell images were acquired and the cells were then exposed to brief pulses of light (up to 2 seconds in duration) at 470 nm from the device. The speed and duration of the evoked Ca^{2+} was dependent upon the light power and pulse length, demonstrating that the tunable system is suitable for deployment in live animals.**SUPPORT:** This research was supported by NIH grant # 1R43 AG046030.

Disclosures: **D.A. Johnson:** A. Employment/Salary (full or part-time):; Pinnacle Technology, Inc. **S. Gabbert:** A. Employment/Salary (full or part-time):; Pinnacle Technology, Inc. **H.P. Harmon:** A. Employment/Salary (full or part-time):; Pinnacle Technology, Inc. **E. Naylor:** A. Employment/Salary (full or part-time):; Pinnacle Technology, Inc. **D.A. Johnson:** A. Employment/Salary (full or part-time):; Pinnacle Technology, Inc. **P.G. Haydon:** F. Consulting Fees (e.g., advisory boards); Pinnacle Technology, Inc.. **R. Doyle:** None. **D.J. Hines:** None. **P.A. Petillo:** A. Employment/Salary (full or part-time):; Pinnacle Technology, Inc..

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.14/TT89

Topic: G.04. Physiological Methods

Support: DARPA REPAIR Program (N66001-10-C-2010)

NSF EFRI Program (0937848)

Title: A transparent ZnO optoelectrode array for probing neural circuits through spatiotemporally controlled light delivery and simultaneous multisite electrophysiology

Authors: ***J. LEE**[1], I. OZDEN[1], Y.-K. SONG[2], A. NURMIKKO[1]
[1]Sch. of Engin., Brown Univ., Providence, RI; [2]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Within the last decade, optogenetics has established itself as a powerful technique to study brain function. However, its applicability to a rich variety of neuroscience problems can be enhanced by further development of optoelectronic devices. For example, it has been rarely utilized in studies requiring simultaneous multi-site modulation and recording of neural activity. Earlier, we introduced a transparent micro-optoelectrode array (MOA), which would allow

simultaneous intracortical multisite light-delivery and electrophysiological recordings with high temporal resolution. The MOA was made from a single crystal of the transparent semiconductor zinc oxide (ZnO), in geometry and physical dimensions mimicking that of a silicon Blackrock/Utah array. For initial rodent studies, it consisted of 4×4 transparent electrodes, "optoelectrodes". Due to high transparency, neural activity could be easily recorded without light induced artifacts even at the sites of light delivery. We report here the utilization of the MOA in a transgenic (Thy1-ChR2) mouse model to characterize the effects of light power and spatiotemporal delivery on neural population activity and behavior. To perform light delivery through the MOA, the MOA was inserted acutely into either motor or barrel cortex, and broadband neural activity was recorded in response to light delivered through one or more optoelectrodes by scanning laser beam. At very low light power levels (~1 μW, 473 nm), modulated neural activity was observed, but only at the optoelectrodes from which light was delivered. This very low power regime was quite useful for precise spatiotemporally controlled stimulation of underlying neural circuitry. As an example, we reproduced barrel cortical activity in response to whisker stimulation by patterned optical stimulation which has not been practically possible with other means. As optical power began to increase closer to more typical excitation levels in the literature (up to 1 mW), modulated activity began to spread over the 4×4 array due to network connectivity (and some additional light diffusion). In our "medium power regime" (several 10 μW), we could deduce network connectivity and connection strength by response latency and firing rate analysis. At higher power levels (several 100 μW), we could observe behavioral responses in both awake and anesthetized animals which helped us to map the motor cortex functionally below the array. Ongoing work is focusing on the use of the ZnO MOAs to study the motor cortical neural dynamics in response to rodent behavior, while also characterizing and improving device features, in preparation for chronic implant and larger size arrays.

Disclosures: **J. Lee:** None. **I. Ozden:** None. **Y. Song:** None. **A. Nurmikko:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.15/TT90

Topic: G.04. Physiological Methods

Support: Max Planck Society

Title: A system for neural circuit mapping at single cell resolution using two-photon excitation of soma-targeted channelrhodopsin

Authors: *C. A. BAKER[1], A. PARRA-MARTIN[2], M. M. BOLTON[1]
[1]Disorders of Neural Circuit Function, [2]Functional Architecture of Cerebral Cortex, Max Planck Florida Inst., Jupiter, FL

Abstract: Psychiatric disorders are being increasingly linked to dysfunctions in molecules involved in the development or maintenance of functional neural circuit connectivity. Delineating the effects of disease-associated genetic changes on the fine structure of neuronal networks will be crucial to understanding these disorders and for the development of therapeutics. Optogenetics using channelrhodopsin (ChR2) and selective targeting of genetically-defined cell types has revolutionized the study of long range projections at moderate resolution, but conventional optical stimulation paradigms likely excite cells above and below the cell of interest and thus limit their use in mapping neural circuits at single-cell resolution. Two-photon microscopy can stimulate a diffraction-limited volume deep within tissue, but may not simultaneously excite enough ChR2 molecules to depolarize a neuron to action potential threshold. We utilize recent developments in temporal focusing of two-photon excitation to generate a disk-like field of excitation in acute brain slices, simultaneously exciting multiple ChR2 molecules in a single focal plane off-target activation of cells above or below the neuron of interest. Moreover, we have further reduced the potential for off-target effects by spatially restricting ChR2 expression to the neuronal soma and proximal dendrites. Under these conditions we elicit action potentials only when stimulating the soma of ChR2-expressing cells; the lack of responses to stimulating fibers of passage or at axially displaced locations highlights the utility of the technique for fine mapping. These protocols can also be combined with optical calcium or voltage indicators to verify the stimulation effects and examine the responses of neurons throughout the network. We apply these techniques to examine local circuit connectivity in the neocortex of mice harboring mutations associated with autism in humans.

Disclosures: C.A. Baker: None. **M.M. Bolton:** None. **A. Parra-Martin:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.16/TT91

Topic: G.04. Physiological Methods

Support: Center for Integrated Smart Sensors funded by the Ministry of Science, ICT & Future Planning as Global Frontier Project(CISS-2012366054204)

National Research Foundation of Korea Grant funded by the Korean Government (NRF-2010-H1C6A1)

BK21 plus program (Smart Humanity Convergence Center, Department of Transdisciplinary Studies, Graduate School of Convergence Science and Technology. Seoul National University) funded by the Ministry of Education (MOE, Korea)

Title: Tuning optical characteristics of Channelrhodopsin-2 by conjugating plasmonic nanoparticles

Authors: *Y. BANG[1,2], S. LEE[1], Y. KIM[1,2], A.-H. LEE[1], Y.-K. SONG[1,2]
[1]Dept. of Transdisciplinary Studies, Program in Nano Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Advanced Inst. of Convergence Technol., Suwon, Korea, Republic of

Abstract: Channelrhodopsin-2(ChR2) is one of the major light-gated ion channels which can depolarize neurons with an optimal action spectra at 473nm wavelength. Even in this precise cell-type specific photo-stimulation technique, it generally requires a high power laser or a high brightness LED as an optical excitation source, due to relatively small optical density of ChR2 [1,2]. Moreover, in intact brain, blue light tends to scatter more than red light and have limited penetration depth issues [3]. Thus, in current optogenetic fields, many researchers have focused on genetic approaches to develop ChR variants with various optimal action spectra ranging from 450nm to 630nm, and to acquire faster kinetics and more efficient membrane trafficking [3]. These conventional genetic methods have succeeded but it takes lots time and resources for finding the right genetic mutations and optimizing their expressions in mammalian neurons. With consideration of effective nanotechnology approaches rather than genetic approaches, we applied AviTag bio-conjugation method in order to induce Localized Surface Plasmonic Resonance (LSPR) effect through metallic plasmonic nanoparticles near ChR2 channels. Especially, silver nanoparticle has stronger LSPR peaks than any other metallic nanoparticles, ranging from 400nm to 500nm, depending on their sizes. It also enables inducing enhancement of light intensity [4]. The DNA sequence for 15 amino acids representing AviTag was cloned to Synapsin1-hChR2-EYFP lentiviral plasmids, allowing the expression of AviTag at the N-terminal of ChR2 for conjugating biotinylated plasmonic nanoparticles. In order to confirm the tagging strategy of AviTag in primary rat hippocampal neuron, biotin conjugated 655nm Qdots were used to visually localize N-terminal side of ChR2 channel through fluorescence and electron microscopy imaging. In addition, application of biotinylated silver nanoparticles and its optical/kinetic analysis were conducted. Thus, by conjugating different sizes of silver nanoparticles to ChR2, we could tune its optical characteristics and also increase its photosensitivity, which could enable photo-modulation of neurons with much less optical power and various wavelength of light. References [1] E. Boyden et al., Nature Neuroscience,

8(9):1263-1268 (2005) [2] K. Deisseroth et al., Nature Methods, 8(1):26-29 (2011) [3] J. Lin et al., Nature Neuroscience, 16:1499-1508 (2013) [4] M. Rycenga et al., Chemical Reviews, 111, 3669-3712 (2011)

Disclosures: **Y. Bang:** None. **S. Lee:** None. **Y. Kim:** None. **A. Lee:** None. **Y. Song:** None.

Poster

271. Optogenetics: Tool Development

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 271.17/TT92

Topic: G.04. Physiological Methods

Support: UK BBSRC grant BB/L018268/1

Title: Multiscale computational tools for optogenetics

Authors: ***K. NIKOLIC**[1], S. JARVIS[2], S. SCHULTZ[2]
[1]Electrical & Electronic Engin., [2]Bioengineering, Imperial Col. London, London, United Kingdom

Abstract: Optogenetics is rapidly becoming a key technology for neuroscience and deconstruction of brain circuits, and new applications are rapidly emerging throughout biology. At the same time new variants of opsins are being synthesized. There are several families of opsins, each of which has unique temporal and spectral properties. There is a substantial effort to characterize opsins for each cell population, and a continual drive to improve their efficacy. While the effect of an opsin can be quantified at the level of individual cells (e.g. neurons), it currently remains practically impossible to experimentally test each opsin for each cell type or biological system of interest. This significantly limits the effectiveness of optogenetics as a biological tool. Hence we propose a set of computational tools for optogenetics. Firstly, we developed a tool for characterizing opsins, allowing us to link from the underlying biophysical photocycle that defines kinetic model of opsins (molecular-complex scale) - tool 1. This level is significant to obtain a functional understanding of each opsin and hence guide not only opsin choice for a given system, but potentially also guide opsin development. From the biophysical model, the corresponding state-model representations (of ChR2 and NpHR, a few more opsins to follow) have been included into ion-channel representation in NEURON (tool 2). This tool allows the inclusion of opsins within single-compartment and multi-compartment representations of single neurons. Finally, we implement opsins within “point neurons”, such as Leaky Integrate

and Fire (LIF), for inclusion in network level simulations - tool 3. We demonstrate the tools on the example of ChR2 and NpHR-expressing neocortical layer 5b pyramidal cells. We first identify a six-state functional model for ChR2 and a three-state model for NpHR and then implement it in NEURON. Then we present results for typical illumination strategies, back-propagation of action potentials, threshold dynamics, etc. We demonstrate how the gain and threshold of the transfer characteristics of a transfected neuron (input: current, output: spikes) can be modulated by selectively targeting whole cell, or apical and basal dendrites. This approach will allow the inclusion of realistic models of optogenetics in existing simulations, allowing the use of virtual opsins to identify the correct experimental opsin choice. Together, these tools will improve the use of optogenetics as an effective and refined tool, enabling its potential to transform the biological sciences.

Disclosures: **K. Nikolic:** None. **S. Jarvis:** None. **S. Schultz:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.01/UU1

Topic: G.07. Data Analysis and Statistics

Title: Characterizing EEG artifacts with matching pursuit time-frequency decomposition

Authors: *K. W. WHITAKER, V. LAWHERN, P. J. FRANASZCZUK
Translational Neurosci. Br., U.S. Army Res. Lab., Aberdeen Proving Ground, MD

Abstract: Electroencephalography has the potential to be adapted and utilized outside of the lab, but this will require new computational approaches to understanding the data. Matching pursuit is a data-driven approach to signal analysis and decomposition that is not dependent on expert judgment and expertise. A dictionary of Gabor functions (atoms) in time -frequency space was used to characterize EEG data with specific categories of common movement-based artifacts. The data was acquired with three different EEG acquisition systems worn by the same five subjects who performed a predefined set of movements after an auditory cue. All of the EEG acquisition systems have been used in real world environments such as shopping centers, office buildings and parks. Extremely minimal preprocessing, down selection of channels and data filtration, was performed on the data. The parameters of the atoms in the decomposition were found to be specific to categories of movement artifacts. These atoms are characterized by a set of five parameters (phase, modulus, frequency, time and octave (scale)). Only frequency, time

and modulus were necessary and sufficient to discriminate between different categories of artifacts. This analysis of EEG artifacts revealed useful information for providing context to interpret and understand real world brain activity. In addition to enabling the development of novel classification schemes for movement artifacts, this experiment supports EEG acquisition technology with minimal data pre-processing prior to analysis.

Disclosures: K.W. Whitaker: None. **V. Lawhern:** None. **P.J. Franaszczuk:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.02/UU2

Topic: G.07. Data Analysis and Statistics

Title: Real time algorithms for sharp-wave ripple detection

Authors: *A. SETHI, C. KEMERE
Rice Univ., Houston, TX

Abstract: Neural activity during sharp wave ripples (SWR), short bursts of co-ordinated oscillatory activity in the CA1 region of the rodent hippocampus, is believed to be responsible for a variety of memory functions from consolidation to recall. Detection of these events using real time methods, has thus far relied on simple heuristic thresholds. This investigation tests and improves the current methods for detection of SWR events in neural recordings. We propose novel methods and profile current methods, in time and frequency domain, to reduce latency in ripple detection. Proposed algorithms are tested on simulated data. The findings show that simple real-time algorithms can improve upon existing power thresholding methods and can detect ripple activity with latencies in the range of 10-20 ms with acceptable false positive rates.

Disclosures: A. Sethi: None. **C. Kemere:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.03/UU3

Topic: G.07. Data Analysis and Statistics

Title: Automatic eye-movement artifact removal using short fast fourier transform and nonnegative matrix factorization

Authors: *H. TSUBAKIDA[1], T. SHIRATORI[1], A. ISHIYAMA[1], Y. ONO[2]
[1]Sch. of Advanced Sci. and Engin., Waseda Univ. c/O Prof. Tsushi Ishiyama, Shinjukuku, Japan; [2]Meiji Univ., Kanagawa, Japan

Abstract: This paper introduces a novel method to automatically remove electro-oculogram (EOG) artifact using short Fast Fourier Transform (sFFT) and nonnegative matrix factorization (NMF). This method requires at least single channel Electroencephalogram (EEG) and concurrently measured EOG. First, sFFT is separately applied to EEG and EOG data to construct nonnegative matrix data Xeye and Xch, respectively, which correspond to the time-frequency distribution of the respective signals. We set the frequency band for extraction from 1 to 22Hz, the frequency band that is frequently used in EEG analysis. Second, NMF decomposed Xeye into two matrices, basis matrix Aeye and coefficient matrix Seye. Third, NMF further decomposed Xch into A = [Aeye Ach] and S = [Seye Sch], under the constraint that A and S should include Aeye and Seye as subspaces. Forth, reconstruct nonnegative matrix data X'ch by multiplying Ach and Sch. Finally, inverse sFFT was applied to reconstruct noise-removed EEG signal. We tested the feasibility of this method using EEG signals measured from eight healthy young volunteers. Participants kept rest for a period of 3 s when a cue appeared on a computer screen while EEG and EOG signals were recorded. The cue appeared 20 times per one session. EEG signals were recorded from 15 electrodes placed on the scalp of a subject and EOG was from electrode placed near the left eye, sampled at 125Hz. Our method removed EOG artifact more effectively than the conventional NMF-related method using only Aeye or Seye as a constraint for generating Ach and/or Sch. Our method removed the EOG artifact as effectively as independent component analysis (ICA), which has been reported to be an efficient signal processing to remove EOG artifact from EEG. Our method is superior to ICA in the sense that it can remove EOG artifact without any subjective criteria and hyper-parameters which ICA requires. Figure1 shows raw EEG data measured from a representative subject and the results of noise removal using our method, conventional method, and ICA. These results demonstrate the effectiveness of our new method.

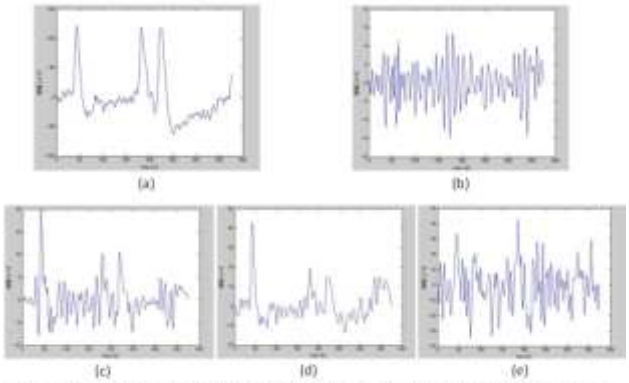


Fig.1 (a) raw EEG data (b) the result of noise removal using our method (c) the result of noise removal using conventional NMF-related method under the constraint that ***A*** should include ***Aeye*** as subspace (d) the result of noise removal using conventional NMF-related method under the constraint that ***S*** should include ***Seye*** as subspace (e) the result of noise removal using ICA

Disclosures: H. Tsubakida: None. **T. Shiratori:** None. **A. Ishiyama:** None. **Y. Ono:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.04/UU4

Topic: G.07. Data Analysis and Statistics

Support: NIH Grant MH080309

NSF GRFP

Title: Spatiotemporal adaptive filter for cleaning MR artifacts in simultaneous EEG-fMRI recordings

Authors: *A. M. GORDON[1], A. GONZALEZ-BARBOSA[2], J. M. ALES[1], A. M. NORCIA[1], A. D. WAGNER[1,3]
[1]Dept. of Psychology, [2]Dept. of Electrical Engin., Stanford Univ., STANFORD, CA;
[3]Neurosciences Program, Stanford Univ., Stanford, CA

Abstract: Simultaneous recording of electroencephalographic (EEG) and functional MRI (fMRI) data can provide spatiotemporal precision not achievable by using either technique individually. One limitation of simultaneous EEG/fMRI recordings is that the EEG signal is contaminated with MR-related artifacts. There are two main noise sources added to the EEG signal: 1) gradient-based artifacts related to MR data acquisition, and 2) biophysical artifacts

amplified by the large B0 field in the MR environment. While there has been an increase in the number of studies in which EEG and fMRI data are acquired simultaneously, how to optimally remove these artifacts remains unresolved for task-related designs. Here, we propose a new technique that uses spatial and temporal analysis to adaptively extract artifacts from the EEG signal. This technique finds the optimal basis (via Singular Value Decomposition) that loads on the spatial (channels) and temporal (time-points locked to artifact onset) data matrix. Additionally, this filter uses information--the MR slice ID and heart rate (monitored through a photoplethysmogram)--to better fit the data. To quantify performance, we examined the signal-to-noise ratio (SNR) of task-related measures, including visually evoked potentials (VEPs) and motor response evoked potentials (MEPs), across subjects and recording sessions (N=95). After gradient artifact removal, we found a significant increase in SNR for VEPs and MEPs compared to the most commonly used technique in the literature (FASTR, Niazy 2005). Subsequent BCG cleaning yielded further improvements on SNR for VEPs but not MEPs. This work provides a novel artifact removal technique that outperforms the current standard in task-related measurements of artifact removal performance.

Disclosures: **A.M. Gordon:** None. **A. Gonzalez-Barbosa:** None. **J.M. Ales:** None. **A.M. Norcia:** None. **A.D. Wagner:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.05/UU5

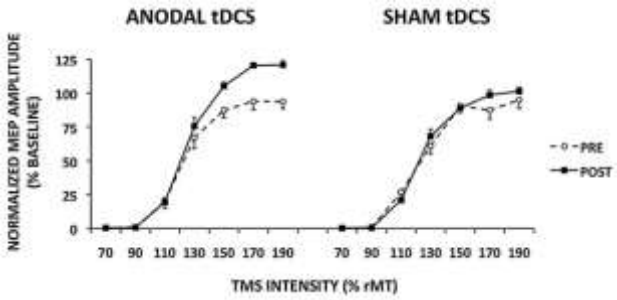
Topic: G.07. Data Analysis and Statistics

Title: Statistics: Minimal effort but maximum profit!

Authors: *H. THIJS[1], R. MEESEN[2], K. CUYPERS[2]
[1]I-Biostat, Diepenbeek, Belgium; [2]REVAL, Hasselt Univ., Hasselt, Belgium

Abstract: In what follows a more detailed description is provided about how the investment of time in such collaboration of clinical researchers within the field of brain research with a statistician contributed in optimizing the results of the experiments. More precisely, as described in CUYPERS et al (2013), applying anodal tDCS in humans has shown to influence CS excitability. Within this experiment anodal tDCS and sham tDCS were both applied to the primary motor cortex for 20 min and TMS was used to measure the influence on CS excitability. In a first statistical approach the area under the recruitment curves were compared for both

anodal tDCS and sham tDCS showing a significantly increase after application of anodal tDCS while after application of sham tDCS no increase was detected. In contrast, a second statistical analysis used a sigmoidal curve-analysis which indicated a higher level of CS output after anodal tDCS. The key aspect of this second approach is the use of a mathematical model to describe the individual curves in much more detail. While the area under the curve may not be able to discriminate between two patients this second approach has a much better discriminative power. Finally, both results lead to the same conclusion that anodal tDCS caused an increase in CS output and might can therefore be used to facilitate motor recovery in MS patients, but from the second approach more insights with respect to the behavior of the CS output could be derived. In conclusion simple methods should be used where possible but the drive for the correct analysis is equally important. In the end it are the Multiple Sclerosis patients who might benefit most from the combination of an excellent experiment with a correct statistical analysis. **Reference:** CUYPERS K., LEENUS D., VAN WIJMEERSCH B., THIJS H., LEVIN O., SWINNEN S. and MEESEN R. (2013) '*Anodal tDCS increases corticospinal output and projection strength in multiple sclerosis'.* NEUROSCIENCE LETTERS, 554, p.151-155.



Disclosures: **H. Thijs:** None. **R. Meesen:** None. **K. Cuypers:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.06/UU6

Topic: G.07. Data Analysis and Statistics

Support: NIDCD Grant DC04548 to ERG

Title: Fractal analysis of spontaneous activity in single neurons

Authors: L. H. FAVELA[1], C. A. COEY[2], *E. R. GRIFF[3], M. J. RICHARDSON[2]
[1]Philosophy and Psychology, [2]Psychology, Univ. of Cincinnati, Cincinnati, OH; [3]Univ. Cincinnati, Cincinnati, OH

Abstract: The spontaneous activity of single neurons was analyzed via fractal methods. Time-series data in the form of interspike intervals (ISIs) from single-unit recordings were obtained from reliably identified mitral cells recorded *in vivo* from the main olfactory bulb of freely breathing anesthetized rats (Nica, Matter, & Griff, 2010). The anesthetic plane was adjusted such that a toe pinch desynchronized the EEG without causing limb withdrawal. These data were originally analyzed via standard linear statistics in order to compare mean spike rates. That analysis suggested different subclasses of mitral cells. Results from other previous experiments compared the spontaneous activity of mitral and tufted cells (Stakic, Suchanek, Ziegler, & Griff, 2011). A goal of the current research was to further examine the different subclasses of mitral cells by utilizing nonlinear methods to distinguish subclasses based on the dynamical structure of their spontaneous activity. Detrended fluctuation analysis (DFA), a type of nonlinear, fractal analysis was utilized to examine the ISIs from 29 single unit mitral cell recordings (Nica et al., 2010). The spontaneous activity of these cells was recorded for an average of 540 s (range 100-1243 s). DFA identifies the structure in the fluctuations in a measurement variable over time by assessing the scaling relation between the size and time scale of variation in behavior. The Hurst exponent (H) is a measure of fractal scaling (Ihlen, 2012). Results of the DFA suggested two subclasses of mitral cells, referred to here as A and B. There was no significant difference in the fractal scaling of cell groups A and B at shorter timescale fluctuations, with cells in both groups exhibiting dynamical structure near random variation ($H \approx 0.5$). There was a significant difference between the groups at longer timescales, with group B exhibiting variation near $H \approx 1.0$, whereas group A did not. The results of our DFA analysis highlight the power of nonlinear methods to capture the dynamical structure of neural behavior. This approach is being extended to multifractal techniques and to a comparison between mitral and tufted cell spontaneous activity.

Disclosures: L.H. Favela: None. **C.A. Coey:** None. **E.R. Griff:** None. **M.J. Richardson:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

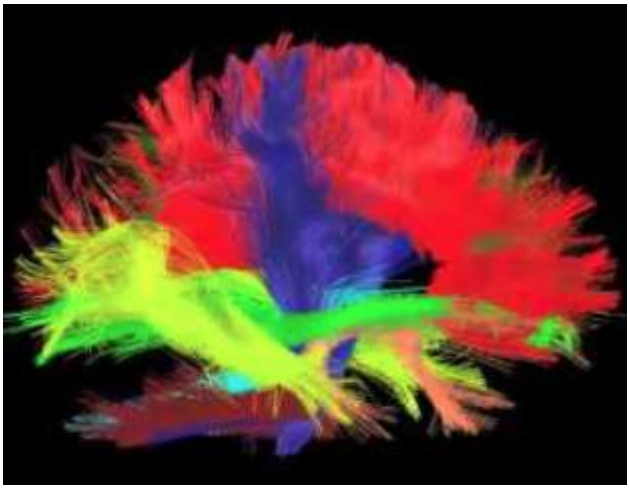
Program#/Poster#: 272.07/UU7

Topic: G.07. Data Analysis and Statistics

Title: Automatic white matter bundle dissection in ad and pd - results from adni and ppmi

Authors: *M. DESCOTEAUX[1,2], J.-C. HOUDE[1,2], J.-R. BÉLANGER[2], P.-M. JODOIN[1,2], F. MORENCY[2]
[1]Computer Sci., Univ. De Sherbrooke, Sherbrooke, QC, Canada; [2]Imeka, Sherbrooke, QC, Canada

Abstract: Introduction: This work presents a technique to automatically dissect the white matter bundles of the human brain from non-invasive diffusion Magnetic Resonance Imaging (MRI) fiber tracking datasets from the ADNI (Alzheimer's Disease NeuroImage Initiative) and PPMI (Parkinson's Progression Markers Iniative) public databases. Methods: We performed fiber tractography using Dipy (www.dipy.org) on the ADNI and PPMI databases from available subjects with diffusion tensor imaging (DTI) datasets. Using anatomical *FreeSurfer* labels from T1-weighted images of each subject, we can automatically and robustly extract 27 major white matter bundles (corticospinal tract, arcuate fasciculus, cingulum, amongst many others). The white matter query language tool is used to define specific queries for the ADNI and PPMI databases, where careful exclusion and inclusion definitions are defined to obtain white bundles free of spurious tracts. Finally, the mean and standard deviation fractional anisotropy (FA), diffusivities (axial, radial, mean), and apparent fiber density diffusion metrics are computed along each bundle. Results: All 27 bundles are successfully extracted from more than 90% of the subjects in the ADNI and PPMI databases. A quality assurance procedure was run to identify the white matter bundles with spurious tracts. The majority of bundles are robustly dissected. This dissection is done in the native space of every subject and thus avoids having to register to a template space. Mean and standard deviation of the diffusion metrics show large variations across populations and more statistical analysis needs to be done. Conclusions: We used a novel white matter query language to dissect detailed white matter bundles using state-of-the-art tractography. This novel analysis could permit automatic construction of brain atlases on dedicated populations, automatic delineation of eloquent structures of the brain and most importantly the detection of abnormalities in white matter bundles, which we are confident could improve the diagnostics Alzheimer's and Parkinson's disease.



Disclosures: **M. Descoteaux:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Imeka Solution Inc (www.imeka.ca). **J. Houde:** A. Employment/Salary (full or part-time):; Imeka Solution Inc (www.imeka.ca). **J. Bélanger:** A. Employment/Salary (full or part-time):; Imeka Solution Inc (www.imeka.ca). **P. Jodoin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Imeka Solution Inc (www.imeka.ca). **F. Morency:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Imeka Solution Inc (www.imeka.ca).

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.08/UU8

Topic: G.07. Data Analysis and Statistics

Support: National Institutes of Health R01 NS078396-01

Stanford NeuroVentures Program to J.P.

Belgian-American Educational Foundation – Henri Benedictus

Rotary Medical Foundation of Liège

Wellcome Trust

FRS-FNRS Belgian Research Funds

Title: Introducing Multiple Kernel Learning for automatic selection of features in intracranial EEG

Authors: *J. V. SCHROUFF[1,2], M. ÖZKER[3], M. DASTJERDI[1], B. FOSTER[1,2], V. RANGARAJAN[1,2], J. MOURÃO-MIRANDA[4], C. PHILLIPS[5], J. PARVIZI[1,2]
[1]Lab. of Behavioral and Cognitive Neurosci., [2]Stanford Human Intracranial Cognitive Electrophysiology Program (SHICEP), Stanford Univ., Palo Alto, CA; [3]Hlth. Sci. Ctr., Univ. of Texas, Houston, TX; [4]Dept. of Computer Sci., Univ. Col. London, London, United Kingdom; [5]Cyclotron Res. Ctr., Univ. of Liège, Liège, Belgium

Abstract: Introduction Machine learning models have been successfully applied to neuroimaging data to make predictions about behavioral/cognitive states of interest based on the pattern of activation or anatomy over a set of features. While these multivariate methods have greatly helped the neuroimaging community, their application to electrophysiological data has been scarce, particularly for invasive techniques (e.g., electrocorticography, ECoG). In the present work, we propose a novel approach to decode electrophysiological recordings based on multiple kernel learning (MKL). This technique combines the signal from different channels and/or frequency bands hierarchically. In addition, the considered algorithm is sparse, which eases model interpretation. We illustrate the success of this approach using our own previously published ECoG data. Material and Methods The dataset used in this work comprises ECoG recordings from 3 subjects implanted with grid and strip electrodes for clinical treatment of refractory epilepsy. The patients performed an experimentally controlled task involving numerical and autobiographical episodic memory conditions. The ECoG signal used for further classification was the instantaneous power in 6 frequency bands (δ:1-4Hz, θ: 4-8Hz, α: 8-12Hz, β: 15-25Hz, low-γ: 30-55Hz, high-γ a.k.a. high frequency broadband, HFB: 70-110Hz). For classification, we used the simple MKL algorithm, which linearly combines multiple models defined by kernels. From each kernel (here channel and/or frequency band), a multivariate model was built, distinguishing between the two task conditions. The MKL algorithm then linearly combines the different models obtained by computing a weight for each kernel. The algorithm considered in this work is sparse, i.e. some of those weights will be perfectly null and the corresponding channel and/or frequency band will hence not contribute to the model. Results For each subject, considering each channel in the HFB range led to high classification accuracies (S1: 96.9%, S2: 75.8%, S3: 85.8%). This result was expected in view of the previous univariate analyses performed on this dataset (Dastjerdi et al, Nature Communications 2013). Furthermore, the channels most contributing to the obtained model were the same as previously reported in the aforementioned study. However, considering the different frequency bands as different kernels led to higher classification accuracies for all subjects (S1: 96.9%, S2: 77.4%, S3: 90.2%). This result shows that, although the HFB was always selected in the final model, other bands were also contributing to the classification (S1: HFB and low- γ, S2: β, HFB, α, δ, θ, S3: HFB, α, δ).

Disclosures: **J.V. Schrouff:** None. **M. Özker:** None. **M. Dastjerdi:** None. **B. Foster:** None. **V. Rangarajan:** None. **J. Mourão-Miranda:** None. **C. Phillips:** None. **J. Parvizi:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.09/UU9

Topic: G.07. Data Analysis and Statistics

Title: Six month reproducibility of diffusion spectrum imaging

Authors: ***A. B. YU**[1], J. M. VETTEL[1], S. T. GRAFTON[2], T. D. VERSTYNEN[3]
[1]Translational Neurosci. Br., United States Army Res. Lab., Aberdeen Proving Ground, MD; [2]Dept. of Psychological and Brain Sci. and UCSB Brain Imaging Ctr., Univ. of California Santa Barbara, Santa Barbara, CA; [3]Dept. of Psychology, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Advancements in diffusion weighted imaging methods enable novel approaches to quantify individual differences in structural connectivity, providing a means to understand how much variability in structure can account for functional and behavioral processes. However, in order to understand structural differences between individuals or structural changes within an individual, an understanding of the reproducibility of the measurement is essential. We obtained diffusion spectrum imaging (DSI) scans for 14 subjects at 6 month intervals to examine the reproducibility of diffusion-based metrics at three different levels of analysis: voxel-based, tractography-based, and network-based analyses. The primary white matter fiber tract directions within each voxel were reconstructed in MNI-space using q-space diffeomorphic reconstruction (QSDR). QSDR yields an orientation distribution function (ODF) at each voxel, giving the magnitude and direction of anisotropy of multiple independent fiber tracts in a standardized space across subjects. Voxel-based analyses of quantitative anisotropy (QA) showed that white-matter regions were variable across subjects and yet highly reproducible within subjects. Across the brain, we found varied reproducibility in the ODF, where some areas were more sensitive to distortions. Whole-brain tractography was then used to examine reproducibility of estimated structural connectivity across voxels. The length, counts, and overall directions of fibers were reproducible across time, though individual tracts showed variable levels of reproducibility. This variability in the tracts was confirmed in the network-based analyses where we found substantial individual variability in the degree of reproducibility. Networks for each individual scan were created using tract counts between region parcellations from a T1-weighted scan. Region-

specific statistics including betweenness and degree distribution showed highly variable reproducibility across subjects, suggesting that network measures may be less stable in the presence of noise and compounded errors in pre-processing. Overall, we find that DSI can reliably capture individual variability in white matter pathways, with high within-subject reproducibility at relatively short (e.g., 6 month) time frames. Our findings guide expectations for how well diffusion imaging can detect longitudinal changes in white matter integrity.

Disclosures: **A.B. Yu:** None. **J.M. Vettel:** None. **S.T. Grafton:** None. **T.D. Verstynen:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.10/UU10

Topic: G.07. Data Analysis and Statistics

Title: Most informative graph theory metrics for unsupervised classification of cognitive states

Authors: L. C. BUCHANAN, *J. GONZALEZ CASTILLO, C. W. HOY, D. A. HANDWERKER, P. A. BANDETTINI
SFIM/LBC/NIMH/NIH, Bethesda, MD

Abstract: Background: Previous studies [1,2] have shown how whole-brain functional connectivity can differentiate cognitive states. One remaining challenge is to compress the large dimensionality of the connectome's feature space to ease computational hurdles and uncover drivers of distinct mental states while maintaining classification power. Here, we evaluate if graph theory network metrics can dramatically reduce the dimensionality of the data without negatively affecting classification accuracy. We also rank graph theory metrics by their ability to discriminate cognitive states. Finally, we find a subset of metrics that convey the most cognitively relevant information about the network structure of the brain. Methods: We collected 25mins of fMRI data (TR=1.5s, voxel size=8mm3, 7T) on 22 subjects as they perform and transition between 4 tasks: rest, math, 2-back, and visual attention. Each task was performed for 3mins during 2 different blocks within the scan. After pre-processing, time-series for 150 ROIs from the Craddock Atlas [3] were extracted and segmented into 90s non-overlapping windows aligned with task blocks. For each window, we computed 20 graph theory metrics using the Brain Connectivity Toolbox [4]. Some metrics were computed at the whole brain level, while others were computed for each ROI. Metrics were then sorted based on how well they discriminate between tasks. Finally, vectors with an increasing number of metrics entered a k-

means clustering algorithm (k=4). Agreement between k-means results and the window groupings according to task was quantified using the Adjusted Rand Index (ARI) [5]. Results: (1) The discriminative values of the evaluated set of metrics varied substantially across metrics. (2) Locally computed metrics (i.e., per ROI) are more informative than global metrics in discriminating cognitive states. (3) Optimal combinations of metrics produced moderate accuracy when classifying windows according to task (ARI = 0.71 +/- 0.20). These accuracy levels are lower than those obtained with classification algorithms based on whole-brain connectivity matrices for the same set of ROIs and tasks [2]. Conclusions: We show how only a subset of graph theory metrics conveys relevant information about functional brain reorganization when performing different tasks. Optimally combining these metrics helped us achieve moderate classification accuracy when attempting to discriminate between cognitive states established by the task paradigm. [1] Shrier et al. 2012 Cer Cortex; [2] Gonzalez-Castillo et al. OHBM 2013; [3] Craddock et al. 2012 Hum. Brain Mapp.; [4] Rubinov et al. 2010 NeuroImage. [5] Hubert et al. 1989 J. Classification.

Disclosures: L.C. Buchanan: None. **J. Gonzalez Castillo:** None. **C.W. Hoy:** None. **D.A. Handwerker:** None. **P.A. Bandettini:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.11/UU11

Topic: G.07. Data Analysis and Statistics

Support: LLNL Laboratory Directed Research and Development #14-SI-001

Title: Extracellular recordings of human dorsal root ganglion cells using the *in vitro* chip-based human investigational platform (ichip)

Authors: *S. H. FELIX, M. W. MCNERNEY, H. A. ENRIGHT, E. V. MUKERJEE, N. O. FISCHER, J. J. OSBURN, A. S. CHANG, S. E. BAKER, F. QIAN, J. V. CANDY, K. S. KULP, E. K. WHEELER, S. S. PANNU
Lawrence Livermore Natl. Lab., Livermore, CA

Abstract: The envisioned *In Vitro* Chip-Based Human Investigational Platform (iCHIP) system will integrate multiple representative cell types with the objective of dramatically reducing the time needed to develop countermeasures against toxic agents and bring new therapies to market.

We have demonstrated that the iCHIP can sustain human neural cultures for over 29 days *in vitro*. Electrophysiological recordings from a multielectrode array (MEA) provide information about cell health and responses to different agents. To extract useful information from the MEA, it is important to understand the significance of synchronous signals that are detected on multiple channels. We have analyzed various scenarios of synchronous spiking in order to distinguish between duplicated spikes and local propagation in the human neural culture. The MEA consisted of a four by four array of electroplated platinum electrodes, 20µm in diameter with a pitch of 250µm. Human primary dorsal root ganglion (DRG) cells were obtained from Anabios, Inc. Various chemical challenges and rinses were introduced into the cell culture using an automated FloPro fluidic handling system while monitoring electrophysiological recordings. Statistical signal processing algorithms were used to detect spikes that exhibit a relatively low signal to noise ratio. We analyzed the occurrence of correlated spiking on multiple channels. Some of the spikes are duplicated indicating strong electrical coupling between the electrodes. Other instances exhibit shifted waveforms between channels, indicating that the MEA had resolved local propagation among the DRG cells. Additional studies to be presented will include fluorescent changes with an intracellular Ca^{2+} indicator dye (Fluo8). The results provide insight on spatial and temporal resolution of the iCHIP MEA, coupling of the cells to the electrodes, and the morphology of the human DRG culture. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52- 07NA27344. Document #LLNL-ABS-654038

Disclosures: **S.H. Felix:** None. **M.W. McNerney:** None. **H.A. Enright:** None. **E.V. Mukerjee:** None. **N.O. Fischer:** None. **J.J. Osburn:** None. **A.S. Chang:** None. **S.E. Baker:** None. **F. Qian:** None. **J.V. Candy:** None. **K.S. Kulp:** None. **E.K. Wheeler:** None. **S.S. Pannu:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.12/UU12

Topic: G.07. Data Analysis and Statistics

Title: Assessing signal quality of a dry EEG system across multiple signal domains common in EEG research

Authors: *W. HAIRSTON, G. A. APKER
Translational Neurosci. Br., US Army Res. Lab., Aberdeen Proving Ground, MD

Abstract: As neuroscientists challenge the boundaries of the conventional laboratory, there has been an increased demand for easy-application "dry", high-density, mobile EEG systems. However, the research community has been reluctant to adopt newly developed systems over concerns regarding their signal quality relative to the standard gel (wet) electrode technologies. This skepticism in part is due to a lack of in-depth demonstration of viability across the compendium of classically studied EEG signals. Here we present a method for standardized validation of any EEG-system for laboratory quality recording of brain signals and demonstrate its application to a 32-channel dry EEG system with the goal of providing a quantitative assessment of its signal reproduction quality. In our method, a multi-section waveform is generated with 1 & 2 Hz triangle waves, known oscillatory signals spanning 0.1-200 Hz, as well as pre-recorded EEG signals recorded in the laboratory during typical experimental conditions (VEP, eyes open/closed, movement/artifact generation) laboratory grade gold standard. These signals are passed into a 'phantom head' device on which an EEG system is mounted and the signal is repeated several times to assess the long term reliability of the recordings. The recordings of the known signal are compared between the test system and a laboratory grade system (also driven by signals from the phantom) for each section of input signal using typical EEG analysis techniques (i.e. signal correlation, RMS comparison, spectral analysis, ERP amplitude, etc). Simple correlation analysis between laboratory standard and input signal in each phase of the signal were generally high (average R2 of 0.63, maximum of 0.96) with the exception of extremely low frequencies (< 1 Hz). We found similarly strong correlation of the test system's performance with both the laboratory standard system and the input signal itself in nearly all phases of the recording (average R2 of 0.62 and 0.58, respectively, with a maximum of 0.96 for both). Further, these relationships did not significantly change over the course of several hours of recording with the dry-system, a known limitation of gel-based systems. We did observe a significant delta-band deviation of the test system signal, presumably due to differences in embedded filter characteristics. While these results suggests that signal reproduction quality of the test system performs comparably to the laboratory standard in most cases, additional tests using human subjects will be needed to ensure performance robust to human elements (e.g. hair penetration, varied head sizes, sweating, etc).

Disclosures: W. Hairston: None. **G.A. Apker:** None.

Poster

272. Data Analysis and Statistics II

Location: Halls A-C

Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.13/UU13

Topic: G.07. Data Analysis and Statistics

Title: An approach to studying neural signals underlying acute and chronic Stress in the real-world

Authors: T. DOTY[1], *B. KELLIHAN[2], J. CANADY[2], G. APKER[1], W. HAIRSTON[1], K. MCDOWELL[1]
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Abstract: Stress has a significant societal and health impact. Of particular importance is how acute stress events affect chronic stress levels. Previous research has revealed that stress in the real world has a much larger effect on the body than stress induced in laboratory settings, but previous studies of real-world stress have been limited to the study of physiology and self report. Due to technological limitations, researchers have been unable to study the neural signature of real-world stress. Additionally, no research to date has studied the interaction of acute and chronic stress in the real-world. Therefore, we have developed a novel, real-world neuroimaging system, called the Multi-Aspect Real-world Integrated Neuroimaging (MARIN) system, optimized to study physiological phenomena in the real-world and particularly suited to the study of acute and chronic stress. This system integrates neurological data from a gel-free, wireless EEG device with physiological data from wireless cardiac and skin conductance sensors, as well as self-reports of activity and stress. Coordination of the system is managed through an Android handheld mobile device. The system provides the capability for users to indicate an acute stressful event has occurred by pressing an alert button on the mobile device. Users may also add additional information about the event, e.g. if the event happened five minutes beforehand. This capability allows for acute stress information to be time locked to the physiological and neurological data streams for post hoc processing. Additionally, several times per day the system prompts the user to perform a baseline eyes open rest task , which includes stress and mood inventories. The exact time of these inventories and baseline periods is recorded on the mobile device for post hoc processing. The level of chronic stress associated with those baseline periods is assessed via self-report, which allows for an investigation of the neural and physiological signals associated with varying levels of chronic stress. In addition, the interplay between neural and physiological signals associated with acute and chronic stress can be studied in the same user when they wear this system over multiple days in their daily life. We believe our system is an important step in demonstrating that neuroimaging can be transitioned from the laboratory into the real world and while the system is optimized for the study of stress, the advances of this approach can be applied to study other naturally occurring psychological phenomena.

Disclosures: **T. Doty:** None. **B. Kellihan:** None. **J. Canady:** None. **G. Apker:** None. **W. Hairston:** None. **K. McDowell:** None.

Poster

272. Data Analysis and Statistics II

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Time: Sunday, November 16, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 272.14/UU14

Topic: G.07. Data Analysis and Statistics

Title: Fully-Bayesian inference of time-varying synaptic weights from neural spike trains

Authors: *S. LINDERMAN[1], C. STOCK[2], R. ADAMS[1]
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Abstract: Learning and memory in the brain are implemented by complex, time-varying changes in neural circuitry. Recent innovations in neural recording technology offer unprecedented views into the brain, and motivate the development of novel statistical tools to formulate and test hypotheses about synaptic dynamics. Here we present a fully Bayesian method of discovering time-varying synaptic weights from spike train data. We build upon the generalized linear model (GLM), a widely applied model for functional interactions underlying neural spike trains. We exploit the particle MCMC algorithm to infer unobserved synaptic weight trajectories. Though our approach can handle arbitrary plasticity models, for the purposes of this abstract we focus on the well-known spike timing dependent plasticity rule (STDP). We produced synthetic data of a two-neuron system undergoing STDP (Fig. 1). Given only the spike trains and the STDP learning rule, our particle MCMC inference algorithm is able to infer an accurate posterior distribution over the hidden weight trajectories (Fig 2). For comparison, we show how a traditional GLM with static weights would compromise by either ignoring the connection and increasing the background firing rate, or by taking an average of the weight trajectory. Our probabilistic framework lays the foundation for application to biological recordings, Bayesian model comparison of learning rules, and nonparametric inference of the learning rule itself.

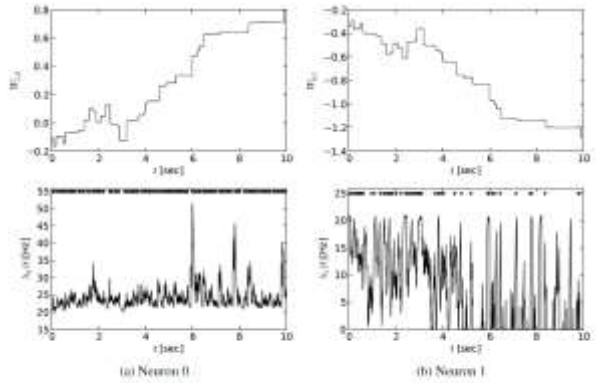

Figure 1: Example of simulated spike trains from a GLM with two recurrently connected neurons undergoing spike-timing dependent synaptic plasticity. The top row shows the evolution of the synaptic weight for neuron 1 to 0 (left) and 0 to 1 (right). The bottom row shows the induced firing rates (black line) and the spikes (black dots). The excitatory connection from 1 to 0 strengthens over time, causing increases in neuron 0's firing rate. Simultaneously, the inhibitory synapse from neuron 0 to 1 strengthens and suppresses firing of neuron 1.

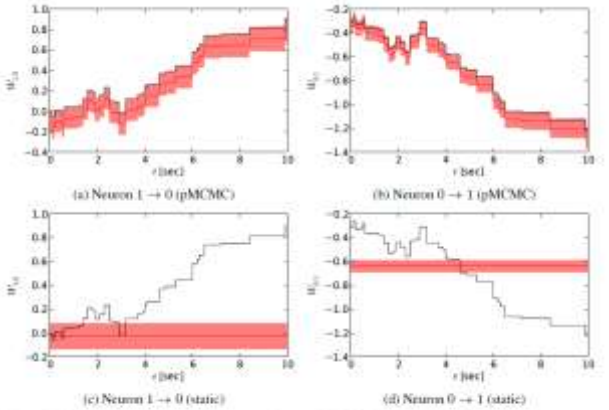

Figure 3: Inferred synaptic weight trajectory using particle MCMC with the STDP learning rule (top row). For comparison, we show the inferred weights using MCMC and a static weight model (bottom row). Both inference algorithms observe only the spike trains shown in Figure 1. Black line: true weight trajectory. Red line: mean of inferred weight trajectory. Red shaded area denotes one standard deviation about the mean.

Disclosures: **S. Linderman:** None. **C. Stock:** None. **R. Adams:** None.

Poster

272. Data Analysis and Statistics II

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Program#/Poster#: 272.15/UU15

Topic: G.07. Data Analysis and Statistics

Title: MELD: Mixed effects for large datasets

Authors: *D. M. NIELSON[1], P. B. SEDERBERG[2]
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Abstract: Technical advances are providing ever increasing amounts of data and analysis techniques have not kept pace. Large datasets, where the features far outnumber observations, are difficult to analyze because the sheer number of statistical comparisons reduces power and increases processing time. In EEG and fMRI, current statistical methods rely on the randomness of noise in time and space to circumvent multiple comparisons problems. In doing so, you lose the ability to detect tightly focussed but significant signals. Our mixed effects for large datasets (MELD) method combines the power of linear mixed effects regression (LMER) with approaches inspired by partial least squares for maximizing variance in the dimensions of interest. MELD is much faster than an element wise LMER analysis, and on simulated data MELD is more sensitive at lower subject numbers and smaller signal extents than standard

techniques, such as GLMs. When evaluated on publicly available EEG and FMRI datasets, MELD produces comparable results to standard analytical techniques.

Disclosures: D.M. Nielson: None. **P.B. Sederberg:** None.

Poster

272. Data Analysis and Statistics II

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Title: Conserved spatiotemporal motifs during evoked reverberation in cultured neuronal networks

Authors: *W. WU[1,2], L. QI[1], Y. LI[3], P.-M. LAU[1], G.-Q. BI[1,2]
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Abstract: Persistent reverberatory activity in brain circuits has been proposed to serve as a neural substrate underlying working memory and motor planning. Previously, we have reported reverberatory activities in small networks of cultured neurons (Lau and Bi, PNAS 2005). To further study the spatiotemporal dynamics of such collective neuronal activity, we grew hippocampal neurons on multi-electrode arrays (MEAs) and evoked network reverberation via electrical stimuli from single electrode. Based on the distance metric in high-dimentional feature space, we developed a new method to find conservd motifs of network activity with precise spatiotemporal patterns and to estimate statistical significance of their occurrences in a non-parametric way. After calibrating on various simulated data sets, we demonstrate that the method is reliable and robust for detecting precise neuronal firing sequences in different conditions. Using this method, we analyzed experimental data sets of network reverberation from the MEA recordings. In networks exhibiting evoked reverberation, the activity following each stimulus of a specific input site often contained a dominating motif with precision of a few milliseconds that repeated many times throughout the whole reverberation period. Interestingly, reverberation evoked at different input sites in the same network contained motifs of distinct patterns, although

sometimes they could share overlapping subpopulations of neurons in the network that might have formed a core circuit. These results demonstrate that activity motifs with precise spatiotemporal patterns could be maintained within a neuronal network during reverberation, and could potentially serve as a precise and efficient way of information coding.

Disclosures: W. Wu: None. **L. Qi:** None. **Y. Li:** None. **P. Lau:** None. **G. Bi:** None.

Poster

272. Data Analysis and Statistics II

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Program#/Poster#: 272.17/UU17

Topic: G.07. Data Analysis and Statistics

Title: Ecological fallacy in neuroscience studies: A systematic review and simulation study

Authors: *J. CRAGG[1,2], J. K. KRAMER[3], D. PATRICK[1], J. BORISOFF[2,4], M. RAMER[1,2]
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Abstract: “How does it work?” Basic neuroscience is largely concerned with establishing causal links between biological phenomena or processes in the nervous system. Accordingly, top-tier biomedical journals such as Nature Neuroscience are increasingly demanding mechanistic details connecting experimental intervention and outcome. Providing these will require avoiding inferences from ‘ecological’ analyses - those based on aggregate measures - which are at present, highly prevalent, according to our systematic review of the neuroscience literature. We use simulated data to define and demonstrate ‘ecological fallacy’ within the context of basic neurosciences sciences, and propose a simple solution to avoid this common error.

Disclosures: J. Cragg: None. **J.K. Kramer:** None. **D. Patrick:** None. **J. Borisoff:** None. **M. Ramer:** None.

Poster

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Support: Federal Ministry of Education and Research (BMBF) Germany, Grant Number 01GQ1005B

EU-FP7 MSCA IEF 330792 (DynViB)

Title: First neuronal connectomics challenge: From imaging to connectivity: From design to result harvesting and crowdpublishing

Authors: *J. G. ORLANDI[1], B. RAY[2], M. SAEED[3], J. SORIANO[1], A. STATNIKOV[2], O. STETTER[4,5], I. GUYON[6], D. BATTAGLIA[7,5]
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Abstract: We have organized a crowdsourcing challenge to reverse engineer the structure of neuronal networks from patterns of activity recorded with calcium fluorescence imaging. Unraveling the connectivity of neuronal circuits involving hundredths to tenths of thousands of neurons or more is an important step in neuroscience applications, relevant for the investigation of animal and human cognition and learning, as well as for the understanding of brain function and dysfunction. However, a direct assay of such a large number of synaptic connections is still unfeasible, and probably unpractical. Even connectomics methods based on axonal tracing are time consuming and often applicable only post-mortem. This challenge proposes to approach the problem from a different angle, by reconstructing an approximation as precise as possible to the structural connectivity of a large neuronal circuit algorithmically inferring it from the simultaneous observation of the neuronal activity of thousands of neurons, which can be achieved via state-of-the-art calcium fluorescence imaging. To stimulate the development of novel connectivity-from-activity inference algorithms by providing at a same time a way to benchmark and compare their efficiency, we resorted to competition-driven crowdsourcing. We generated simulated calcium fluorescence traces from simulated neuronal cultures of different topologies and with a realistic bursting dynamics, accounting even for artefacts such as light scattering. Participants to the challenge were ranked based on the achieved performance in reconstructing selected competition instances, with know ground-truth structural connectivity. Many of the top participants made then available their code for extensive post-challenge

validations and verifications. This allowed us to gather a rich suite of highly diverse algorithms, all with a performance significantly advancing current state of the art, thanks to a genuine knowledge transfer between usually segregated research communities. A review article, written collectively with a "crowdpublishing" approach phylosophically coherent with the challenge, will comment on challenge results and act as a reference for the use of publicly released codes.

Disclosures: J.G. Orlandi: None. **B. Ray:** None. **M. Saeed:** None. **J. Soriano:** None. **A. Statnikov:** None. **O. Stetter:** None. **I. Guyon:** None. **D. Battaglia:** None.

Poster

272. Data Analysis and Statistics II

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Topic: G.07. Data Analysis and Statistics

Title: Neural networks with combination of individual and template-based input information improves attenuation correction in quantitative PET/MR hybrid scanner settings

Authors: E. ROTA KOPS[1], *H. HAUTZEL[2], A. SANTOS RIBEIRO[3], G. ANTOCH[4], H. HERZOG[1], H.-W. MÜLLER[2], N. J. SHAH[1]
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Abstract: Aim: PET imaging for quantitative brain receptor modeling is inevitably depending on adequate attenuation correction of the raw emission data. With the introduction of hybrid PET/MR scanners attenuation data as previously derived from rotating radioactive transmission rods or CT scans is no longer available. Therefore, one approach is the use of a transmission or CT-based attenuation coefficient (AC) template. However, the use of standard AC maps is limited with regards to anatomical variability between subjects. An alternative for acquiring AC-maps with increased individual precision is the inclusion of anatomical MRI data for segmentation of skull, soft tissue and air. In this study a feed forward neural network (FFNN) algorithm for calculating AC maps is presented which uses individual MRI data and an AC template either without (FFNN1) or with (FFNN2) adding information from one corresponding individual CT scan during the training step. FFNN1 yields maps with predefined ACs for each segmented tissue while continuous AC maps are obtained by FFNN2. Methods: Up to now,

MRI, FDG PET and CT data were acquired in 13 subjects. FFNN1 used ultrashort echo time (UTE) MRI together with template-based AC maps as input data for the training (n=1) and the classification step (n=12). For training FFNN2 the network weights of FFNN1 were optimized by guiding the network's output with the corresponding CT-based AC map (n=1), leading to continuous AC maps in the classification step (n=12). The FFNN1/2 results were compared with the CT-based ones (reference standard). All classification results were evaluated by using dice coefficients D. After reconstruction of the FDG PET emission data with the AC maps derived either from FFNN1/2 or CT, the influence of the FFNNs' results was assessed in volumes of interest (AAL-atlas in MNI space). Finally, relative differences (RD) were calculated. Results: The resulting Ds showed similar values for FFNN1 and FFNN2 in whole-head bone regions (D1=0.71, D2=0.73) and in skull regions only (D1=0.79, D2=0.81). In contrast, FFNN1 demonstrated a less accurate quantification of the corresponding PET signal as compared to FFNN2 (mean RD of all ALL VOIs: FFNN1 4.6%, FFNN2 3.9%). Conclusions: Our preliminary results indicate that neural networks combining individual data from UTE MRI with general template-based information improve quantitative analyses in PET/MR hybrid scanner settings. This approach is capable for optimizing brain receptor modeling and quantification in paradigms with cognitive tasks or neuro-/psychopharmacological challenges which sought to measure cerebral blood flow by fMRI and changes in neurotransmission by PET in parallel.

Disclosures: E. Rota Kops: None. **H. Hautzel:** None. **A. Santos Ribeiro:** None. **G. Antoch:** None. **H. Herzog:** None. **H. Müller:** None. **N.J. Shah:** None.

Poster

272. Data Analysis and Statistics II

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Topic: G.07. Data Analysis and Statistics

Title: Improving fMRI data test-retest reliability using independent component analysis (ICA) denoising technique

Authors: *Y. TONG[1], K. E. HEALY[1], L. H. STERNBERG[1], E. XIAO[2], Y. XU[3], S. DAS[1], K. F. BERMAN[1], D. R. WEINBERGER[2], V. S. MATTAY[2]
[1]NIH/NIMH, Bethesda, MD; [2]The Lieber Inst. for Brain Develop., Baltimore, MD; [3]NIH/NIDCD, Bethesda, MD

Abstract: Studies have shown that fMRI time series data can be decomposed into maximally independent components (ICs) that either reflect stimulus-induced or spontaneous brain activity, or reflect scanner-related noise, head movement and physiological artifacts. Identifying and removing noise-related ICs from an individual subject's time series could potentially improve the signal to noise ratio of fMRI data. In this study, we examine whether a ICA-based noise removal method can improve the test-retest reliablity as measured by individual subject's intraclass correlation (ICC) between two fMRI sessions obtained under similar conditions. Three BOLD fMRI tasks were used to test the effectiveness of the denosing technique: 1) a modified Flanker task (Sambataro, 2013) with 36 subjects (11 male); 2) an affective face matching task (FMT; Hariri, 2002) with 36 subjects (10 male); 3) a picture encoding and retrieval task (PEAR; Hariri, 2003) with 34 subjects (9 male). Healthy subjects (age = 18 - 48 yrs old) underwent BOLD fMRI on the same GE 3T MRI scanner, while they performed these tasks under similar conditions on two separate occassions. Images were preprocessed in SPM8. A pre-selected contrast image was generated per subject per session for each task: the response inhibition contrast map for the Flanker task; the face matching contrast map for the FMT task; and the neutral picture encoding contrast map for the PEAR task. Denoising of the fMRI time series data was performed using an ICA approach. Using the GIFT toolbox, each task related fMRI time series was decomposed into 60 ICs. The systematic classification of artifactual and neuronal activity related ICs was based on their degree of spatial clustering, location of major positively weighted clusters and neighborhood connectedness between positively and negatively weighted clusters (Xu, submitted). The noise components identified through an automated procedure were subtracted from the original dataset. To assess the effect of denoising on test-retest reliability, a whole brain voxel-wise ICC was computed between each subject's contrast maps for each task from the two sessions (Kristo, 2014). We also compared the temporal signal-to-noise ratio (TSNR) between the denoised and non-denoised data. As expected, the ICA denoising method significantly improved within-subject whole brain ICC values for these tasks (Flanker: $p < 0.03$; FMT: $p < 0.005$; PEAR: $p < 0.01$). The TSNR values were also greatly improved in the denoised data ($p < 0.000$ for all tasks). These results indicate that the ICA noise removal approach can be a useful tool to improve the test-retest reliability of fMRI data.

Disclosures: **Y. Tong:** None. **K.E. Healy:** None. **L.H. Sternberg:** None. **E. Xiao:** None. **Y. Xu:** None. **S. Das:** None. **K.F. Berman:** None. **D.R. Weinberger:** None. **V.S. Mattay:** None.